Supporting Information

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SI Materials and Methods

Cell Culture. Human osteosarcoma U2OS, human foreskin fibroblast (HFF), Huh7.5, HeLa, and HT1080 cells were cultured in DMEM supplemented with 10% (vol/vol) FBS and antibiotics. HFF cells were provided by Jon Boyle (Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA). Human umbilical vein endothelial cells (HUVECs) and human uterine microvascular endothelial cells (HUtMVECs) were cultured in phenol red–free Endothelial Cell Growth Medium-2 (EGM-2; Lonza) supplemented with 5% (vol/vol) FBS and antibiotics. Primary placental fibroblasts were isolated and maintained in DMEM supplemented with 10% (vol/vol) FBS, Hepes, L-glutamine, and antibiotics. Vero African green monkey kidney cells were maintained in DMEM supplemented with 5% (vol/vol) FBS and antibiotics. BeWo cells were maintained in F12K Kaighn's modified medium supplemented with 10% (vol/vol) FBS and antibiotics. Caco-2 (ATCC clone) human intestinal epithelial cells were cultured in MEM supplemented with 10% (vol/vol) FBS, nonessential amino acids, sodium pyruvate, and antibiotics.

RNA Isolation, Quantitative RT-PCR, and RNAseq. For cellular mRNA analysis, total RNA was extracted using TRI reagent (MRC) or RNeasy (Qiagen) according to manufacturer's protocol. RNA samples were treated with RNase-free DNase (Qiagen). Total RNA was reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad), high-capacity cDNA kit (Life Technologies) or RT^2 First Strand kit (SABiosciences). For each sample, 1 μg RNA was used for cDNA synthesis. Quantitative RT-PCR (qRT-PCR) was performed using iQ SYBR Green Supermix (Bio-Rad) in an Applied Biosystems StepOnePlus real-time PCR machine or ViiA 7 system (Life Technologies), according to the manufacturer's instructions. Gene expression was calculated using the 2-ΔΔCT method (1), normalized to human β-actin. Autophagy and Toll-like receptor (TLR) qRT-PCR arrays (SABiosciences) were performed with 1 μg RNA per 96-well plate and subjected to qRT-PCR using SYBR/ROX RT^2 qPCR 2× master mix (SABiosciences) according to the manufacturer's protocol. Gene expression was defined from the threshold cycle (Ct), and relative expression levels were calculated using SABiosciences RT^2 Profiler PCR array analysis automated software (http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php).

RNA library construction and microRNA (miRNA) sequencing was performed by Ocean Ridge Biosciences using extracted RNA as noted above. The small RNA libraries were aligned to the NCBI-37 human reference genome using Bowtie and then intersected with the mature miRNA sequenced annotated by miRBase (v.18) using BEDtools. The miRNA counts in each library were normalized using established algorithms (2). The chromosome 19 miRNA cluster (C19MC) miRNAs and the non-C19MC miRNAs in the six libraries were Laplace smoothed by adding 1 to the normalized counts, log2 transformed, and clustered by the agglomerative hierarchical clustering, using the complete linkage method. Heat maps were then generated separately for the clustered C19MC miRNAs and non-C19MC miRNAs. To quantify the differences in miRNA expression between U2OS cells that were exposed to primary human trophoblast (PHT) conditioned medium vs. cells that were exposed to nonconditioned medium, we applied the differential expression test that assumes that, in all of the libraries, miRNA counts follow negative binomial distributions (2), and used a shrinkage estimator for the dispersion parameters of the miRNAs. The *P* values of the tests were adjusted using the Benjamini and Hochberg's method (3) to control for false discovery rate. Statistical analyses were performed using statistical computing software R and the DESeq package of R (4).

Electron Microscopy. Cells were washed, fixed with 2.5% glutaraldehyde in PBS for 1 h, and then processed for electron microscopy as previously described (5). Sections were imaged using a JEOL JEM 1011 transmission electron microscope (JEOL) at 80 kV fitted with a side mount AMT 2k digital camera (Advanced Microscopy Techniques). At least 5–10 individual cells were captured per condition per replicate. The number of autophagosomes per cell (including amphisomes, autophagosomes, autophagic vacuoles, and autolysosomes) was manually quantified.

C19MC Bacterial Artificial Chromosome Preparation and Transfection. The bacterial artificial chromosome (BAC) RP11-1055O17 containing 160,970 bp of genomic DNA from region q13-42 of chromosome 19 was obtained from the BACPAC Resource Center located at the Children's Hospital Oakland Research Institute (CHORI). The BAC clone harbors the entire C19MC miRNA cluster spanning nearly 100 kb and contains an additional 60 kb of flanking sequences. Recombineering of the BAC was performed as described (6). A GFP::zeocin cassette from the pSELECT-GFPzeo-mcs plasmid (InvivoGen) was PCR amplified and cloned into the HindIII and BamHI sites of pBluescript II SK(+) (pBS-SK). BAC specific homology arms (each ∼500 bp) were PCR amplified and cloned into pBS-SK using the restriction sites flanking the GFP::zeocin cassette (5′arm: XhoI and HindIII; 3′arm: BamHI and XbaI). The targeting cassette was then PCR amplified, gel purified, and electroporated into the recombinogenic SW106 bacterial strain containing the recipient BAC. A control BAC was also constructed that harbors a deletion of the entire C19MC coding sequence. Choosing a 5′ homology arm located upstream of the miRNA cluster and keeping the same 3′ homology arm, recombination led to a deletion (BAC "trimming") of the entire miRNA locus (∼100 kb), leaving the ∼60 kb of genomic DNA flanking the GFP::zeocin cassette intact. Bacteria harboring the BAC with the desired alteration were selected with chloramphenicol (12.5 mg/mL) and zeocin (25 mg/mL; Nitrogen Life Technologies). The correct BAC constructs, confirmed by restriction mapping and PCR, were transformed back into DH10B bacterial cells for propagation. BAC DNA for transfection was prepared using the PhasePrep BAC DNA kit, following the manufacturer's instructions (Sigma).

Pharmacological Agents. Cells were pretreated with 3-methyladenine (3-MA; 5 mM; Sigma) for 30–60 min before infection, and the drug was incubated throughout the duration of infection. As a positive control for cell death, cells were treated with staurosporine (1 μM; Sigma) for 16 h. For mRFP-LC3b punctae assays, 3-MA was added for 30 min before nonconditioned or conditioned media exposure and was present throughout. Rapamycin (5 μM; Calbiochem) treatment or serum starvation with Hank's balanced salt solution (HBSS; Gibco, Life Technologies) for 4 h was used as a positive control for autophagy.

Antibodies. Mouse anti–VSV-G, mouse anti–hCMV gB glycoprotein, rabbit anti-IRF3, rabbit anti-p65, and goat anti–β-actin antibodies were obtained from Santa Cruz Biotechnology. Mouse anti-enterovirus VP1 (NCL-Entero) was purchased from

Novacastra Laboratories (Leica Biosystems). Rabbit anti-LC3b antibodies were purchased from Abcam. Rabbit anti-p62 and rabbit anti–beclin-1 antibodies were obtained from Cell Signaling Technology.

Immunofluorescence and Confocal Microscopy. Cell monolayers were cultured in eight-well chamber slides (Nunc LabTek) at 37 °C. Cells were then washed and fixed as indicated with either ice cold methanol, 3:1 methanol-acetone, or 4% paraformaldehyde (PFA) in PBS and permeabilized with 0.25% Triton X-100 in PBS. Fixed monolayers were incubated with the primary antibody, washed, incubated with Alexa Fluor 488–or 594–conjugated secondary antibodies (Invitrogen), washed, and then mounted with Vectashield (Vector Laboratories) containing DAPI. Cholera toxin B (CTB) conjugated to Alexa Fluor 488 (8 μg/mL; Invitrogen) and transferrin conjugated to Alexa Fluor 594 (Invitrogen) uptake was performed essentially as previously described (7). Images were captured with an IX81 inverted microscope equipped with a motorized stage or with an Olympus FluoView 1000 laser scanning confocal microscope. Images of infected cells were taken using an Olympus UPlanApo 10×/0.4 NA dry or UApo 20×/0.75 NA dry objective, whereas all other images were taken with an UPlanSApo 60×/1.35 NA oil objective.

For virus imaging, cells were fixed and stained for markers of virus infection [coxsackievirus B3 (CVB), poliovirus (PV) (VP1), vesicular stomatitis virus (VSV) (VSV-G), human cytomegalovirus (hCMV) (gB)] or assessed for fluorescence expression [vaccinia virus (VV)-YFP, herpes simplex virus-1 (HSV-1)-GFP, VSV-GFP)] A minimum of three independent fields per condition were counted (at least 500 cells total) per replicate. Infection levels are reported as the percentage of virus positive cells among the total number of cells, determined by DAPI staining using ImageJ (National Institutes of Health) analysis. For LC3b autophagy assays, at least 20 individual cells from a minimum of four independent fields were captured per condition. The total number of mRFP-LC3b–positive punctae was quantified per cell using ImageJ analysis with identical settings per condition. Analysis of the extent of VSV and mRFP-LC3b punctae colocalization was performed using ImageJ.

Measurements of Cell Viability. U2OS cells exposed to nonconditioned or conditioned PHT medium for ∼24 h, or cells expressing the entire C19MC or control BAC, were subjected to trypan blue or 7-aminoactinomycin D (7-AAD) exclusion assays. Cells were incubated with either trypan blue (0.2%) or 7-AAD (5 μg/mL) for 5–10 min and washed with PBS, and images were captured using an IX81 inverted Olympus microscope (described in detail above). Relative cell viability was quantified using a TC10 automated cell counter (Bio-Rad) in cells incubated with trypan blue.

Reporter Gene Assay. Activation of IFNβ or IFN-stimulated response element (ISRE) promoters was measured by reporter assay. Cells were transfected with 1 μg of DNA per well of a 24-well plate, a 30:1 ratio of IFNβ, or ISRE firefly luciferase reporter plasmids to pRL-null (Renilla control) as per the manufacturer's protocol. Cells were lysed in 100 μL of lysis buffer, and the levels of firefly and renilla luciferase levels were quantified using the Dual-Luciferase Reporter Assay System (Promega) with a dual injector equipped Synergy 2 SL Luminescence Microplate Reader (BioTek). Levels of firefly luciferase were normalized to control renilla luciferase levels. For poly(I:C) treatment, cells were transfected with 1 μg poly(I:C) per well using XtremeGene-9 for 16 h as per the manufacturer's protocol.

Immunoblots. Cells were grown in six-well plates, and all lysates prepared with RIPA buffer [50 mM Tris·HCl (pH 7.4); 1% Nonidet P-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM phenylmethanesulfonyl fluoride; 1 mg/mL aprotinin, leupeptin, and pepstatin; 1 mM sodium orthovanadate]. For beclin-1 immunoblotting, cells were lysed on ice in a 50 mM Tris·HCl, pH 7.5, buffer containing 150 mM NaCl and 0.5% Nonidet P-40. Insoluble material was precipitated by brief centrifugation. Protein concentration of lysates was determined by BCA protein assay (Thermo Scientific). Lysates containing equal amounts of protein were loaded onto 10% (beclin-1) or 15% (LC3b) gels (Bio-Rad) and transferred to PVDF or nitrocellulose membranes. Membranes were blocked in 5% nonfat dry milk, probed with the indicated antibodies, and developed with HRP-conjugated secondary antibodies (Santa Cruz Biotechnology) and SuperSignal West Pico or Dura chemiluminescent substrates (Thermo Scientific). Densitometry was performed using Image J.

Virus Entry Assays. Virus entry assays in PHT cells were performed with CVB and PV as described (8, 9). VV and HSV-1 internalization assays were performed by incubating PHT cells with virus [multiplicity of infection (MOI) = 25] at 37 °C until fixation at various time points (30, 60, and 90 min). VSV entry assays in U2OS cells exposed to either nonconditioned or conditioned PHT medium for 24 h was performed by incubating cells with virus (MOI = 500) for 1 h at 37 °C until fixation in 4% PFA followed by permeabilization in 0.25% Triton X-100. VSV particles were visualized with anti–VSV-G antibody.

qPCR Primers. Primers used were as follows: actin (5′-ACTGGGACGACATGGAGAAAA-3′; 5′-GCCACACGCAGCTC-3′), VSV (5′-TGCAAGGAAAGCATTGAACAA-3′; 5′-GAGGAGTCACCTGGACAATCACT-3′), GFP (5′-CACATGAAGCAGCACGACTTCT-3′; 5′-AACTCCAGCAGGACCATGTGAT-3′), CMV Towne strain (5′-GCGGTGGTTGCCCAACAGGA-3′; 5′-ACGACCCGTGGTCATCTTTA-3′), Tk (5′-ACC CGC TTAACAGCGTCAACA-3′; 5′-CCAAAGAGGTGCGGGAGTTT-3′), VV rpo35 early (5′-GCCAATGAGGGTTCGAGTTC-3′; 5′-AACAACATCCCGTCGTTCATC-3′), CVB3 (5′-ACGAATCCCAGTGTGTTTTGG-3′; 5′-TGCTCAAAAACGGTATGGACAT-3′), p62 (5′-TGTGAATTTCCTGAAGAACG-3′; 5′-TCGATATCAACTTCAATGCC-3′), and ISG56 (5′-CAACCAAGCAAATGTGAGGA-3′; 5′-GGAGACTTGCCTGGTGAAAA-3′).

Modified TCID50 Virus Titering Assays. Vero or PHT cells were seeded to confluence in 96-well plates. Cells were incubated with serial dilutions of the indicated viruses for ∼40–45 h and then stained with 0.05% crystal violet (in 10% ethanol). For experiments performed with conditioned medium, Vero cells were incubated in nonconditioned or conditioned medium 24 h before incubation with virus. Serial dilutions of virus were made in either nonconditioned or conditioned medium, and cells were incubated and developed with crystal violet as described above.

Neutralizing Virus Plaque Assays. VSV virus stock was diluted 1:20 in either nonconditioned or conditioned PHT medium and then incubated at 37 °C for 1 h. Plaques assays were performed on Vero cells. Plaques were visualized after 36 h by staining with crystal violet.

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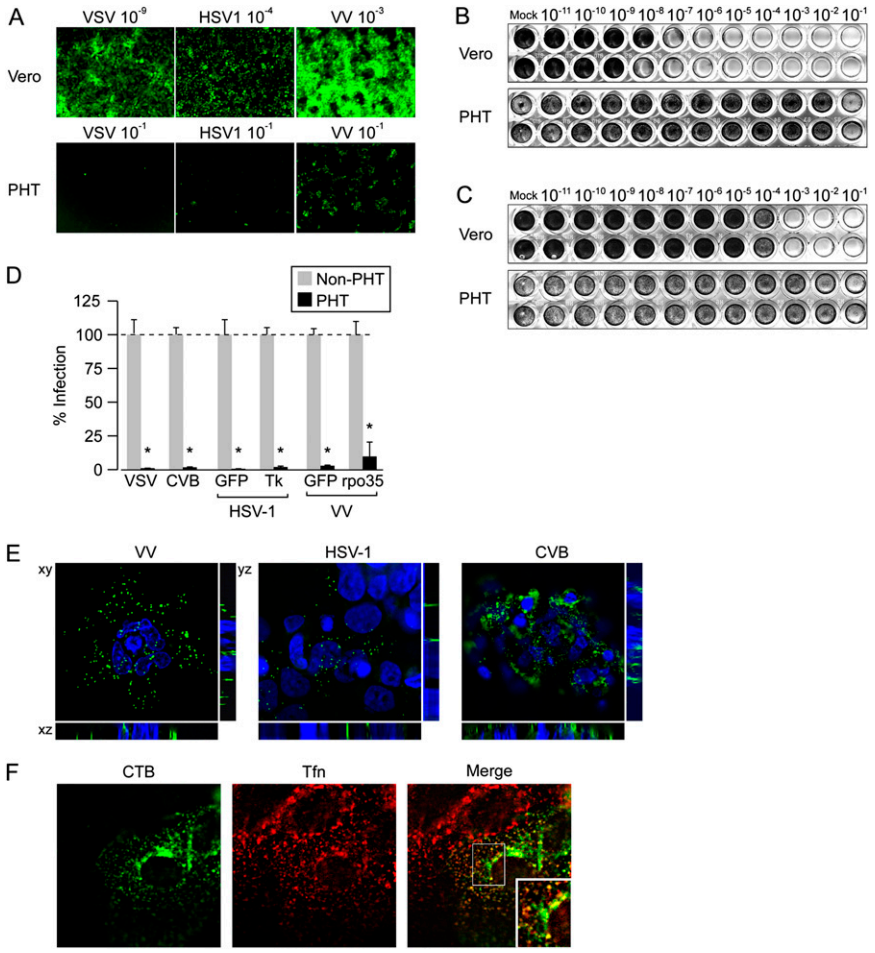

Fig. S1. PHT cells are resistant to viral infections by a mechanism independent from defects in virus binding and/or entry. (*A*) Vero (*Upper*) or PHT (*Lower*) cells were infected with the indicated dilutions of GFP-VSV, GFP-HSV-1, or YFP-VV for ∼40–45 h. Shown are representative immunofluorescent images. (*B*) TCID50 assays for VSV in either Vero (*Upper*) or PHT (*Lower*) cells. Cells were infected with the indicated serial dilutions of virus for ∼40–45 h and then stained with crystal violet. (*C*) TCID50 assays for CVB in either Vero (*Upper*) or PHT (*Lower*) cells. Cells were infected with the indicated serial dilutions of virus for ∼40–45 h and then stained with crystal violet. (*D*) PHT and U2OS cells were infected in parallel with CVB, VSV, HSV-1, or VV for ∼6–7 h. Relative CVB, VSV, HSV-1 (VP26-GFP or early gene Tk), or VV (GFP or early gene rpo35) RNA was assessed by qRT-PCR (*$P < 0.0001$). (*E*) Virus entry assays were performed in PHT cells with PV, HSV-1, VV, or CVB (60 min after infection). DAPI-stained nuclei are shown in blue, and viruses are shown in green. (*F*) CTB (green) and transferrin (Tfn) (red) uptake in PHT cells. PHT cells were exposed to Alexa Fluor 488–conjugated CTB and Alexa Fluor 594–conjugated Tfn for 60 min at 37 °C, fixed, and assessed using confocal microscopy. Shown are representative images of internalized CTB and Tfn. (*Inset*) 3× zoom.

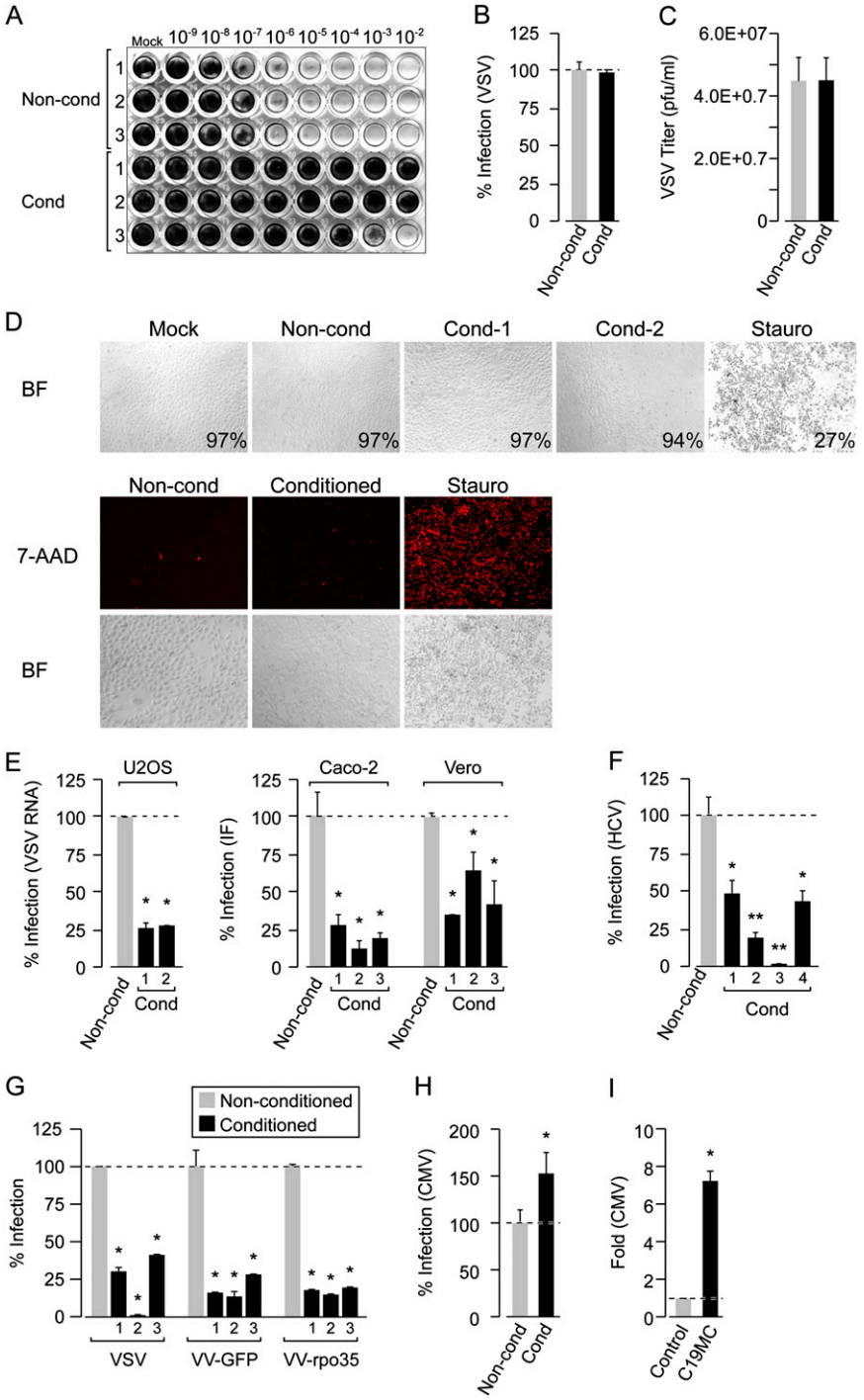

Fig. S2. Medium from different preparations of PHT cells confers an antiviral effect on recipient cells. (*A*) TCID50 assays for VSV in Vero cells pretreated for 24 h in (*Upper*) nonconditioned (Noncond) medium (in triplicate) or (*Lower*) three independent preparations of conditioned (Cond) PHT medium. Cells were infected in the indicated dilution of virus in the presence of nonconditioned or conditioned medium for ∼40–45 h and then stained with crystal violet. (*B*) Vero cells were exposed to nonconditioned (Noncond) or conditioned (Cond) medium isolated from BeWo cells for 24 h and then infected with VSV. Shown is the percent of infected cells [as assessed by immunofluorescence (IF)]. (*C*) VSV was incubated in nonconditioned (Noncond) or conditioned (Cond) PHT medium (in the absence of cells) for 1 h at 37 °C, then plaque assays performed. Shown are VSV titers (in PFU/mL). (*D*) (*Upper*) Brightfield images captured from U2OS cells exposed to nonconditioned (Noncond) or conditioned (Cond) PHT medium (two independent preparations) or treated with staurosporine as a positive control and incubated with trypan blue. Shown is the percent cell viability (as quantified using a TC10 automated cell counter) in the bottom right corner. (*Lower*) U2OS cells exposed to nonconditioned or conditioned PHT medium or staurosporine as a positive control were incubated with 7-AAD. (*Upper*) 7-AAD fluorescence. (*Lower*) Corresponding brightfield (BF) images. (*E*) (*Left*) U2OS cells were exposed to nonconditioned (Noncond) or conditioned media (Cond) from two independent PHT preparations and infected with VSV. Relative VSV RNA was assessed by qRT-PCR ($*P < 0.0001$). (*Right*) Caco-2 or Vero cells were exposed to nonconditioned or conditioned PHT medium isolated from three independent preparations of PHT cells for 24 h before infection with VSV. Shown is the percent of infected cells (as assessed by IF; $*P < 0.0005$). (*F*) Huh7.5 cells were exposed to nonconditioned (Noncond) or conditioned (Cond) medium isolated from four independent preparations of PHT cells for 24 h before infection with HCV. Shown is percent infection as assessed by luciferase assay ($*P < 0.005$, $**P \leq 0.0005$). (*G*) U2OS cells exposed to nonconditioned or conditioned PHT medium were infected with VSV or VV for ∼6 h. Relative VSV or VV

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(early gene rpo35 or early gene GFP). RNA was assessed by qRT-PCR (*$P < 0.0001$). (*H*) HFF cells were exposed to nonconditioned (Noncond) or conditioned (Cond) PHT media for 24 h before and during infection with CMV. Shown is the percent of infected cells (assessed by IF; *$P < 0.05$). (*I*) U2OS cells stably expressing control or C19MC BAC were infected with CMV, and infection levels were assessed by qRT-PCR. Data are shown as fold change over control (*$P < 0.0001$). In *B*, *C*, and *E–I*, data are displayed as mean ± SD and are representative of experiments performed a minimum of three times.

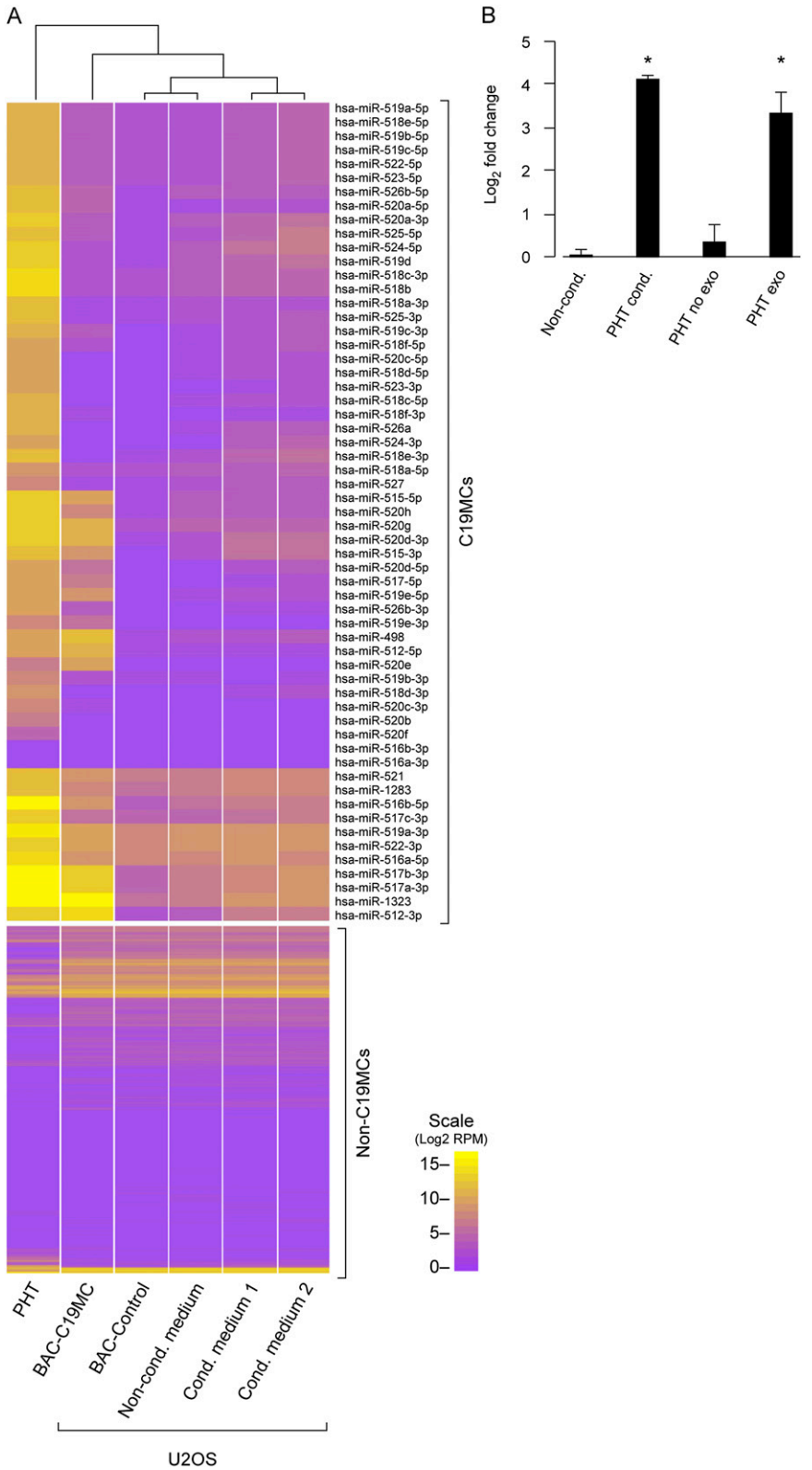

Fig. S3. (*A*) Heatmap depicting C19MC miRNA expression in either PHT or U2OS cells. U2OS cells stably expressing C19MC-encoding BAC or exposed to PHT conditioned (Cond) medium, as described in *Materials and Methods* and *SI Materials and Methods*. (*Upper*) Expression of C19MCs. (*Lower*) Expression of non-C19MC miRNAs. The graded colors are proportional to the expression levels (see scale at bottom right), expressed as reads-per-million (RPM). Data clustering for C19MC miRNAs, performed as described in *SI Materials and Methods*, is shown above the upper panel. The differences in miRNA expression between U2OS cells exposed to nonconditioned or conditioned medium was statistically analyzed in Table S1. (*B*) The expression of miR-517-3p in U2OS cells exposed to nonconditioned, conditioned, exosome-depleted conditioned medium, or exosomes purified from PHT medium, as described in *Materials and Methods*. miRNA expression was determined using qRT-PCR in three independent experiments, and mean ± SD fold change of all three experiments is shown using a $\log_2$ scale compared with control, nonconditioned medium.

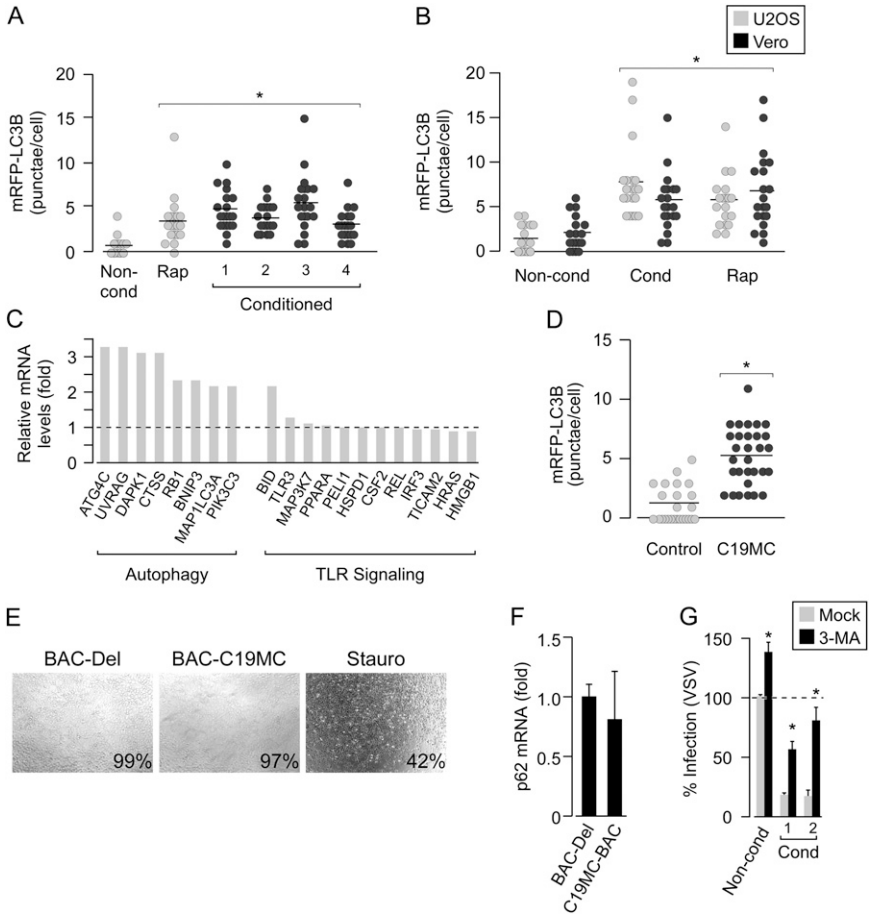

Fig. S4. Medium from PHT cells induces autophagy in recipient cells. (*A*) Vero cells were transfected with mRFP-LC3b and at 24 h posttransfection were exposed for 24 h to either nonconditioned (Noncond) or conditioned medium isolated from four independent PHT preparations. Cells were exposed to rapamycin (Rap) as a positive control. Shown are the levels of autophagic induction as determined by quantification of mRFP-LC3b–positive punctae by confocal microscopy (*$P < 0.0001$). (*B*) Vero and U2OS cells were transfected with mRFP-LC3b and then exposed to nonconditioned (Noncond) or conditioned (Cond) PHT medium 24 h posttransfection. Cells were exposed to rapamycin (Rap) as a positive control. Shown are the levels of autophagic induction as determined by quantification of mRFP-LC3b–positive punctae by confocal microscopy (*$P < 0.0001$). (*C*) Relative mRNA levels in U2OS cells exposed to nonconditioned or conditioned PHT medium for 24 h and analyzed using autophagy or TLR-targeted qRT-PCR arrays. (*D*) U2OS cells stably expressing a control or C19MC BAC were transfected with mRFP-LC3b, fixed after 48 h, and analyzed for mRFP-LC3b punctae by confocal microscopy (*$P < 0.0001$). (*E*) Brightfield images captured from control (BAC-Del) or C19MC-expressing (BAC-C19MC) U2OS cells or in cells treated with staurosporine as a positive control and incubated with trypan blue. Percent cell viability (as quantified using a TC10 automated cell counter) is shown in the bottom right corner. (*F*) RNA levels of p62 in control (BAC-Del) or C19MC-expressing (C19MC-BAC) U2OS cells as assessed by qRT-PCR. (*G*) HT1080 cells were exposed to two preparations of conditioned PHT medium in the absence (mock) or presence of 3-MA before and during VSV infection. Relative VSV RNA was analyzed by qRT-PCR (*$P < 0.05$).

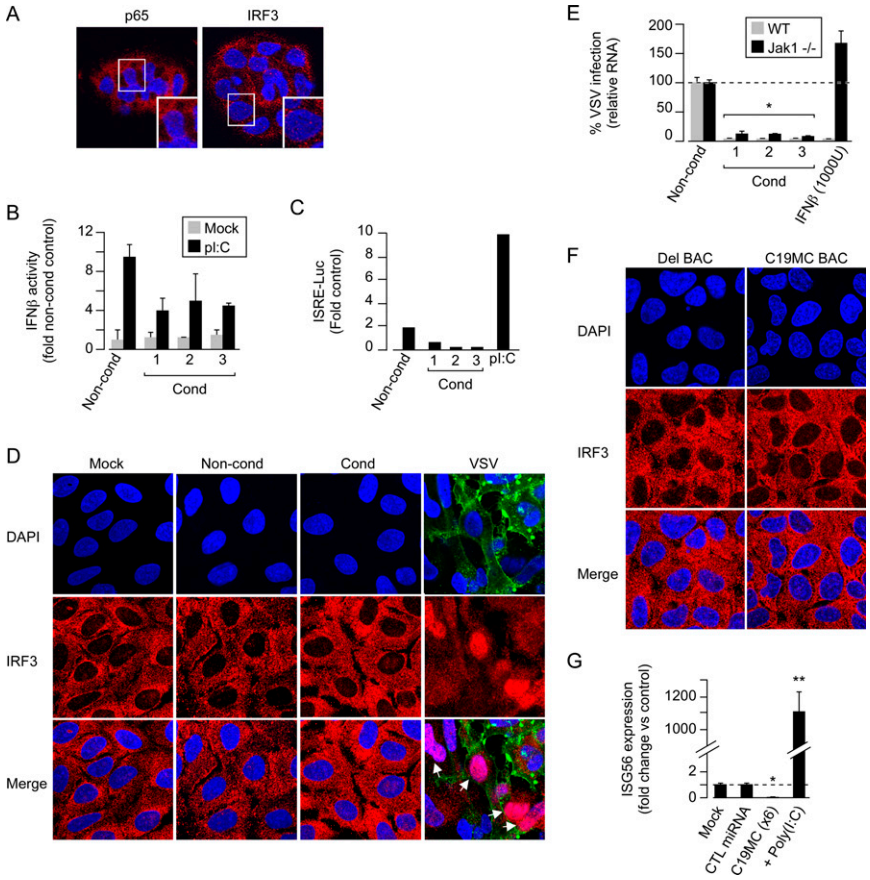

Fig. S5. Antiviral effects of conditioned PHT medium is not due to type I–mediated IFN signaling. (*A*) Confocal micrographs of PHT cells stained with anti–NF-κB p65 subunit or IRF3 antibodies. DAPI-stained nuclei are shown in blue. (*B*) IFN-β luciferase assays in U2OS cells exposed to nonconditioned (Noncond) medium or three independent preparations of conditioned (Cond) PHT medium. In parallel, exposed cells were transfected with 1 μg poly I:C. (*C*) ISRE luciferase assays in U2OS cells exposed to nonconditioned (Noncond) medium or three independent preparations of conditioned (Cond) PHT medium. As a positive control, naïve cells were transfected with 1 μg poly I:C. (*D*) Confocal microscopy for IRF3 (red) in control (mock-exposed) U2OS cells or cells exposed to non-conditioned or conditioned (Cond) PHT medium. As a positive control, naïve cells were infected with VSV (far right, green). DAPI-stained nuclei are shown in blue. (*E*) Control (WT, 2fTGH) or JAK-deficient (U4A) HT1080 cells were exposed to nonconditioned (Noncond) PHT medium or three independent preparations of conditioned (Cond) PHT medium and infected with VSV. In parallel, cells were exposed to 1,000 U of purified human IFNβ. Shown are the relative amounts of VSV RNA as assessed by qRT-PCR and normalized to nonconditioned–treated cells (*$P < 0.0001$). (*F*) Confocal microscopy of IRF3 (red) and DAPI-stained nuclei (blue) in control Del-BAC or C19MC-expressing BAC U2OS cells. (*G*) ISG56 mRNA levels as assessed by qRT-PCR in control (nontransfected) U2OS cells or cells transfected with control (CTL) miRNA mimics or mimics of the highest six expressed C19MC-associated mimics (*$P < 0.05$, **$P < 0.0001$). In parallel, cells were transfected with 1 μg poly(I:C). In *B*, *C*, *E*, and *G*, data are displayed as mean ± SD and are representative of experiments performed at least three times.

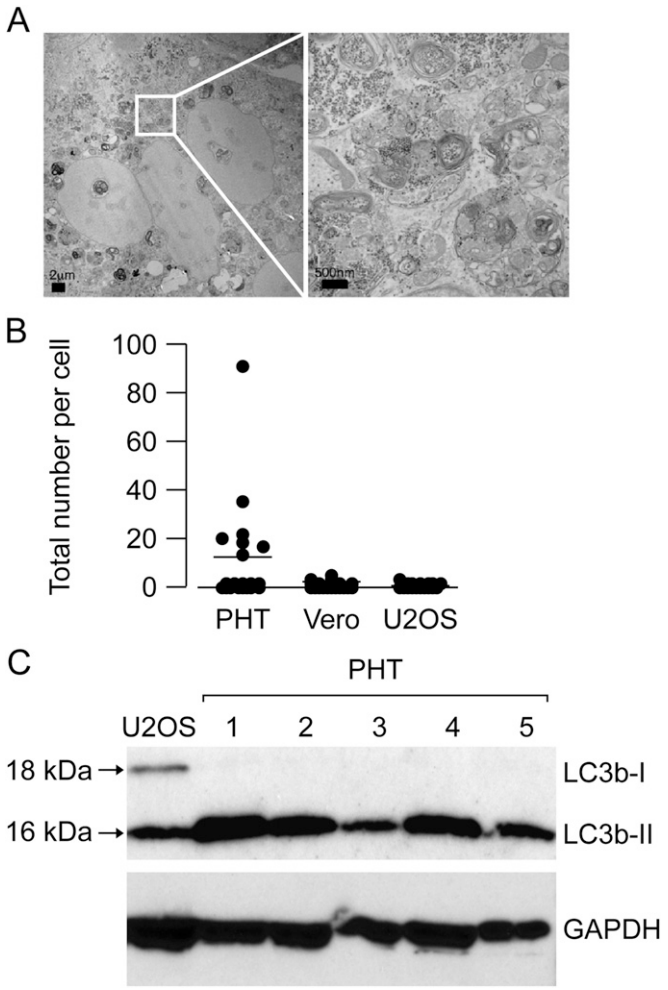

Fig. S6. PHT cells exhibit high levels of autophagy. (*A*) Electron micrographs of PHT cells. (*Left*) Magnification: 4,000×. (*Right*) Zoomed image of area shown in white box at left. (*B*) Quantification of electron micrographs for autophagosomes/autolysosomes in naïve PHT, Vero, or U2OS cells. (*C*) Immunoblot analysis for LC3b from lysates of U2OS cells or five independent preparations of PHT cells or GAPDH as a loading control.

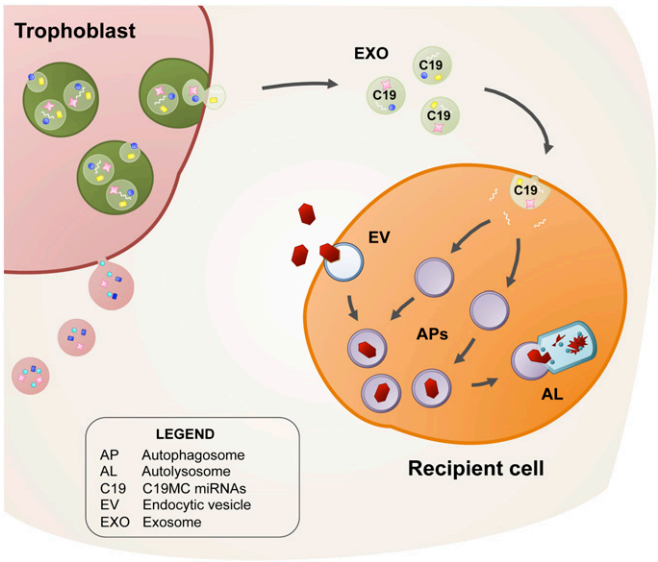

Fig. S7. A schematic figure depicting the exosome-mediated transfer of C19MC miRNAs (C19) to recipient cells and the induction of viral resistance. Primary placental trophoblasts release exosomes (EXO) containing C19MC miRNAs, which are taken up by recipient cells, thereby mediating C19MC miRNA-dependent autophagy. Incoming viral particles (in red) are likely trafficked in endocytic vesicles (EV) from the endosomal pathway into preexisting autophagosomes (APs), which then fuse with lysosomes to form autolysosomes (AL), as a mechanism to degrade these virus-containing vesicles.

Table S1. Differences in miRNA expression between U2OS cells exposed to conditioned or nonconditioned medium, analyzed by RNAseq

C19MC miRNA	Conditioned medium	Nonconditioned medium	Fold change (CM/FM)	Log2 fold change	*P*	*P* adjusted (BH)
miR-517–3p	2,374.403	765.215	3.103	1.634	2.66E−32	4.68E−30
miR-519a-3p	1,738.835	1,144.108	1.520	0.604	9.81E−07	4.60E−05
miR-522–3p	1,662.638	1,245.641	1.335	0.417	0.001083036	0.021856025
miR-1323	1,454.781	459.377	3.167	1.663	8.06E−35	3.31E−32
miR-516a-5p	1,093.955	635.203	1.722	0.784	7.75E−09	4.34E−07
miR-521	631.942	494.047	1.279	0.355	0.014010033	0.215579384
miR-1283	565.757	341.747	1.655	0.727	4.06E−06	0.000166442
miR-516b-5p	424.862	188.208	2.257	1.175	1.08E−13	9.49E−12
miR-512–3p	337.442	44.576	7.570	2.920	3.44E−38	2.48E−35
miR-524–5p	269.386	37.146	7.252	2.858	2.06E−33	6.34E−31
miR-515–3p	249.009	17.335	14.365	3.844	4.03E−38	2.48E−35
miR-517c-3p	228.786	116.392	1.966	0.975	2.02E−07	1.03E−05
miR-525–5p	216.140	26.002	8.312	3.055	2.73E−30	4.20E−28
miR-520d-3p	200.409	21.050	9.521	3.251	4.39E−30	6.01E−28
miR-520a-3p	141.669	35.908	3.945	1.980	2.43E−14	2.50E−12
miR-518e-3p	122.653	16.097	7.620	2.930	4.67E−19	5.75E−17
miR-519d	113.300	38.385	2.952	1.562	4.05E−09	2.37E−07
miR-518b	105.055	58.196	1.805	0.852	0.000622055	0.013434194
miR-518c-3p	76.726	32.194	2.383	1.253	2.34E−05	0.000823018
miR-518a-5p	70.166	34.670	2.024	1.017	0.002164332	0.040989113
miR-520g	65.535	61.911	1.059	0.082	0.848007306	1
miR-518e-5p	65.366	26.002	2.514	1.330	5.44E−05	0.001594773
miR-519a-5p	65.366	26.002	2.514	1.330	5.44E−05	0.001594773
miR-519b-5p	65.366	26.002	2.514	1.330	5.44E−05	0.001594773
miR-519c-5p	65.366	26.002	2.514	1.330	5.44E−05	0.001594773
miR-522–5p	65.366	26.002	2.514	1.330	5.44E−05	0.001594773
miR-523–5p	65.366	26.002	2.514	1.330	5.44E−05	0.001594773
miR-524–3p	57.113	2.476	23.063	4.527	1.85E−13	1.42E−11
miR-520h	54.554	34.670	1.574	0.654	0.048469819	0.547397683
miR-526b-5p	48.663	28.479	1.709	0.773	0.020670659	0.277189278
miR-515–5p	43.639	34.670	1.259	0.332	0.311332029	1
miR-498	38.128	17.335	2.199	1.137	0.003592299	0.06503118
miR-527	34.719	17.335	2.003	1.002	0.032552612	0.396755106
miR-526a	33.466	8.667	3.861	1.949	0.000139813	0.00374151
miR-519c-3p	32.153	4.953	6.492	2.699	3.16E−06	0.000138861
miR-520a-5p	26.509	11.144	2.379	1.250	0.014724591	0.223777425
miR-518f-5p	25.322	4.953	5.113	2.354	0.000166051	0.004258507
miR-520d-5p	24.485	3.715	6.591	2.721	4.17E−05	0.001426038
miR-518a-3p	24.118	17.335	1.391	0.476	0.300976907	1
miR-525–3p	20.871	14.859	1.405	0.490	0.270466957	1
miR-519e-5p	17.257	4.953	3.484	1.801	0.008673863	0.14013828
miR-518d-5p	16.733	3.715	4.505	2.171	0.005073798	0.086747848
miR-520c-5p	16.733	3.715	4.505	2.171	0.005073798	0.086747848
miR-518c-5p	14.673	7.429	1.975	0.982	0.150512908	1
miR-523–3p	14.119	3.715	3.801	1.926	0.016108611	0.241825612
miR-518d-3p	11.457	1.238	9.252	3.210	0.006515171	0.108380746
miR-517–5p	9.017	1.238	7.282	2.864	0.018394499	0.263298001
miR-512–5p	8.716	8.667	1.006	0.008	1	1
miR-518f-3p	7.288	2.476	2.943	1.557	0.215985658	1
miR-519b-3p	7.132	4.953	1.440	0.526	0.60445537	1
miR-526b-3p	6.258	1.238	5.054	2.338	0.13991013	1
miR-520e	2.789	3.715	0.751	−0.414	0.91188741	1
miR-519e-3p	1.506	0.000	Inf	Inf	0.579448235	1
miR-520b	1.458	1.238	1.177	0.235	1	1
miR-520c-3p	1.157	0.000	Inf	Inf	0.94380054	1
miR-520f	0.428	0.000	Inf	Inf	1	1
miR-516a-3p	0.000	0.000	NA	NA	NA	NA
miR-516b-3p	0.000	0.000	NA	NA	NA	NA

Counts were normalized by the median of ratio of the observed counts in each library to the geometric mean of the observed counts of all libraries, as described in *Materials and Methods*. Columns of conditioned and nonconditioned medium represent the mean of the normalized miRNAs counts in those samples. Inf, infinity; NA, not available.

Table S2. Groups of mimics to C19MC miRNAs used in our experiments

Sixteen C19MC miRNAs	Subgroup 1: Nine C19MC miRNAs	Subgroup 2: Seven C19MC miRNAs	Six highest expressed C19MC miRNAs	Seven lowest expressed C19MC miRNAs
miR-517–3p	miR-517–3p		miR-517–3p	
miR-1323		miR-1323	miR-1323	
miR-516b-5p		miR-516b-5p	miR-516b-5p	
miR-525–5p		miR-525–5p	miR-525–5p	
miR-512–3p		miR-512–3p	miR-512–3p	
miR-515–3p	miR-515–3p		miR-515–3p	
miR-518e	miR-518e			
miR-515–5p		miR-515–5p		
miR-517c	miR-517c			
miR-519c-3p	miR-519c-3p			miR-519c-3p
miR-520h	miR-520h			miR-520h
miR-519d	miR-519d			miR-519d
miR-518b	miR-518b			miR-518b
miR-512–5p		miR-512–5p		miR-512–5p
miR-520c-3p	miR-520c-3p			miR-520c-3p
miR-518a-5p		miR-518a-5p		miR-518a-5p

The arrow indicates relative expression level.

Table S3. Summary of expression changes in autophagy-related transcripts

Gene	Fold change	Gene	Fold change	Gene	Fold change	Gene	Fold change
ATG4C	3.2861	*MAP1LC3B*	1.2209	*HMGB1*	0.9027	*ATG10*	0.724
UVRAG	3.2696	*CHU.K.*	1.2054	*TNFRSF1A*	0.901	*FADD*	0.7218
CCL2	3.1764	*HSPA1A*	1.1822	*ELK1*	0.8971	*BAX*	0.7145
DAPK1	3.119	*ATG12*	1.1791	*IL1B*	0.8966	*PIK3R4*	0.7073
CTSS	3.1154	*RIPK2*	1.1446	*UBE2V1*	0.8948	*MAP2K3*	0.6992
EIF2AK2	2.5184	*ATG16L1*	1.1417	*GABARAP*	0.8913	*HSPA8*	0.6945
RB1	2.3295	*PTGS2*	1.1354	*IKBKB*	0.8859	*MAPK8*	0.68385
BNIP3	2.3171	*TOLLIP*	1.124	*PTEN*	0.8814	*ATG7*	0.6794
MAP1LC3A	2.1561	*EIF2AK3*	1.1192	*EIF4G1*	0.8792	*MAPK8IP3*	0.6767
PIK3C3	2.1554	*IL8*	1.1002	*GABARAPL2*	0.8772	*NFRKB*	0.6665
BID	2.149	*UBE2N*	1.0968	*MAP4K4*	0.8748	*TAB1*	0.6663
AMBRA1	1.9193	*MAP3K7*	1.0954	*CD180*	0.8699	*ULK1*	0.6621
ARSA	1.849	*TLR4*	1.0872	*CLN3*	0.8339	*TICAM1*	0.6597
BCL2L1	1.7855	*APP*	1.0846	*PRKAA1*	0.8324	*CDKN1B*	0.6285
PRKRA	1.7526	*PPARA*	1.0624	*FAM176A*	0.8304	*TGM2*	0.6192
ATG4D	1.7454	*PELI1*	1.0265	*TRAF6*	0.8257	*DRAM2*	0.6166
SQSTM1	1.7187	*PRKAA2*	1.0185	*MAP2K4*	0.8234	*RPS6KB1*	0.6072
NFKBIA	1.6335	*FAS*	1.0149	*NR2C2*	0.8234	*RGS19*	0.6005
ATG4A	1.6288	*BECN1*	1.0139	*BCL2*	0.8218	*NFKB2*	0.5946
LY96	1.4979	*HSP90AA1*	1.0107	*HTT*	0.8135	*TMEM74*	0.576
NFKB1	1.45885	*HSPD1*	0.9957	*TLR6*	0.809	*ATG9A*	0.5758
TBK1	1.3469	*CSF2*	0.9879	*ECSIT*	0.804	*CASP8*	0.57545
TP53	1.3446	*HGS*	0.9745	*BAD*	0.8011	*JUN*	0.5644
MAP3K1	1.3294	*REL*	0.9743	*BAK1*	0.7987	*SARM1*	0.5564
DRAM1	1.3204	*CXCR4*	0.9529	*MYD88*	0.7962	*NFKBIL1*	0.5528
ATG3	1.289	*MAPK14*	0.9497	*GAA*	0.7936	*IRAK1*	0.5404
AKT1	1.2812	*IRF3*	0.9437	*IRF1*	0.7825	*FOS*	0.5091
TLR3	1.2655	*TICAM2*	0.9347	*CASP3*	0.7749	*TP73*	0.5061
ATG16L2	1.2457	*ATG4B*	0.9261	*GABARAPL1*	0.7581	*TNFSF10*	0.4193
TGFB1	1.2354	*ATG5*	0.9159	*HDAC1*	0.7541	*IRGM*	0.2952
ULK2	1.2352	*HRAS*	0.9159	*RAB24*	0.7351	*TNF*	0.2475
SNCA	1.2286	*CTSB*	0.9132	*RELA*	0.7325	*ATG9B*	0.2436
						IFNA4	0.1286