Supplemental Methods

Study Design

This study was conducted to identify differential gene expression in megakaryocytes and platelets after viral infection, specifically dengue and influenza infection and to identify anti-viral proteins expression on megakaryocytes and platelets, which regulate viral infection. All subjects provided written, informed consent and all study protocols were IRB approved. Patients with acute, primary or secondary dengue infection (n=29) were recruited from two sites: (1) the San Pablo Hospital in Bayamón San Juan, Puerto Rico and (2) the Instituto Nacional de Infectologia Evandro Chagas (INI) – FIOCRUZ in Rio de Janeiro, Brazil. Dengue virus infection was confirmed serologically and/or molecularly by RT-PCR, in accordance with current standards. Primary and secondary infections were distinguished using the IgM/IgG antibody ratio as previously described1-3. The mean time between the onset of illness and sample collection was 4.2 (±1.5) days. Patients (n = 24) with influenza A/H1N1 infection were recruited from one of three academic medical centers in Salt Lake City, Utah. Influenza A infection was confirmed by PCR on a respiratory tract specimen. Age-, gender-, and race-matched healthy subjects (n=31) were medication free and without acute or chronic illnesses. Clinical laboratory tests were determined from EDTA anti-coagulated blood samples that were drawn in parallel with the ACD samples used for platelet isolation. Laboratory parameters were measured according to established protocols and with the appropriate quality controls.

The dengue vaccine study was performed under an FDA-reviewed investigational new drug application and was approved by the Institutional Review Boards of the University of Vermont. Written informed consent was obtained in accordance with federal and international regulations (21CFR50, ICHE6) and Good Clinical Practices (GCP) were followed throughout. The NIAID Data Safety Monitoring Board reviewed all safety data every 6 months and external independent monitoring was performed. This Phase 1 randomized, double-blind, placebo-controlled trial was conducted in Burlington, Vermont. Study subjects were enrolled under study protocol CIR 323 (ClinicalTrials.gov NCT03416036). Healthy adults, aged 18–50, were recruited and enrolled. Eligibility criteria were as previously described4, which used an exclusion criterion of evidence of exposure to any of the following flaviviruses: DENV1-4, yellow fever virus, Japanese encephalitis virus, West Nile virus, St. Louis Encephalitis virus, or tick-borne encephalitis virus.
either by serology or documentation of vaccination. Eligible subjects were also required to be seronegative for hepatitis B, hepatitis C, and human immunodeficiency viruses and were required to have normal blood hematology, serum chemistry, and physical examination findings. The live attenuated dengue vaccine (TV003) contains all four dengue serotypes (DENV1-4) in a tetravalent admixture and was generated as previously described4. Subjects were randomly selected to receive 0.5 mL of vaccine or placebo, administered subcutaneously on day 0. Subjects were then evaluated with clinical assessments and examinations every other day for the first 14 days. Platelets were isolated on day 0 and day 14 of the study as described above and IFITM3 expression was examined by real-time PCR. All assays were done prior to unblinding.

For the influenza vaccine studies, seven healthy adults were recruited from the University of Utah. Patients were recruited during the 2012-2013 influenza season. The components of the trivalent influenza vaccine were: A/California/7/2009 (H1N1)pdm09, the pandemic strain, A/Victoria/361/2011 (H3N2), and the B/Wisconsin/1/2010. Platelets were isolated at day 0, immediately before administration of the influenza vaccine and again 14 days after the influenza vaccine was administered. Total RNA was isolated from day 0 and day 14 platelets and IFITM3 expression was measured by quantitative real-time PCR as described above.

Cell isolation, differentiation, and nomenclature

CD45 leukocyte-depleted human platelets were isolated as previously described5,6 from whole blood from dengue patients and healthy controls. These protocols yield a platelet suspension that generally has <2 leukocytes per 10⁷ platelets. For culture of CD34⁺ hematopoietic stem cell-derived MKs, human CD34⁺ cells from human umbilical cord blood were isolated as described previously5,7. The CD34⁺ cells were placed in X-Vivo 20 media that contained 40 ng/mL recombinant human stem cell factor (SCF; Invitrogen, Carlsbad, CA), 50 ng/mL recombinant thrombopoietin (TPO; Invitrogen), and 10 ng/mL recombinant human interleukin-3 (IL-3; Invitrogen). Every 2-3 days, the cells were re-suspended in media with fresh growth factors, with the exception that IL-3 was removed at day 5. DNA was isolated from CD34⁺-stem cells on day 0 and Sanger Sequencing performed to determine if cells expressed the rs12252 SNP for
IFITM3\(^8,9\). For dengue infection of HSCs, primary adult mobilized CD34\(^+\) stem cells were purchased from the Cell Therapy and Regenerative Medicine Center at the University of Utah.

Dengue virus serotype 2 strain (DENV2) 16881 was propagated in C6/36 Aedes albopictus mosquito cells and in rhesus monkey kidney epithelial cells (LLC-MK2) to ensure infectivity in mammalian cells. Dengue virus was always propagated finally in LLC-MK2 cells before infecting MKs or stem cells\(^10\). CD34\(^+\) derived MKs were incubated on culture days 11-12 with DENV2 at a Multiplicity of Infection (MOI) of 1.0 for 18 hours at 37\(^{\circ}\)C in a 5% CO\(_2\) atmosphere. In some experiments, CD34\(^+\) derived MKs were pre-treated with interferons for up to 18 hours at various concentrations as indicated in each experiment below. To measure DENV infection after interferon treatment, CD34\(^+\) derived MK mock or DENV infected supernatants were incubated with LLC-MK2 cells for 24 hours. The cells were then fixed with Paraformaldehyde (PFA 4% [2% final]) and stained for dengue virus as described below.

**Plaque Assay**

CD34\(^+\) derived MKs were infected with DENV2 (MOI 0.1) as described above. After 24 hours DENV2-infected supernatants were removed and added to LLC-MK2 cells for 2 hours. Following the infection, 1.6% agarose in Nutrient Solution (1X Earl Balance Salt Solution, lactalbumin hydro- lysate-yeast extract, fetal bovine serum, gentamicin, and NaHCO\(_3\)) was placed over the LLC-MK2 cells. Plates were incubated for 10 days at 37\(^{\circ}\)C in 5% CO\(_2\). After 10 days, 3.7% formaldehyde was added to each well for 1 hour. The agar was then removed and 1% crystal violent in 10% ethanol was added to each well. Plates were then washed with PBS and plaques were counted.

**RNA Preparation and Sequencing**

Next-generation RNA sequencing (RNA-seq) and analysis were performed on total RNA extracted from platelets isolated from confirmed, dengue-infected patients from Brazil and age-, gender-, and race-matched healthy subjects recruited from the same location. To independently determine the reproducibility of platelet RNA-seq data, we also performed RNA-seq on a second cohort of confirmed, prospectively studied, dengue infected patients from Puerto Rico and age- and gender-, and race-matched healthy controls.
recruited from the same location. In select experiments, RNA-seq was also performed on CD34+ derived MKs incubated with DENV2 (culture day 11, t=18 hours) or, as a control, mock virus.

For platelet and MK RNA-seq, isolated platelets or MKs were lysed in Trizol. Total RNA was isolated and DNase treated according to the manufacturer's protocol (Invitrogen) and as previously described\textsuperscript{11-14}. RNA integrity number scores did not differ significantly between patients and controls (not shown). Libraries were prepared with Illumina TruSeq RNA Preparation kit v2 with poly (A) selection. Libraries were bar-coded and sequenced for 50 cycles, single-end, on an Illumina HiSeq genome analyzer (1 sample sequenced for each patient). Sequence reads were processed by the University of Utah Bioinformatics Core. FASTQ sequence reads were aligned with Novocraft’s Novo alignment program (Novocraft Technologies; \url{www.novocraft.com}), as before\textsuperscript{15-18}. Reads were aligned to the GRCh38/Hg38 Dec. 2013 build. Reads were assigned to transcripts with DefineRegionDifferentialSeq (DRDS) (DESEQ) in the USeq analysis package, and differential expression analyzed using the DESeq2 analysis package\textsuperscript{19}. Read coverage was visualized in Integrative Genomics Viewer (BROAD institute\textsuperscript{20}). To make these data publicly available, a complete dataset of the RNA-seq studies was uploaded to Gene Expression Omnibus. RNA-seq raw data files are accessible through NCBI's SRA under bioproject IDs PRJNA517714 (SAMN10841493-SAMN10841506: Dengue Platelets, Dengue Healthy Control Platelets, H1N1 Platelets, mock treated and infected Megakaryocytes) and PRJNA397446 (SAMN07457428, SAMN07457429, SAMN07457432: H1N1 Healthy Control Platelets).

\textit{Real-time PCR}

For mRNA real-time PCR, isolated RNA was reverse transcribed using the Superscript III first-strand cDNA synthesis kit (Invitrogen). cDNA was used in a quantitative reverse-transcription–polymerase chain reaction (qRT-PCR) reaction using the PerfeCTa\textsuperscript{TM} SYBR green supermix (Quanta Biosciences, Gaithersburg, MD) on a Bio-Rad iCycler machine (Hercules, CA) adapted to detect \textit{IFITM3} mRNA. qRT-PCR was performed containing 10uM primers, Quanta PerfeCTa\textsuperscript{TM} SYBR\textsuperscript{®} Green Supermix, and cDNA diluted in nuclease free water. The amplification program was as follows: (i) initial melt at 95°C for 2
minutes; (ii) 40 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 45 seconds at 72°C; (iii) 10 min at 72°C; and (iv) 80 cycles with 0.5°C incremental increases from 55°C to 95°C for 10 seconds each to enable melting curve visualization. mRNA was normalized to housekeeping transcript glyceraldehyde 3-phosphate dehydrogenase (GADPH), beta tubulin (TUBB), or beta-2-microglobulin (B2M) and quantified using the $2^{-\Delta\DeltaCT}$ method. Forward and reverse primers were as follows, respectively: (1) IFITM3: 5’-CTAGGGACAGGAAGATGGTTGG-3’ and 5’-ATCCATAGGCCTGGAAGATCAG-3’, (2) DENV2: 5’-ATGGACCTTGGTAATTGTGTA and 5’-TGGAAATGTGTGTTCTATG-3’, (3) B2M: 5’-AGATGAGTATGCCTGCCGTGT-3’ and 5’-AGCTACCTTTGAGCAACCTG-3’, (4) GAPDH: 5’-GAACATCATCCCTGCTCTACTG-3’ and 5’-AGCTTGACAAAGTGGTCGTTGAG-3’, (4) TUBB: 5’TTCCTGTCTCGATGTGGTTACG-3’ and 5’-GTGTGGGTACGCTTCAGAGTGC-3’.

MK supernatant switch experiments

CD34+ hematopoietic progenitor cells were cultured for 4 days, as described above. On culture day 4, one half of the culture was infected with DENV2 at a MOI of 10 while the other half of the culture was exposed to mock infection, as a control. On culture day 6, the cells were pelleted and recovered supernatants were then used to re-suspend cells from the opposite treatment group (i.e. mock infected cells plus supernatants harvested from dengue infected cells or DENV2 infected cells plus supernatants harvested from mock infected cells). Cells were then infected with DENV2 (MOI of 1.0). On culture day 10, MKs were fixed and processed for assessment of DENV2 and IFITM3 protein expression, as described below. For dengue infection of primary mobilized CD34+ stem cells, cells were thawed and placed in Ex vivo media with growth factors as described above. The next day, CD34+ cells were pelleted and resuspended in 50% fresh media and 50% cultured supernatant from day 12 CD34+ derived megakaryocytes infected with mock or DENV2 (MOI 0.1) for 24 hours. The next day CD34+ cells were then washed and re-suspended in fresh and infected with DENV2 (MOI 0.1) for two hours. After two hours, cells were washed three times media and re-suspended in fresh media for 24 hours. The next day the cells were lysed in Trizol LS and DENV2 infection measured by real-time PCR.
Reagents and antibodies

The following reagents and antibodies were used for switch experiments: PFA 4% [2% final]), TO-
PRO-3 (1:1000, Lifetechnologies, Eugene, OR), mouse anti-DENV2 (EMD Millipore, Billerica, MA),
rabbit anti-IFITM3 (Abcam, Cambridge, MA), goat anti-mouse IgG biotin labelled, goat anti-rabbit IgG
Alexa 546-conjugate, streptavidin Alexa 488 conjugate (all Lifetechnologies, Eugene, OR), interferon-alpha
and beta antibodies (Abcam, San Francisco, California). The following reagents and antibodies were used
for the co-localization studies: Duolink® In Situ system (OLINK Bioscience, Uppsala, Sweden), rabbit anti-
IFITM3 (Proteintech, Rosemont, IL), mouse anti-PDI and mouse anti-LAMP1 (Abcam, Cambridge, MA).
For IFN stimulation studies, IFNα and IFNβ were purchased from Millipore (Burlington, MA) and IFNγ
was purchased from Thermofisher Scientific (Waltham, MA).

Immunocytochemistry

On select culture days, MKs were fixed in suspension by adding PFA (2% final concentration).
Fixed MKs were subsequently layered onto vectabond™ (Vector Laboratories, Burlingame, CA) coated
coverslips using a cytospin centrifuge (Shandon Cytospin, Thermo Fisher Scientific, Waltham, MA). MKs
were then permeabilized and dengue virus and IFITM3 were detected using the aforementioned antibodies.
Specificity of staining for IFITM3 was confirmed with isotype-matched, non-immune IgG. For platelet
dengue infection, 1x10⁷ platelets were incubated with dengue virus (MOI 0.2) and incubated for 2 hours.
After two hours, the platelets were washed with media and re-suspended in M199 for 24 hours before being
fixed with 4% PFA. Platelets were centrifuged onto vectabond™ coverslips and stained with mouse anti-
DENV2 and rabbit anti-CD41 (Abcam, Cambridge, MA).

Microscopy

High-resolution, confocal reflection microscopy was performed using an Olympus IX81, FV300
(Olympus, Melville, NY) confocal-scanning microscope equipped with a 60x/1.42 NA oil objective for
viewing platelets and MKs. An Olympus FVS-PSU/IX2-UCB camera and scanning unit and Olympus
Fluoview FV 300 image acquisition software version 5.0 were used for recording. Monochrome 16-bit images were further analyzed and quantified using Adobe Photoshop CS6 and ImageJ (NIH). In addition, an EVOS FL Auto Cell imaging system with integrated dual camera system, system specific software and equipped with a 60x/1.42 NA oil objective was used (Supplemental Figure 3). Monochrome 16-bit images were further analyzed and changes quantified using Adobe Photoshop CS6, ImageJ (NIH), and CellProfiler (www.cellprofiler.org).22

Single recognition and co-localization studies

Microscopy based co-localization studies were performed using Duolink® In Situ system (OLINK Bioscience, Uppsala, Sweden) as previously described12.

MEG-01 transfection and dengue infection

The pZIP-mEF1a vector (shERWOOD-UltramiR Lentiviral shRNA) containing green fluorescent protein (GFP) was purchased from Transomics (Huntsville, Alabama). IFITM3 was cloned into the pZIP empty vector by replacing GFP using single blunt end ligation which destroyed the AgeI but maintained the EcoRI sites. Plasmids were purified by Qiagen MaxiPrep kits (Germantown, Maryland) and sequenced over the modified region to ensure proper placement and orientation of IFITM3. Verified plasmids were packaged into lentivirus by System Biosciences (Palo Alto, California). MEG-01 (ATCC, Manassas, Virginia) cells were transduced using an MOI of 50 plus polybrene for both IFITM3 and empty constructs. Medium was exchanged 24 hours after transduction. To select positively transduced cells, puromycin was added to growth medium for one week. Cells were then allowed to grow without puromycin for an additional week.

MEG-01s were subjected to dengue infection in the presence of antibody against dengue at subneutralizing concentrations as previously described23. Briefly, MEG-01s (2.5 × 10^5) were re-suspended in 100μl of RPMI with 2% FBS. A mouse monoclonal antiflavivirus antibody (4G2, Novus Biologicals, Littleton, Colorado) (200ng 4G2) was mixed with dengue virus (MOI 1) and added to cells, and the resulting mixture was incubated for 2 hours at 37°C. Cells were then washed (four times) to remove residual free virus, growth medium (RPMI with 10% FBS) was replaced, and antibody (200ng of 4G2) was added.
Cells were incubated for 96 hours at 37°C prior to cell harvest to determine level of dengue infection.

For sH-RNA knockdown of IFITM3, MEG-01s were transfected with SMARTvector Human Lentiviral IFITM3 sH-RNA or control vector (Dharmacon, Lafayette, CO) with a murine EF1a promoter and a turboRFP marker. After transfection, MEG-01s were flow sorted by RFP to enrich for sH-IFITM3 or control vector cells. MEG-01s were stimulated with 500 U/mL IFNα for 24 hours before proceeding with dengue infection as described above.

**Plasma interferon-alpha measurements**

Interferon-alpha (IFNα) concentrations were measured in platelet-poor plasma harvested on the same blood drawn for the platelet isolations using a commercially available ELISA (R&D System, Minneapolis, Minnesota).

**Statistical Analyses**

For all analyses, continuous variables were assessed for normality with skewness and kurtosis tests. Summary statistics were used to describe the study cohort and clinical variables are expressed as the mean ± standard deviation or as a number and percentage (%). Parametric two-tailed t-tests or ANOVA were used for continuous variables and the chi-square and Fisher’s exact test for categorical variables. Statistical analyses were performed by using GraphPad Prism (version 7, San Diego, CA). A two-tailed p-value < 0.05 was considered statistically significant.
Supplemental Tables

Supplemental Table 1. Characteristics of dengue-infected patients and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n=16)</th>
<th>Mild Dengue (n=17)</th>
<th>Dengue with WS(^1) + Severe Dengue(^2) (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>30.1 (±5.6)</td>
<td>35.4 (±13.0)</td>
<td>29.4 (±13.6)</td>
</tr>
<tr>
<td>Gender, male</td>
<td>7 (43.8%)</td>
<td>10 (67.7%)</td>
<td>8 (58.3%)</td>
</tr>
<tr>
<td>Platelet Count, K/(\alpha) L</td>
<td>–</td>
<td>89 (±41)</td>
<td>75 (±42)</td>
</tr>
<tr>
<td>White Blood Cells, K/(\alpha) L</td>
<td>–</td>
<td>3.8 (±1.6)</td>
<td>3.6 (±1.7)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>–</td>
<td>41 (±2.9)</td>
<td>43 (±5.1)</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>–</td>
<td>3.6 (±0.5)</td>
<td>3.3 (±0.5)</td>
</tr>
<tr>
<td>TGO/AST, IU/L</td>
<td>–</td>
<td>100 (±79)</td>
<td>105 (±60)</td>
</tr>
<tr>
<td>TGP/ALT, IU/L</td>
<td>–</td>
<td>120 (±136)</td>
<td>107 (±51)</td>
</tr>
<tr>
<td>Dengue with WS(^1)</td>
<td>–</td>
<td>–</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>Severe dengue(^2)</td>
<td>–</td>
<td>–</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Hemorrhagic manifestations(^3)</td>
<td>–</td>
<td>1 (6%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Secondary Infection</td>
<td>–</td>
<td>3 (17.6%)</td>
<td>6 (50%)</td>
</tr>
<tr>
<td>PCR positive</td>
<td>–</td>
<td>11 (65%)</td>
<td>8 (67%)</td>
</tr>
<tr>
<td>IgM+</td>
<td>0 (0.0%)</td>
<td>16 (100%)</td>
<td>11 (92%)</td>
</tr>
<tr>
<td>NS1+</td>
<td>0 (0.0%)</td>
<td>2/10 (20%)</td>
<td>5/12 (42%)</td>
</tr>
</tbody>
</table>

\(^1\) Warning signs: abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleed or increased hematocrit concurrent with rapid decrease in platelet count; according to WHO criteria (2009).

\(^2\) Severe plasma leakage, fluid accumulation and severe bleeding; according to WHO criteria (2009).

\(^3\) Gingival bleed, vaginal bleed, gastrointestinal bleed, petechiae and/or exanthema.

Data are expressed as mean (±SD) or number (%).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TGO, glutamicoxalacetic transaminase; TGP, glutamic-pyruvic transaminase; IgM, dengue-specific immunoglobulin M; NS1, nonstructural protein 1 from DENV.
Supplemental Table 2. Significant differentially expressed transcripts in isolated platelets from patients with dengue infection.

Please see uploaded data file designated “Supplemental Table 2”.
Supplemental Table 3. Characteristics of influenza-infected patients and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n=31)</th>
<th>H1N1/A Influenza Patients (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>46.3 (±19.6)</td>
<td>42.7 (±13.1)</td>
</tr>
<tr>
<td>Gender, male</td>
<td>9 (29.0%)</td>
<td>12 (50%)</td>
</tr>
<tr>
<td>APACHE II(^1)</td>
<td>–</td>
<td>18.7 (±7.3)</td>
</tr>
<tr>
<td>Shock(^2), n (%)</td>
<td>–</td>
<td>12 (50%)</td>
</tr>
<tr>
<td>In-Hospital Mortality, n (%)</td>
<td>–</td>
<td>8 (33.3%)</td>
</tr>
<tr>
<td>Platelet Count, K/μL</td>
<td>–</td>
<td>177 (±107)</td>
</tr>
<tr>
<td>White Blood Cells, K/μL</td>
<td>–</td>
<td>8.2 (±4.3)</td>
</tr>
<tr>
<td>Hemoglobin, mg/dL</td>
<td>–</td>
<td>11.4 (±1.9)</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>–</td>
<td>1.1 (±0.55)</td>
</tr>
</tbody>
</table>

\(^1\)APACHE II: Acute Physiology and Chronic Health Evaluation II
\(^2\)Shock defined as SBP<90 and need for vasopressors
Supplemental Table 4. Significant differentially expressed transcripts in isolated platelets from patients with influenza infection.

Please see uploaded data file designated “Supplemental Table 4”.
Supplemental Table 5. Shared significant differentially expression transcripts in isolated platelets from patients with dengue and patients with influenza infection.

Please see uploaded data file designated “Supplemental Table 5”.
Supplemental Table 6. Significant differentially expressed transcripts from human MKs infected with dengue virus.

Please see uploaded data file designated “Supplemental Table 6”.
Supplemental Figures

Fig. S1. Plasma IFNγ levels increase in patients with Dengue and H1N1/A Influenza. Plasma levels of IFNγ in healthy controls subjects (n=16, Dengue and n=9, Influenza) and patients with Dengue (n=22) (A) or H1N1/A Influenza (n=21) (B).
Fig. S2. IFITM3 increases in platelets isolated from patients with Dengue recruited prospectively from Puerto Rico. Platelets were isolated from patients with confirmed Dengue virus infection (Dengue, n=5) or age, gender, and race--matched healthy controls from the same region of Puerto Rico (Healthy, n=2). (A) *IFITM3* mRNA expression was measured by RT-PCR and normalized to the housekeeping transcript tubulin. (B) Platelet lysates were also probed by immunoblot for IFITM3 protein expression with β-actin as a loading control. Shown is a representative western blot for platelets IFITM3 in patients from Puerto Rico infected with Dengue or a healthy control subject.
Fig. S3. IFITM3 in human MKs is located within late-stage endosomes and lysosomes.

Using the Duolink® In Situ system, human CD34⁺ derived MKs (culture day 11) were stained with the nuclear stain DAPI (blue), actin (purple), and with antibodies against IFITM3 plus protein disulfide isomerase (PDI, a late-stage endosome marker, panel B) or IFITM3 plus lysosomal-associated membrane protein 1 (LAMP1, panel C). Panel A shows control staining in the absence of PDI or LAMP1 antibodies. Yellow staining identifies where both IFITM3 and LAMP1 (B) or IFITM3 and PDI (C) co-localize within MKs (yellow arrows). The bottom panels are enlarged insets. Representative of n=3 independent experiments.
Fig. S4. Interferon-gamma (IFNγ) upregulates IFITM3 in MKs. CD34+ derived MKs (culture day 12) were stimulated with 100 U/mL IFNγ (a type II IFN) or left alone (NT) for 18 hours. (A, B) IFITM3 mRNA expression was measured by qRT-PCR and normalized to the housekeeping transcript GADPH. (C) MKs were probed by immunoblot for IFITM3 protein expression with β-actin as a loading control. Shown is a representative immunoblot (left) and quantification by densitometry normalized to β-actin (right). Data are from n=3-6 independent experiments.
**Fig. S5. Human MKs are permissive to dengue infection.** (A) CD34+ derived MKs (culture day 12) were incubated (t=18 hours) with mock virus (mock) or dengue virus serotype 2 (DENV2, MOI 1.0). Following incubation, MKs were stained by immunocytochemistry for DENV2 antigens (green, yellow arrows) and IFITM3 (red, white arrows). Nuclei are stained with TOPRO (blue). This image is representative from 5-6 independent experiments per group. (B-D) CD34+ derived MKs (culture day 12) were incubated (t = 2 hrs) with mock virus (mock) or dengue virus serotype 2 (DENV2, MOI 0.1). After 2 hours, the MKs were washed three times with PBS and incubated overnight (t=18 hrs). The next day the supernatants were collected and placed on LLMCK2 cells for 2 hours. (B, C) For the plaque assay, agar was placed on top of the LLMCK2 infected cells and allowed to incubate for 10 days before fixation and staining with crystal violet. Viral plaques were counted and infectivity determined (n=5). (D) LLCMK2 infected cells were washed three times with PBS before the addition of fresh media. The next day (t=18 hrs) the cells were lysed in Trizol LS and DENV2 expression was determined by qRT-PCR (n=5 independent experiments).
Fig. S6. Dengue infections in human MKs increases the expression and secretion of type I IFNs. (A-C) IFNA2, IFNB1 and IFNG expression (FPKM, fragments per kilobase per million reads, a measure of RNA expression) measured by next-generation RNA-sequencing (RNA-seq) in CD34+ derived MKs (culture day 12) incubated with mock virus control (n=3) or DENV2 (n=2). Note that IFNG expression is very low-to-undetectable in both mock and DENV2 conditions (e.g. FPKM < 0.3, a cut-off below which mRNA expression is considered negligible). P-values shown are calculated from RNA-seq analytical software tools (Deseq2, described in methods) and adjusted for multiple comparisons. (D) Dengue infected MKs secrete type I IFNs. CD34+ derived MKs (culture day 12) were incubated (t=18 hours) with mock virus control (mock) or DENV2 (MOI 1.0). Following incubation, cell-free supernatants were acid precipitated and IFNα and β were detected by immunoblot. This immunoblot is representative of n=2 independent experiments.
Supplemental References

1. Falconar AK, de Plata E, Romero-Vivas CM. Altered enzyme-linked immunosorbent assay immunoglobulin M (IgM)/IgG optical density ratios can correctly classify all primary or secondary dengue virus infections 1 day after the onset of symptoms, when all of the viruses can be isolated. Clin Vaccine Immunol. 2006;13(9):1044-1051.


