Supplementary Information for

Decidual NK cells kill Zika virus-infected trophoblasts

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Materials and Methods

Isolation of human dNK and trophoblasts. Discarded, de-identified healthy human placental and decidual tissue (gestational age 6-12 weeks) were obtained from women undergoing elective pregnancy termination at a local reproductive health clinic with approval from Harvard and Boston Children's Hospital Human Institutional Review Committees, which deemed this study exempt from human subject regulations. Tissue samples that showed signs of inflammation or discoloration were not used. Briefly, decidual and villous tissues were macroscopically identified and separated. Primary trophoblast cells were isolated from villous tissue by gently removing the basal membrane and digesting for 8 min at 37°C with 75 ml of 0.2% trypsin 0.02% EDTA. DMEM/F-12 medium (25 ml) containing 10% Newborn Calf Serum (NCS) and 1% Pen/Strep was added and the digested tissue was filtered over a gauze mesh. The filtrate was washed and layered on Ficoll for density gradient centrifugation (20 min, 800g). Cells were collected from the interface, washed with DMEM/F12 medium with 10% NCS and incubated for 20 min at 37°C in a tissue culture dish to remove adherent macrophages. EVT were obtained after sorting for live large $CD45^-$ cells (and washing away non-adherent cells after overnight culture), or sorting $CD45^-B7\text{-}H3^+CD49a^+$ cells. Sorted cells were plated in 48 well plates coated with 20 μg/ml fibronectin in EVT medium (DMEM/F12 medium supplemented with 10% NCS, 1x Pen/Strep, 1x insulin, transferrin, selenium (ITS), 5 ng/ml EGF, and 400 units human gonadotropic hormone) and allowed to adhere overnight, or resuspended in the ZIKV inoculum and plated for 1 h before adding the same volume of EVT medium as the inoculum, and then allowed to adhere overnight (1, 2). Decidual tissue was washed with PBS, minced and digested with 0.25% collagenase type IV and 0.025% DNase I with gentle shaking in a water bath for 75 min at 37°C. Released lymphocytes were washed with RPMI 10% FBS (8 min, 1800 rpm), filtered through 100, 70 and 40 μm sieves, and the filtrate was resuspended in 20 ml 1.023 g/ml Percoll and layered on a Percoll gradient (10 ml 1.080 g/ml topped with 15 ml 1.053 g/ml) for density gradient centrifugation (25 min/800 g). Lymphocytes were isolated from the 1.080 g/ml/1.053 g/ml interface, washed twice with RPMI 10% FBS and stained and sorted for dNK cells using a previously described gating strategy (3). Human dNK were cultured in X-VIVO 10™ media with gentamicin supplemented with 5% human AB serum and 2.5 ng/ml recombinant human IL-15 for 12-18 h prior to experiments. pNK were isolated using a RosetteSep human NK enrichment cocktail followed by Ficoll density gradient centrifugation (20 min, 800 g).

Cell lines. JEG-3, 721.221, U-251, HFF and Vero were from ATCC. Cell lines were recent passages and were periodically tested for mycoplasma contamination; flow cytometry was used to confirm surface markers. 721.221 were cultured in RPMI 1640 supplemented with 10% FCS, 1% Pen/Strep and 1% L-glutamine. JEG-3, Vero, HFF and U-251 were cultured in DMEM supplemented with 10% FCS, 1% Pen/Strep and 1% L-glutamine.

Viruses. ZIKV strain PRVABC59 (Puerto Rico, 2015) was obtained from the Arbovirus Branch of the Centers for Disease Control and Prevention (CDC). ZIKV strain MR766 and HSV-2 were obtained from ATCC. HCMV AD169 (IE-1-GFP) was a gift from Daniel Coen (Harvard Medical School). ZIKV stocks were propagated in DMEM supplemented with 2% heat-inactivated FBS in Vero cells. Viral titers were determined by plaque assay on Vero cells incubated for 3 days using a 1.2% Avicel overlay. HCMV was propagated in HFF in DMEM supplemented with 3% FBS and supernatants were collected 8 d post infection. Viral titers were determined by counting GFP+ foci on HFF cells 2 d post infection using a 1.2% methylcellulose overlay. HSV-2 was grown in Vero cells and viral titers were determined by plaque assay in Vero cells.

Infection and tunicamycin treatment of cell lines and primary cells. JEG-3 and U-251 were infected with ZIKV for 1 h in DMEM with 2% FCS using the indicated MOI. Fresh culture media was added after the 1 h incubation with the inoculum. Infected cells were cultured for indicated times before harvesting for assays. In some cases, JEG-3 were pre-treated with 10 μM salubrinal for 1 h before infection, and salubrinal was kept in the culture media post infection. $CD45^-$ placental cell

suspensions (Figure S3 and HLA-C expression in Figure 3) or purified EVT (sorted on CD49a$^+$B7-H3$^+$ - all of Figure 3 except HLA-C expression) were infected while still in suspension with 400 μl of 10^{12} PFU/ml ZIKV PRVABC59. After 1 h, the same volume of EVT media as the inoculum was added, and the cells were allowed to adhere for 24 h in fibronectin-coated plates before analysis of infection and MHC expression, or removal of viral supernatant and addition of dNK for functional assays. Analysis of HLA-C expression was performed in infected cell suspensions and not purified EVT because the HLA-C antibody is not conjugated and requires an anti-mouse secondary, which cannot be done in cells sorted by labeling with mouse antibodies. The staining strategy to measure HLA-C only in EVT is described in the Flow Cytometry section. JEG-3 were infected with MOI 0.5 HSV-2 for 1 h, then medium was aspirated and cells were cultured in fresh medium for 1 or 2 d. For HCMV infection, cells were spinoculated with MOI 4 (1 h, 2800 rpm) virus, and the inoculum was maintained during 12 h culture at 37°C. Spinoculation and culture were repeated once or twice with fresh inoculi to enhance infection rates and then medium was aspirated and cells were cultured in fresh medium for 12 h before harvesting or use for NK functional assays. In some experiments, cells were treated with 0.5 μg/ml tunicamycin for 24 h, with or without salubrinal pretreatment 1 h earlier. Salubrinal was kept in the culture throughout tunicamycin treatment.

Culture, infection and imaging of placental villi. Villous trees (3-5 μm length) from 3 donors were dissected and cultured in Transwell inserts (0.4 μm pore, Costar) coated with Matrigel. Villi were allowed to invade the Matrigel for 12-15 h with collection medium (DMEM/F12 with 2.5% FBS, 1% Pen/Strep, 50 mg/ml gentamicin and 1.25 mg/ml amphotericin) only in the bottom well (4). For infection, villi were incubated with 10^8 PFU ZIKV PRVABC59 for 1 h, followed by addition of fresh villous medium on top and bottom (DMEM/F12 with 20% FCS and 1x P/S). The viral inoculum was removed after 24 h and substituted by fresh villous medium. Infection lasted a total of 72 h. 1 million autologous dNK (in the presence of 2.5 ng/ml IL-15) were added or not after the first hour of infection. Viability of villi was verified by MTT assay: villi were weighed (mg) on the first day of culture for normalization, and villi were incubated with MTT reagent (2.5 mg/ml) at each time point for 3 hours. MTT reagent was removed and solvent (4 mM HCl in isopropanol) was added to the tissue. Absorbance of the solvent was measured at 590 nm and 570 nm and normalized to weight. Viability was calculated by dividing the absorbance/mg of the tissue at each time point by the absorbance/mg of the tissue at the day of collection (considered 100%).

NK functional assays. dNK killing of 721.221 and JEG-3, infected or not for 48 h or treated with tunicamycin for 24 h with or without salubrinal pre-treatment as indicated, was analyzed by 8 h ^{51}Cr release assay using E:T ratios indicated in each experiment, performed in the presence of 2.5 ng/ml IL-15. To inhibit granule-mediated killing, NK were pre-treated with 2 mM EGTA for 30 min at 37°C, and EGTA was maintained during the co-culture. To block NK receptors, NK cells were pretreated for 30 min at 37°C with blocking or control antibodies that were maintained during co-culture (10 μg/ml purified antibodies to NKp46 and NKp30 or mouse IgG1 as control). To assess viral inhibition, NK/JEG-3 co-culture supernatants were used to infect Vero cells and viral titers were assessed by plaque assay. To measure degranulation, dNK were co-cultured for 8 h with 721.221 (E:T ratio 1:3) or uninfected or ZIKV-PRVABC59 infected confluent JEG-3 in the presence of CD107a PerCP-Cy5.5 antibody (250 ng/ml) and 2.5 ng/ml IL-15. To measure intracellular cytokine production, 2 μM monensin and 3 μM brefeldin were added after 1 h of co-culture and cells were harvested 7 h later. Cells were stained for surface markers followed by permeabilization with Cytofix/Cytoperm and intracellular cytokine staining. To assess reduction of ZIKV infection in EVT by dNK, a 10-fold excess of dNK were added to ZIKV-infected EVT (after removal of viral supernatant from a 24 h infection) for 12 h in the presence of 2.5 ng/ml IL-15. dNK were washed away and EVT were trypsinized and stained for surface markers and intracellularly for flavivirus E protein and analyzed by flow cytometry.

Flow Cytometry Antibodies and reagents are listed in Supplemental Table S2. For surface staining, cells were stained for 30 min on ice in the dark with LIVE/DEAD-Violet stain (1:1000) and Annexin V-APC (1:100) in Annexin V buffer when applicable, and then with primary antibodies for 15-30 min in PBS, 2% FCS. HLA-C staining was performed on infected placental cell suspensions by staining with purified HLA-C antibody (DT9), followed by PE anti-mouse secondary for 15 min,

and finally with B7-H3 and CD49e conjugated antibodies to allow gating on EVT. Cells were fixed in 1% paraformaldehyde for 10 min before flow cytometry. For intracellular staining, cells were fixed and permeabilized using the BD CytoFix/CytoPerm kit. Analysis was performed on a FACSCanto II (BD) and sorting was done using a BD Aria II. BD FACSDiva 8.0 (BD) software was used for data collection, while analysis was performed with FlowJo v10.3 (BD).

Confocal microscopy. Uninfected or ZIKV-infected JEG-3 cells, cultured on 1.5 mm glass coverslips, were fixed with 4% paraformaldehyde for 10 min and then permeabilized with PBS/0.01% Triton-X for 10 min before blocking with 5% BSA in PBS and staining with purified anti-flavivirus Group Antigen antibody (1:500) for 3 h, followed by staining with donkey-anti-mouse AlexaFluor 488^{TM} for 1 h. Cells were washed and stained with DAPI (4'6'-diamidino-2-phenylindole, 1: 1,000 dilution) for 10 min, and coverslips were mounted using lab-made self-sealing mounting media (Vinol) without DAPI. Villi explants were fixed in 4% paraformaldehyde for 30 min and dehydrated by immersion in 10% sucrose in PBS for 1 h, followed by 20% sucrose for 1 h and 30% sucrose overnight before embedding in OCT medium for flash freezing. Sections (5 µm) were cut using a cryostat (Microm HM 550, ThermoFisher Scientific) and adhered to positive charged glass slides (Denville). OCT was removed by immersion in 1x PBS and tissue was blocked for 30 min in buffer (1% BSA, 22.52 mg/mL glycine, 0.1% Tween 20 in PBS), and then stained overnight at 4ºC with primary antibodies to detect CDH-1 (1:25, mouse), CD49a (1:50, rabbit), SDC-1 (1:25, mouse), ZIKV NS2B (1:200, rabbit) and anti-flavivirus Group Antigen antibody (1:200, mouse) in 1% BSA, 0.1% Tween 20 in PBS. After 3 washes in PBS, slides were stained with secondary antibodies (donkey anti-rabbit AlexaFluor 488^{TM} and donkey anti-mouse AlexaFluor 647^{TM} 1:1000) for 1 h at RT. Samples were counterstained with DAPI and mounted and imaged by fluorescence microscopy. To quantify the distribution of ZIKV-infected cell types or percentage of each cell type that were infected, EVT were identified as CD49a^{+}CDH-1^{+} cells in the tips of villi with large nuclei, CT were CDH-1^{+} cells with smaller nuclei located under ST or EVT and ST were SDC-1^{+} cells with very small nuclei forming a uniform layer on the outside of the villi. Cells were counted in 10-15 imaging fields of 3 different donors (217x magnification).
Cells and sections were imaged by confocal microscopy using an inverted, fully motorized Axio Observer spinning disk confocal microscope (Carl Zeiss Microimaging, Inc., Thornwood, NY) equipped with a cooled electron multiplication charge-coupled device (CCD) camera with 512 × 512 resolution (QuantEM, Photometics, Tucson, AZ) and a CSU-X1 spinning disc (Yokogawa Electric, Tokyo, Japan) with lasers that were excited at 405, 488, 561 and 640 nm (Coherent, Santa Clara, CA) and emission filter ranges of 452/45, 525/50, 607/36 and 680 long-pass, respectively (Semrock, Rochester, NY). Images were processed using SlideBook V5.0 (Intelligent Imaging Inc., Denver, CO).

qRT-PCR. Total RNA was extracted from JEG-3, U-251 or E15.5 mouse placentas, fetuses and spleen using the RNeasy Mini kit, treated with DNase I and reverse transcribed using 2 µg of total RNA, 50 ng random hexamers, 400 nM dNTPs and 200 U SuperScript II reverse transcriptase kit. Primers and diluted cDNA samples were prepared with Power SYBR Green PCR Master Mix. Amplification cycles using the iQ5 system (BioRad) were 95°C for 10 min, then 40 cycles at 95°C for 10 sec and 55°C for 30 sec. Results were normalized to *ACTB*. Primer sequences are provided in Supplemental Table S1.

Plasmid construction and transfection of JEG-3. To express viral proteins, cDNA fragments of PRVABC59 (GenBank accession number KU501215) encoding the individual viral proteins were subcloned into Flag-tagged pcDNA3.1 or pFLAG-CMV4. Primer sequences are in Supplementary Table S1. JEG-3 were transiently transfected in 12-well plates (10^5 cells per well) using 1 µg DNA and FuGENE HD Transfection Reagent following the manufacturer's guidelines. ER stress was measured by detecting Firefly and Renilla luciferase activity in JEG-3 lysates of cells co-transfected with pFLAG-XBP1u-Fluc or p5xATF6-GL3 reporter and plasmids encoding individual viral genes. Luciferase activity was measured by Dual-Luciferase® Reporter Assay System. Firefly luciferase activity was normalized to Renilla luciferase activity.

Mouse experiments. *Ifnar1$^{-/-}$* A129 (B6.129S2-Ifnar1tm1Agt/Mmjax) and C57BL/6 mice were obtained from Jackson Laboratory. Animal use was approved by the Animal Care and Use Committees of Boston Children's Hospital and Harvard Medical School. Virgin female *Ifnar1$^{-/-}$* A129 mice (6-8 weeks old) were mated with male *Ifnar1$^{-/-}$* A129 and the appearance of a vaginal plug was used to mark E0.5. On E6.5, mice were intraperitoneally infected with 10^3 PFU of ZIKV PRVABC59 in 0.2 mL of PBS. Mice were sacrificed at E15.5 and maternal blood and spleen, placenta and fetus were harvested, weighed and homogenized for viral loads on clarified samples (centrifugation at 2,000g at 4ºC for 10 min) measured as above by plaque assay and/or by qRT-PCR as described (5). In some experiments, WT or A129 mice were injected intraperitoneally daily for 3 d beginning on E3.5 and on E10.5 with antibodies to CD8 T cells (0.5 mg/mouse rat anti–mouse CD8 mAb, clone 2.43) or NK cells (0.2 mg/mouse of rabbit anti–mouse asialo GM1) or a control antibody (clone LTF-2). Control antibodies were administered following the same regimen. To deplete B cells, a single dose of rabbit anti-mouse anti-CD20 (0.25 mg/mouse, Clone SA271G2) was injected intraperitoneally on E0.5 and repeated once every week. Pregnant animals were randomly assigned to experimental groups for depletion studies. Cell depletion was verified by flow cytometry of maternal blood mononuclear cells on E5.5.

Mouse placental histology. At least three mouse placentas per treatment condition from separate litters of dams sacrificed on day E15.5 were fixed in 10% neutral buffered formalin for 48 h and embedded in paraffin. Sections (3–5 μm) were stained with haematoxylin and eosin (H&E). For immunofluorescence staining, deparaffinized and permeabilized tissues were blocked in buffer (1% BSA, 22.5 mg/mL glycine, 0.1% Tween 20 in PBS) for 2 h before overnight incubation with primary antibodies. The following primary antibodies were used: pan-cytokeratin (1:500), vimentin (1:500, rabbit) and 1:200 mouse anti-ZIKV serum (prepared in our laboratory from infected mouse sera). After washing three times in PBS, secondary antibody conjugated with either AlexaFluor™ 488 or 647 (1:500) in 0.1% Tween 20 in PBS was added for 1 h at room temperature. Samples were counterstained with DAPI and mounted and analyzed by fluorescence microscopy as described above.

Statistical analysis. Values presented are the mean ± S.E.M. or median ± interquartile range, as indicated. Statistical analysis was performed using Prism 6.0c (GraphPad). Equality of variances was tested by Levene's test. Unpaired (Kruskal-Wallis test followed by Dunn's post-test) or paired (Friedman's test followed by Dunn's post-test) non-parametric one-way analysis of variance was used in experiments for which multiple comparisons were made. Non-parametric tests were used in experiments with human or animal samples because of the non-normal distribution of these data. Otherwise, one-way or two-way ANOVA (for technical replicates) followed by Tukey's or Sidak's post-test was used. For comparison of 2 unpaired groups in experiments with human samples/animals, Mann-Whitney or Kolmogorov-Smirnov test were used for groups with equal or unequal variances, respectively. For comparison of 2 paired groups, Wilcoxon rank sum test was used. Otherwise, unpaired or paired Student's t-tests (for technical replicates) were used. $p < 0.05$ was required for significance. A chi-squared test was used to analyze the data in Figure 5A. Most experiments were not blinded as to group allocation, either while collecting data or assessing the results.

Figure S1

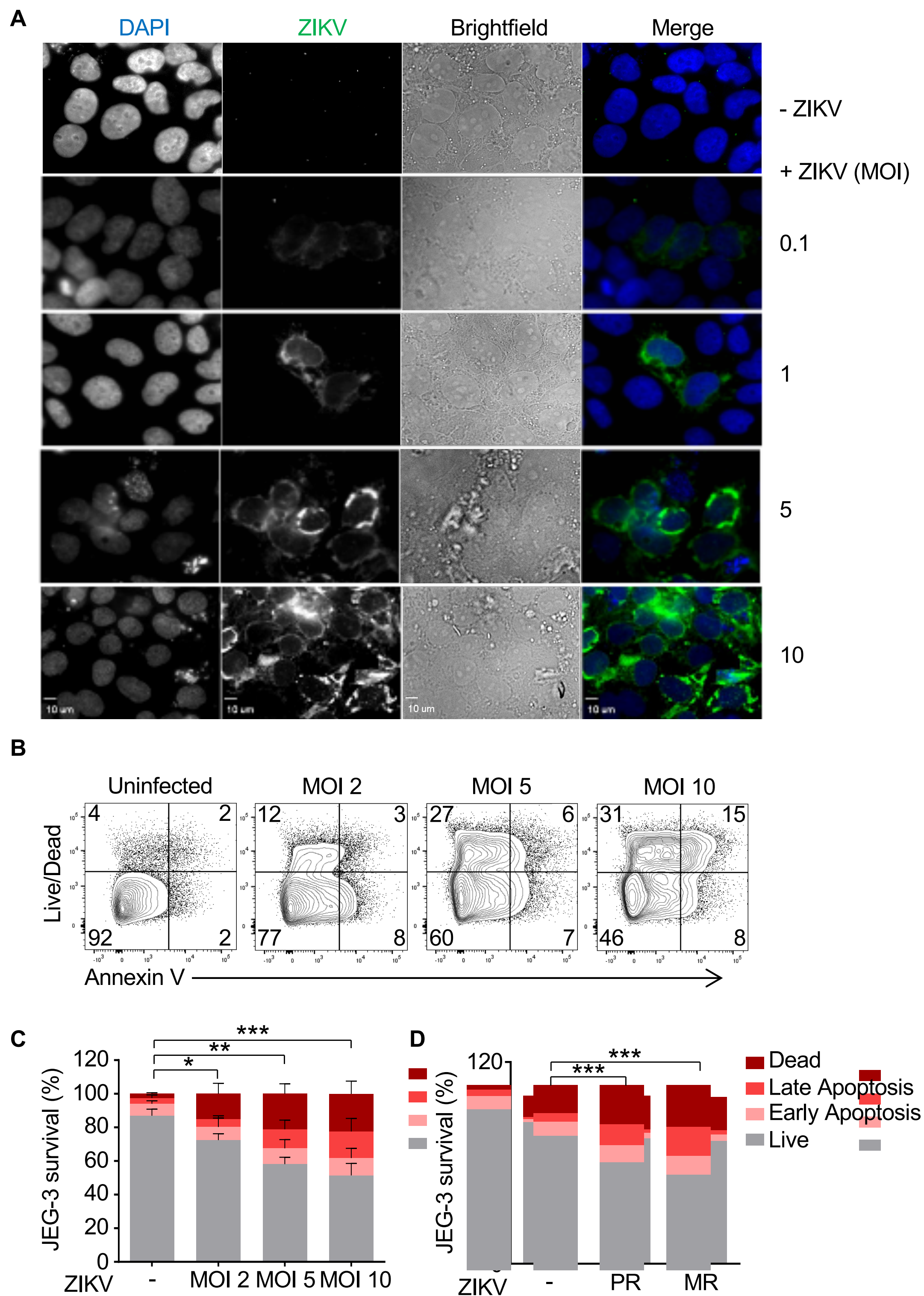


Figure S1. ZIKV efficiently infects JEG-3 and causes cytopathicity. *(A)* Fluorescence microscopy of JEG-3 infected with increasing ZIKV PRVABC59 strain MOI for 48 h and stained for anti-flavivirus group antigen and DAPI. Scale bars, 10 µm. *(B)* Representative flow cytometry plots of LIVE/DEAD and annexin V stain of JEG-3 infected with increasing MOI of ZIKV PRVABC59 strain for 48 h. *(C)* Percentage of dead and apoptotic JEG-3 infected with increasing MOI of ZIVK PRVABC59 strain for 48 h. *(D)* Percentage of dead and apoptotic JEG-3 infected with MOI 2 virus of either PRVABC59 (PR) or MR766 (MR) strain for 48 h. Bars show the mean ± SEM of 3 independent experiments. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ (two-way ANOVA followed by Tukey's multiple comparison test of the dead population). Early apoptotic cells are AnnexinV+LIVE/DEAD-, late apoptotic cells are AnnexinV+LIVE/DEAD+, dead cells are AnnexinV-LIVE/DEAD+.

Figure S2

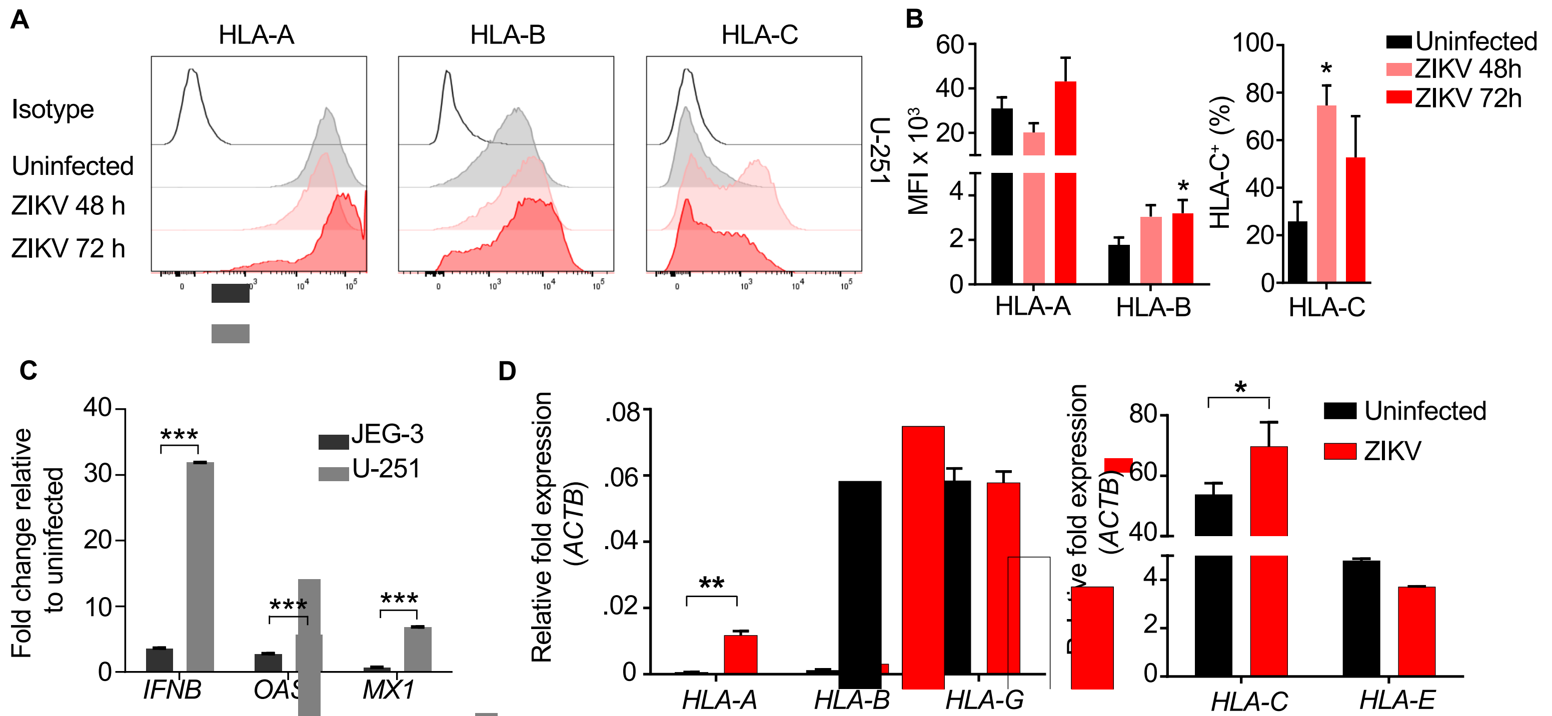


Figure S2. *(A)* Representative HLA-A, HLA-B and HLA-C flow cytometry histograms of U-251 cells (glioblastoma) that were uninfected or infected with MOI 5 of ZIKV PRVABC59 for 48 h or 72 h. *(B)* Mean fluorescence intensity (MFI) of HLA-A and HLA-B (left) and percentage of HLA-C⁺ (right) U-251 cells that were uninfected or infected with MOI 5 of ZIKV PRVABC59 for 48 h or 72 h. *(C)* Fold change in *Ifnb*, *Oas* and *Mx1* mRNA expression in ZIKV-infected JEG-3 (MOI 2) and U-251 (MOI 5) cells after 72 h. *(D)* Fold change in HLA-A, HLA-B, HLA-C, HLA-G and HLA-E mRNA expression in JEG-3 cells 48 h after ZIKV PRVABC59 infection (MOI 2), relative to uninfected cells Bars show mean ± S.E.M. of 3 independent experiments *,p<0.05; **, p<0.01; ***,p<0.001. by non-parametric one-way ANOVA (Friedman's test) with Dunn's post-test, comparing to the uninfected *(B)* and unpaired t-test (*C, D*)

Figure S3

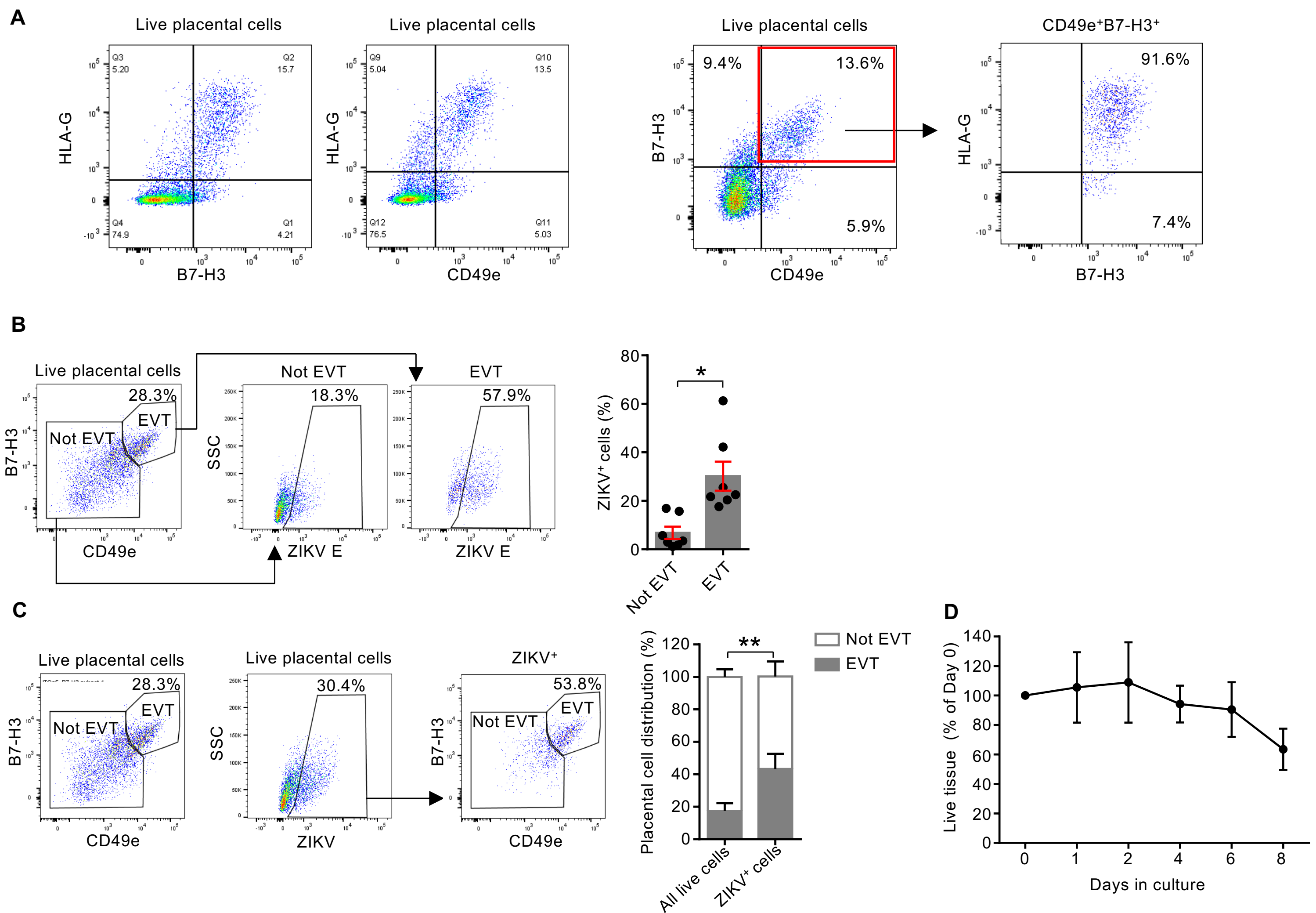


Figure S3. Gating strategy for identification of EVT, infected placental cell distribution and viability of villi explants. *(A)* Gating strategy for identification of extravillous trophoblast within isolated placental cell suspensions without using HLA-G. Cells isolated from human villi were stained with several cell type markers and analyzed by flow cytometry. After gating in live cells, the majority of EVT (HLA-G+ cells) were identified as double positive for CD49e (integrin α5) and B7-H3. *(B)* Representative flow cytometry dot plots showing the percentage of ZIKV infected cells (10^{12} PFU) within EVT and other placental cells (left) and quantification (right) (n=7). (*C*) Representative flow cytometry plots showing the distribution of EVT and other placental cells within the whole suspension or within only infected cells (left) and quantification (left) (n=7). *(D)* Viability of human villi explants in Matrigel culture for up to 8 days, relative to day 0, measured by MTT assay. Tissue amount was normalized by weight (n=2). Bars represent mean±SEM. * p<0.05, ** p<0.01 by Wilcoxon rank sum test *(B)* and two-way ANOVA followed by Sidak's multiple comparison test (C).

Figure S4

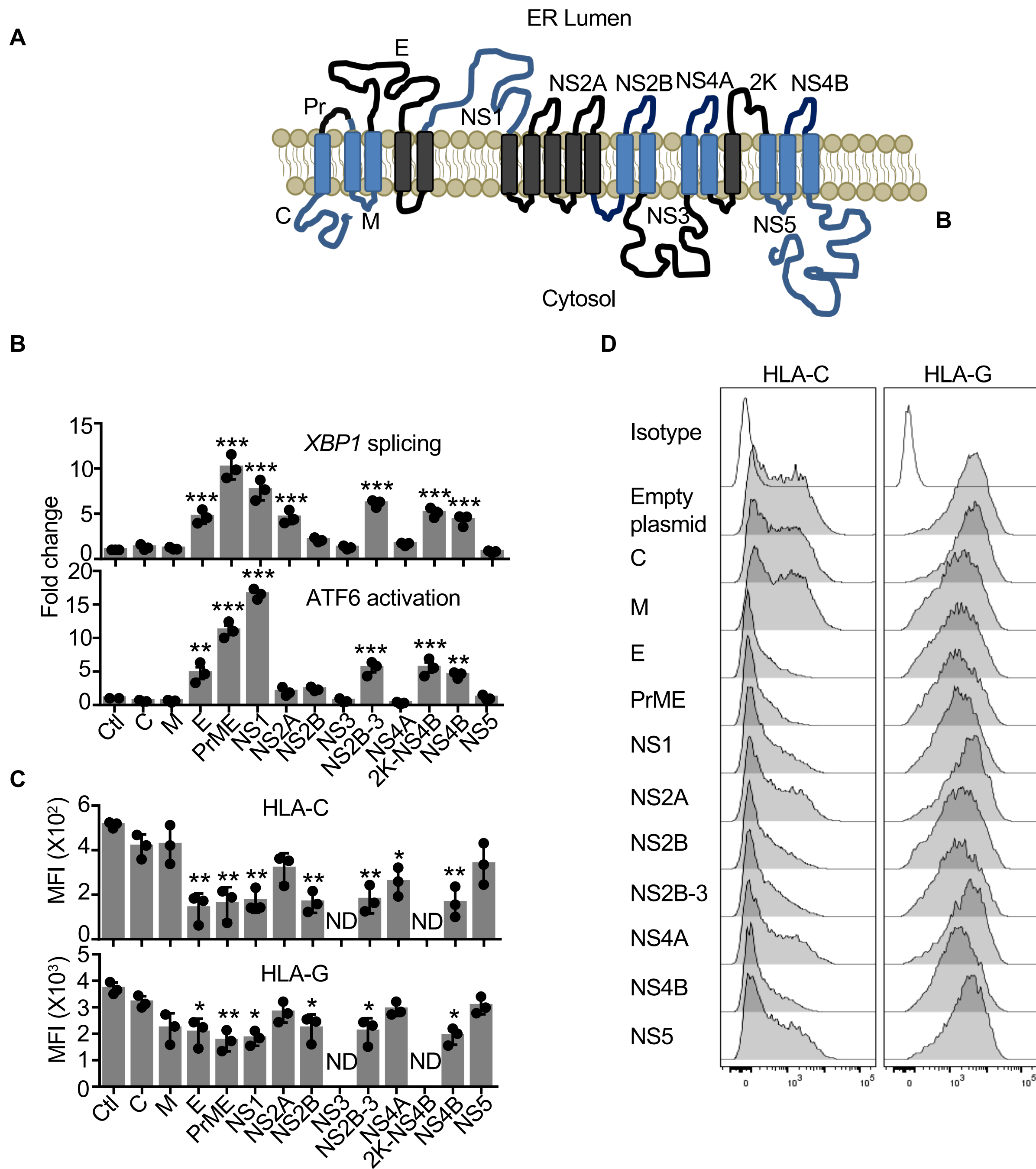
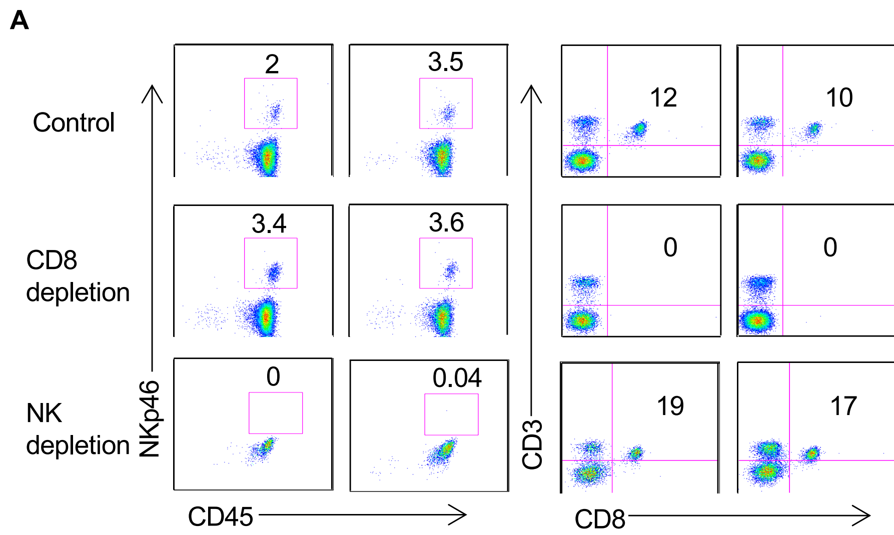


Figure S4. *(A)* Schematic of the localization of the structural and nonstructural proteins of ZIKV in the ER. *(B)* Effect of overexpression of individual ZIKV proteins on ER stress, measured by detecting firefly and Renilla luciferase activities in the lysates of JEG-3 cells that were co-transfected with either pFLAG-XBP1u-Fluc or p5xATF6-GL3 reporter plasmids with plasmids encoding individual viral proteins. Firefly luciferase activity was normalized to Renilla luciferase activity and shown compared to activity in cells transfected with the empty plasmid control (Ctl). (*C*) Effect of overexpression of individual ZIKV proteins in HLA-C and HLA-G expression. ND, not done *(D)* Representative flow cytometry histograms of HLA-C and HLA-G in JEG-3 transfected with plasmids expressing ZIKV proteins or empty plasmid. MFI is shown in B, third and fourth rows. Graphs show mean ± SEM of at least 3 experiments. *,p<0.05; **,p<0.01; ***, p<0.001 Statistics analyzed by one-way ANOVA followed by Tukey's post-test relative to control.

Figure S5



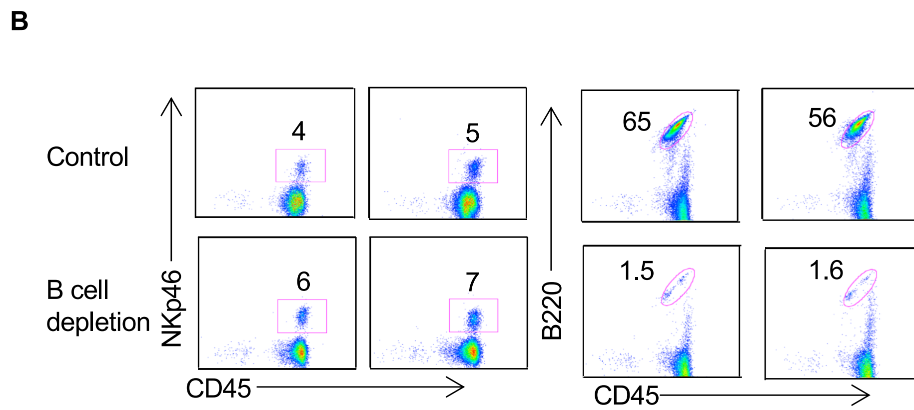


Figure S5. Representative flow cytometry plots (of three experiments) showing (A) NK, CD8 T and (B) B cells in blood from control antibody, NK, CD8 or B cell depleting antibody-treated A129 mice

Supplemental Table S1. Primers used in this study

Gene	Human	Mouse
sXBP1	CTGAGTCCGAATCAGGTGCAG	CTGAGTCCGAATCAGGTGCAG
	ATCCATGGGGAGATGTTCTGG	GTCCATGGGAAGATGTTCTGG
usXBP1	CAGCACTCAGACTACGTGCA	CAGCACTCAGACTATGTGCA
	ATCCATGGGGAGATGTTCTGG	GTCCATGGGAAGATGTTCTGG
ACTB	AAGGCCAACCGCGAGAAGAT	GCAAGTGCTTCTAGGCGGAC
	ACAGCCTGGATAGCAACGTACA	AAGAAAGGGTGTAAAACGCAGC
ATF4	GTTCTCCAGCGACAAGGCTA	GGGTTCTGTCTTCCACTCCA
	ATCCTGCTTGCTGTTGTTGG	AAGCAGCAGAGTCAGGCTTTC
CHOP	AGAACCAGGAAACGGAAACAGA	CCACCACACCTGAAAGCAGAA
	TCTCCTTCATGCGCTGCTTT	AGGTGAAAGGCAGGGACTCA
BiP	TGTTCAACCAATTATCAGCAAACTC	TTCAGCCAATTATCAGCAAACTCT
	TTCTGCTGTATCCTCTTCACCAGT	TTTTCTGATGTATCCTCTTCACCAGT
GRP94	GAAACGGATGCCTGGTGG	AAGAATGAAGGAAAAACAGGACAAAA
	GCCCCTTCTTCCTGGGTC	CAAATGGAGAAGATTCCGCC
ACTB	AAGGCCAACCGCGAGAAGAT	CATTGCTGACAGGATGCAGAAGG
	ACAGCCTGGATAGCAACGTACA	TGCTGGAAGGTGGACAGTGAGG
ZIKV primers and probe	CCGCTGCCCAACACAAG CCACTAACGTTCTTTTGCAGACAT FAM/AGCCTACCT/ZEN/TGACAAGCAATCAGACACTCAA/3IABkFQ/	
C	CTTGAATTCCATGAAAAACCCAAAAAAGAAATCCG CTTGGATCCCTATGCCATAGCTGTGGTCAGCAG	
PrM	CTTGAATTCCATGGCGGAGGTCACTAGACGTG CTTGGATCCCTAGCTGTATGCCGGGGCAATCA	
E	CTTGAATTCCATGATCAGGTGCATAGGAGTCAGC CTTGGATCCCTAAGCAGAGACGGCTGTGGATAAGAAG	
NS2A	CTTGAATTCCATGGGATCAACTGATCACATGGACC CTTGGATCCCTACCGCTTCCCACTCCTTGTGAG	

NS2B	CTTGAATTCCATGAGCTGGCCCCCTAGCGAAG CTTGGATCCCTACCTTTT TCCAGT CTTCACGTATAC
NS3	CTTGAATTCCATGAGTGGTGCTCTATGGGATGTGC CTTTCTAGACTATCTTTTCCCAGCGGCAAACTCC
NS4A	CTTGGATCCCTATCTTTGCTTTTCTGGCTCAGGTATG CTTGAATTCCATGGGAGCGGCTTTTGGAGTGATGG
NS4B	CTT GAATTCC ATG AAT GAA CTC GGA TGG TTG GAG AG CTT GGA TCC CTA ACG TCT CTT GAC CAA GCC AGC G
2KNS4b	CTTGAATTCCATG TCT CCC CAG GACAACCAA ATG G CTT GGA TCC CTA ACG TCT CTT GAC CAA GCC AGC G
NS5	CTT GAATTCC ACC ATG GGA GGT GGA ACA GGA GAG AC CTT GGT ACC CTA CAG CAC TCC AGG TGT AGA CCC
Human *IFNB*	GAATGGGAGGCTTGAATACTGCCT TAGCAAAGATCTTCTGGAGCATCTC
Human *OAS1*	GGTGGAGTTCGATGTGCTG AGGTTTATAGCCGCCAGTCA
Human *MX1*	ACCTGATGGCCTATCACCAG TTCAGGAGCCAGCTGTAGGT
HLA-A	GAC GAC ACG CAG TTC GTG C CAT GTC CGC CGC GGT CCA
HLA-B	ACC AGA GCG AGG CCG GG GTG TCC GCS CGG TCC AG
HLA-C	CGC GCG GAG TCC AAG AGG GTG TCC GCS CGG TCC AG
HLA-E	GCGAGCTGGGGCCCGCCA CCGCCTCAGAGGCATCATTTG
HLA-G	AGCTGTGGTGGTGCCTTC GGGCAGGGAAGACTGCTT

Supplemental Table S2. Sources and catalog numbers of the reagents used in this study

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Pacific Orange mouse anti-human CD45	ThermoFisher Scientific	Clone HI30; Cat.# MHCD4530
PE mouse anti-human CD56	BioLegend	Clone HCD56; Cat.# 318306
Pacific Blue mouse anti-human CD56	BioLegend	Clone HCD56; Cat.# 318326
PE-Cy7 mouse anti-human CD14	BioLegend	Clone M5E2; Cat.# 301814
PerCP-Cy5.5 mouse anti-human CD14	BioLegend	Clone M5E2; Cat.#301824
AlexaFluor 700™ mouse anti-human CD8	BioLegend	Clone SK1; Cat.# 344724
PerCP mouse anti-human CD3	BioLegend	Clone UCHT1; Cat.# 300428
PerCP-Cy5.5 mouse anti-human CD107a	BioLegend	Clone H4A3; Cat.# 328616;
PE-Cy7 mouse anti-human IFN-γ	BioLegend	Clone B27; Cat.# 506518
PE mouse anti-human HLA-A2	BioLegend	Clone BB7.2; Cat.# 343306
Biotin mouse anti-human HLA-A11	Abcam	Clone 8.L.170; Cat.# ab31574
FITC mouse anti-human HLA Class I Bw6	Miltenyi Biotec	Clone REA143; Cat.# 130-123-325
APC-Vio770 mouse anti-human HLA Class I Bw6	Miltenyi Biotec	Clone REA143; Cat.# 130-099-837
VioGreen mouse anti-human HLA Class I Bw6	Miltenyi Biotec	Clone REA143; Cat.# 130-099-849
Purified mouse anti-human HLA-C	Mary Carrington	Clone DT9
PE mouse anti-human HLA-E	BioLegend	Clone 3D12; Cat.# 342604
Purified mouse anti-human HLA-E	BioLegend	Clone 3D12; Cat.# 342602
PE mouse anti-human HLA-F	BioLegend	Clone 3D11; Cat.# 373204
Purified mouse anti-human HLA-F	BioLegend	Clone 3D11; Cat.# 373202
PE mouse anti-human HLA-G	Abcam	Clone MEM-G/9; Cat.# ab24384;
APC mouse anti-human HLA-G	Abcam	Clone MEM-G/9; Cat.# a40915
AlexaFluor 488™ mouse anti-human MICA/MICB	Biolegend	Clone 6D4; Cat.# 320912
AlexaFluor 700™ mouse anti-human MICA/MICB	R&D Systems	Clone 159207; Cat.# FAB13001N-100
PE-Cy7 mouse anti-human B7-H3	BioLegend	Clone MIH42; Cat.# 351008
PE mouse anti-human CD49e	BioLegend	Clone NKI-SAM-1; Cat.# 328010
FITC mouse anti-human CD49e	BioLegend	Clone NKI-SAM-1; Cat.# 328008
Purified mouse anti-human E-Cadherin (CDH-1)	GeneTex	Clone G-4770; Cat.# GTX100443S;
Purified rabbit anti-human CD49e (Integrin α5)	Abcam	Polyclonal; Cat.# ab112183
Purified mouse anti-human SDC-1	BioLegend	Clone MI15; Cat.# 356523
Purified mouse anti-flavivirus E-glycoprotein	Abcam	Clone 3571; Cat.# ab155882

Purified mouse anti-flavivirus Group Antigen	EMD-Millipore	Clone D1-4G2-4-15; Cat.# MAB10216-I-100UG
Purified Rabbit anti-Zika Virus NS2B	GeneTex	Polyclonal; Cat.# GTX133308
AlexaFluor 647™ mouse anti-human NKp46	BioLegend	Clone 9E2; Cat.# 331910
AlexaFluor 647™ mouse anti-human NKp30	BioLegend	Clone P30-15; Cat.# 325212
AlexaFluor 647™ mouse anti-human NKp44	BioLegend	Clone P44-8; Cat.# 325112
FITC mouse anti-human NKp80	Miltenyi Biotec	Clone 4A4.D10; Cat.# 130-094-843
PE mouse anti-human NKG2C	R&D Systems	Clone 134591; Cat.# FAB138P-100
APC mouse anti-human NKG2D	BD Biosciences	Clone 1D11; Cat.# 562064
PE-Cy7 mouse anti-human DNAM-1	BioLegend	Clone 11A8; Cat.# 338316
APC-Cy7 mouse anti-human 2B4	BioLegend	Clone C1.7; Cat.# 329518
Ultra-LEAF™ Purified mouse anti-human NKp46	BioLegend	Clone 9E2; Cat.# 331948
Ultra-LEAF™ Purified mouse anti-human NKp30	BioLegend	Clone P30-15; Cat.# 325224
Purified mouse IgG1	BioLegend	Clone MOPC-21; Cat.# 400102;
Purified mouse IgG2a	BioLegend	Clone MOPC-173; Cat.# 400202
AlexaFluor 488™ mouse IgG1 Isotype control	BioLegend	Clone MOPC-21; Cat.# 400129
PE mouse IgG1 Isotype control	BioLegend	Clone MOPC-21; Cat.# 400113;
AlexaFluor 647™ mouse IgG1 Isotype control	BioLegend	Clone MOPC-21; Cat.# 400130;
PerCP-Cy5.5 mouse IgG1 Isotype control	BioLegend	Clone MOPC-21; Cat.#400150;
PE-Cy7 mouse IgG1 Isotype control	BioLegend	Clone MOPC-21; Cat.# 400126
APC-Cy7 mouse IgG1 Isotype control	BioLegend	Clone MOPC-21; Cat.# 400128
AlexaFluor 488™ mouse IgG2a Isotype control	BioLegend	Clone MOPC-173; Cat.# 400233;
AlexaFluor 700™ mouse IgG2a Isotype control	BioLegend	Clone MOPC-173; Cat.# 400248;
PerCP-Cy5.5 hamster anti-mouse CD3	BD Biosciences	Clone 145-2C11; Cat.# 100328;
PE rat anti-mouse/human B220	BioLegend	Clone RA3-6B2; Cat.# 103208
PerCP-eFluor 710 rat anti-mouse CD8	eBioscience	Clone 53-6.7; Cat.# 46-0081-82
PE-Cy7 rat anti-mouse CD45	BioLegend	Clone 30-F11; Cat.# 103114
PE rat anti-mouse NKp46	BioLegend	Clone 29A1.4; Cat.# 12-3351-82
Purified rabbit anti-mouse/human pan-cytokeratin	Antibodies online	Polyclonal; Cat.# ABIN969074
Purified rabbit anti-mouse vimentin	Abcam	Clone EPR3776; Cat.# ab92547
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647™	ThermoFisher Scientific	Polyclonal; Cat.# A-31571

Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488™	ThermoFisher Scientific	Polyclonal; Cat.# A21202
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488™	ThermoFisher Scientific	Polyclonal; Cat.# A32790
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 647™	ThermoFisher Scientific	Polyclonal; Cat.# A32795
PE goat anti-mouse secondary	BioLegend	Clone Poly4053; Cat.# 405307
Streptavidin, APC	ThermoFisher Scientific	Cat.# SA1005
Streptavidin, FITC	BD Biosciences	Cat.# 554060
Purified rabbit anti–mouse CD20	BioLegend	Clone SA271G2; Cat.#152102
Purified rat anti–mouse CD8α	BioXCell	Clone 2.43; Cat.# BE0061
Purified rabbit anti–mouse asialo-GM1	Wako Chemicals	Antiserum; Cat.# 986-10001
Purified rat IgG2b Isotype control	BioXCell	Clone LTF-2; Cat.# BE0090;
Chemicals, Peptides, and Recombinant Proteins		
DPBS	Gibco	Cat.# 14190250
DMEM	Gibco	Cat.# 11965092
RPMI 1640	Corning	Cat. # 15-040-CV
DMEM/F12	Gibco	Cat.# 11320033
Newborn Calf Serum	Gibco	Cat.# 16010159
Fetal Calf Serum	X&Y Cell Culture	Cat.# FBS-500-HI
Penicillin/Streptomycin	Gibco	Cat.# 15140122
L-glutamine	Gibco	Cat.# 25030081
TrypLE Express	ThermoFisher Scientific	Cat.# 12605036
Trypsin from porcine pancreas lyophilized powder, Type II-S	Sigma	Cat.# T7409-100G
EDTA, disodium, dihydrate	American Bioanalytical	Cat.# AB00500-00100;
Ficoll® Paque Plus	GE Healthcare	Cat.# GE17-1440-02
Human Fibronectin	Corning	Cat.# 354008
Collagenase type IV from *Clostridium histolyticum*	Sigma	Cat.# C5138-5G;
Collagenase D from *Clostridium histolyticum*	Roche	Cat.# 11088882001;
Deoxyribonuclease I from bovine pancreas	Sigma	Cat.# DN25-G5;
Red Blood Cell Lysing Buffer Hybri-Max™	Sigma	Cat.# R7757-100ML
Percoll	GE Healthcare	Cat.# 17089109
Matrigel	Corning	Cat.# 354234
Gentamicin	Gibco	Cat.# 15750060
Amphotericin B	Sigma	Cat.# A2942-20ML
X-Vivo 10 Serum free hematopoietic cell medium	Lonza	Cat.# 04-380Q
Human AB Serum	Corning (Fisher Scientific)	Cat.# MT-35-060-CI
Recombinant human IL-15	BioLegend	Cat.# 715902

Insulin-Transferrin-Selenium (ITS-G)	Gibco	Cat.# 41400045
Recombinant human EGF	Peprotech	Cat.# AF-100-15;
Human chorionic gonadotropin	Sigma	Cat.# C1063-1VL;
Methylcellulose	Sigma	Cat.# M7027
Avicel RC-591 inf	FMC Corporation	N/A
EMEM 2X without Phenol Red	Quality Biologics	Cat.# 115073101
Salubrinal	Sigma	Cat.# SML0951
Tunicamycin	Sigma	Cat.# T7765
MTT Formazan	Sigma	Cat.# M2003
Hydrochloric Acid	Sigma	Cat.# 320331
Chromium-51 Radionuclide	Perkin Elmer	Cat.# NEZ030001MC; CAS: 16284-59-6
Protein Transport Inhibitor (Containing Brefeldin A)	BD Biosciences	Cat.# 555029
Monensin	BioLegend	Cat.# 420701
EGTA	Sigma	Cat.# E3889-100G
Paraformaldehyde in PBS, 4%	Affymetrix	Cat.# AAJ19943K2
Formaldehyde, 16%, methanol free, Ultra Pure	Polysciences, Inc	Cat.# 18814-10; CAS: 50-00-0
Formalin Solution, neutral buffered, 10%	Sigma	Cat.# HT501128
BSA	Sigma	Cat.# A9418-500G CAS: 9048-46-8
Triton X-100	Sigma	Cat.# X100-500ML; CAS: 9002-93-1
DAPI	ThermoFisher Scientific	Cat.# 62248
Airvol ® 205 Polyvinyl alcohol	Air Products and Chemicals	N/A
Sucrose	Sigma	Cat.# S8501 CAS: 57-50-1
O.C.T. Compound	Tissue Tek	Cat.# 4583
Glycine	Sigma	Cat.# 50046; CAS: 7299-33-4
Tween 20	Sigma	Cat.# P1379-500ML CAS: 9005-64-5
Critical Commercial Assays		
Human BD Fc Block	BD Biosciences	Cat.# 564219
Fixation/Permeabilization Solution Kit	BD Biosciences	Cat.# 554714
LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit	ThermoFisher Scientific	Cat.# L34955
Mix-n-Stain™ CF647 Dye Antibody Labeling Kit	Biotium	Cat.# 92238
Annexin V APC	BD Biosciences	Cat.# 550475
Annexin V Binding Buffer, 10X	BD Biosciences	Cat.# 556454
RNeasy Mini Kit	Qiagen	Cat.# 74104
DNase I	ThermoFisher Scientific	Cat.# 89836
SuperScript II Reverse Transcriptase Kit	ThermoFisher Scientific	Cat.# 18064014
Power SYBR Green PCR Master Mix	Applied Biosystems	Cat.# 4368702
FuGENE HD Transfection Reagent	Promega	Cat.# E2311

Dual-Luciferase Reporter Assay System	Promega	Cat.# E1910
RosetteSep Human NK Cell Enrichment Cocktail	STEMCELL Technologies	Cat.# 15065
All primers	IDT	
Plasmids		
Flag-tagged pcDNA3.1	ThermoFisher Scientific	Cat.# V79520
pFLAG-CMV40	Sigma	Cat.# E7158
pFLAG-XBP1u-Fluc	Addgene	Cat.# 31239
p5xATF6-GL3	Addgene	Cat.# 11976

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