Supplemental Information

CEACAM1-Mediated Inhibition of Virus Production

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**Fig. S2**

**A.**

![Graph showing MHC-I expression](image)

**B.**

IRF3 binding site

\[\text{WT: } \text{GAAA} \text{GAAA} \text{GAGAAA} \text{GT}\]

\[\text{IRF3}^{\text{Mut}: } \text{GAAA} \text{GGCGAAA} \text{GCGGT}\]

\[\text{Ex1} \quad -228 \quad -135 \quad 0\]

**C.**

| Species                | Promoter conservation (%) | IRF3 site sequence          | Site position from CDS (bp) |
|------------------------|---------------------------|------------------------------|-----------------------------|
| *H. Sapiens* (Human)   | -                         | GAAA\text{GAAA}\text{GAGAAA} \text{GT} | 228                         |
| *P. Troglydes* (Chimpanzee) | 99.4                     | GAAA\text{GAAA}\text{GAGAAA} \text{GT} | 228                         |
| *G. Gorilla*           | 99                       | GAAA\text{GAAA}\text{GAGAAA} \text{GT} | 228                         |
| *P. Pygmaeus Abelii* (Orangutan) | 96                   | GAAA\text{GAAA}\text{GAGAAA} \text{GT} | 234                         |
| *M. Musculus* (Mouse)  | No significant similarity | No site                     | N/A                         |

**D.**

**E.**

![Graph showing mCEACAM1 expression](image)
Fig. S4

A. **FL4-H** Count

B. **S\textsuperscript{35}Methionine** incorporation (%)

C. **S\textsuperscript{35}Methionine** incorporation (%)

D. **S\textsuperscript{35}Methionine** incorporation (%)

E. **S\textsuperscript{35}Methionine** incorporation (%)

F. **S\textsuperscript{35}Methionine** incorporation (%)

G. **S\textsuperscript{35}Methionine** incorporation (%)

H. **S\textsuperscript{35}Methionine** incorporation (%)

I. Relative IL-2 secretion (fold change)

J. Relative IL-2 secretion (fold change)

K. Relative IL-2 secretion (fold change)
Supplementary Figure Legends

Figure S1 Intrinsic induction of CEACAM1, related to Fig.1

(A) FACS staining for CEACAM1 expression on HFF cells infected (3 days post infection, DPI, black empty histogram) with HCMV derived from a clinical isolate (Clinical isolate). Empty gray histogram was staining for CEACAM1 on mock treated cells (Mock) and filled gray histogram was background staining with isotype matched antibody (Bckgnd). (B) CEACAM1 expression (black empty histogram) on HFF cells that were infected (MOI 3) with a UV-inactivated TB40/E virus at 24 HPI (left histogram, 24 HPI UV-TB40). Supernatants were collected at 24, 48, or 96 HPI, transferred to recipient cultures, co-incubated for 48 hours, and then stained for CEACAM1 expression (three right panels, black empty histograms is staining of CEACAM1 on cells receiving sup. from 24, 48, 96 HPI). Background (Bckgnd) were cells stained with isotype matched IgG (filled gray histogram) and mock treated cells (Mock) that were stained for CEACAM1 are shown as empty gray histograms. (C) Real-time PCR quantification of CEACAM1 mRNA levels in HFF cells treated with 100µg/ml cycloheximide that were mock or TB40/E infected (MOI 3) at 2HPI. ***P<0.001. (D) FACS analysis for fold increase in CEACAM1 expression on HFF cells expressing two anti-IFI16 shRNA clones (αIFI16.1/2) and control shRNA sequence, 3 days following transfection of 1µg/ml of polyI:C. Fold increase was compared to MFI of mock treated cells expressing the control shRNA sequence. Figures presented are a representative out of five (A) or three (B-D) independent experiments performed and an average±SD of three triplicates (C-D). N.S – Not significant, ***P<0.001.
Figure S2 Conserved IRF3 binding site in CEACAM1 promoter, related to Fig.2

(A) FACS analysis of pan MHC class I expression (MHC-I) on mock (mock) or IFN-γ treated ARPE-19IFI16isoB cells that underwent knockdown with two shRNAs targeting IRF3 (αIRF3.1/2) or control shRNA sequence (Control). For background (Bckgnd), IFN-γ treated cells expressing the control shRNA sequence were stained with isotype matched control IgG. (B) Schematic representation of the CEACAM1 promoter region with "Ex1" indicating transcript initiation point at first exon and "ATG", which designates the start of the CEACAM1 open reading frame (ORF). The ORF initiation point was set as position "0" and upstream nucleotide positions were assigned negative numeration in relation to this point. The wild-type IRF3 binding site sequence is indicated (WT) along with the mutation introduced into the IRF3 site (IRF3 mutation). (C) Alignment of sequences obtained from the UCSC genome browser to examine the conservation and position (Site position from CDS) of IRF3 binding site sequence in the human CEACAM1 promoter compared to the indicated mouse and primate species. Promoter conservation indicates the percent similarity of the immediate 1000bp promoter region upstream of each CEACAM1 promoter compared to the human sequence. (D) FACS analysis of mCEACAM1 expression on the cell surface of various murine cells following infection with C3X MCMV virus at MOI 1. Cells were stained for mCEACAM1 at 2 DPI (C3X, black empty histogram) and compared to staining of mock treatment (Mock, gray empty histogram), and control background staining (Bckgnd, gray filled histogram). (E) Staining of mouse CEACAM1 on PD1.6 cells (Anti-mCEACAM1, black empty histogram). Cells were also stained with isotype matched control IgG (Control, empty gray histogram) or stained with secondary antibody alone for background (Bckgnd, filled gray histogram). Figures (A, D,
E) are a representative out of three independent experiments performed with similar results.

**Figure S3 IRF3-mediated cellular sensing pathways and CEACAM1-dependent SHP2 function, related to Fig.2-3**

(A) Diverse innate immune sensing machineries converging on IRF3. A schematic representation of various intrinsic cellular pathogen detection systems that culminate in the activation of IRF3. AdV – Adenovirus; cGAS – Cyclic GMP-AMP synthase; CMV – Cytomegalovirus; DAI – DNA-dependent activator of interferon regulatory factors; DDX – DEAD box polypeptide; DHX – DEAH box polypeptide; EBV – Epstein-Barr virus; ER – endoplasmic reticulum; HCV – Hepatitis C virus; HSV – Herpes simplex virus; IFI16 – Gamma-interferon inducible protein 16; IPS-1 – IFN-β promoter stimulator 1; IRF3 – Interferon response factor 3; KSHV – Kaposi sarcoma associated herpes virus; MDA5 – Melanoma differentiation associated protein 5; NOD2 – Nucleotide binding oligomerization domain containing protein 2; RIG-I – Retinoic acid inducible gene 1; RSV- Respiratory syncytial virus; STING- Stimulator of IFN genes; TBK1- Tank binding kinase 1; TLR – Toll like receptor; VSV – Varicella Zoster virus; VV – Vaccinia virus; WNV – West Nile virus. (B) Examining CEACAM1 induction on A549 cells stably transduced with two RIG-I specific shRNAs (αRIG-I.1/2) and control shRNA (control) that were transfected with 1µg/ml purified UV-inactivated TB40/E DNA (UV-DNA) compared to mock (mock) treatment with transfection reagent alone. Background (Bckgnd) are DNA transfected control shRNA transduced cells stained with an isotype matched, control IgG. (C) IFN-γ and mock treated A549 cells expressing control and two IRF3 specific shRNAs (αIRF3.1/2) that were analyzed for expression of pan MHC class I
Control cells treated with IFN-γ that were stained with isotype matched IgG were used as background (Bckgnd). (D-E) Co-immunoprecipitation of CEACAM1 (αCEACAM1), isotype matched control antibody (IgG), or pre-IP lysate (Input) followed by probing with anti-SHP2 antibody in lysates of HFFs infected with TB40/E virus (D) or A549 cells infected influenza virus (E). (F) Intracellular staining of SHP2 in TB40/E infected ARPE-19 cells stably transduced with a control shRNA (Control) or two different clones of SHP2 targeting shRNAs (αSHP2.1/2). For background (Bckgnd), control cells were stained with isotype matched IgG. (G) Five days post infection, supernatant of cells from (F) was collected and titer of TB40/E was determined. Figures (B-G) are a representative of three experiments performed with similar results, and an average±SD of triplicates (G).

**Figure S4** Over expression of CEACAM1 and SHP2 suppress protein production and CEACAM-Ig blocks CEACAM1 activation for ex vivo model, related to Fig. 4

(A, C) HFF cells stably over-expressing CEACAM1 (A, CEACAM1 OE), SHP2 (C, SHP2 OE) depicted with a black empty histogram that underwent extra and intracellular FACS staining for CEACAM1 and SHP2, respectively. (B, D) Protein production analysis in cells from (A) over expressing CEACAM1 (B) and HCMV infected cells from (C) over expressing SHP2 (D) by assessment of $[^{35}\text{S}]$Methionine incorporation. (E, G) Extracellular CEACAM1 (E) and intracellular SHP2 (G) FACS staining in A549 cells overexpressing CEACAM1 (E, CEACAM1 OE) or SHP2 (G, SHP2 OE) depicted in black empty histogram. (F, H) Quantification of global cellular protein production by
assessment of \[^{35}S\]Methionine uptake in A594 cells from (E) over-expressing CEACAM1 (F) and influenza virus infected cells from (G) over-expressing SHP2 (H).

For figures (A, C, E, G), gray empty histogram is staining of control cells transduced with empty vector (Control) and gray filled histogram are background (Bckgnd) cells stained with isotype matched control antibody. In figures (B, D, F, H), protein production in empty vector transduced control cells was set as 100%. (I) Human decidual tissues were acquired from women undergoing elective termination of first-trimester pregnancies. Decidual tissues were isolated and underwent a washing and sectioning procedure that prepared them for organ culturing and infection by HCMV. Tissues were then immediately infected with a GFP encoding TB40/E\(^{\text{GFP}}\) virus enabling tracking of viral infection in downstream confocal imaging and FACS assays. (J) Staining for CEACAM1 expression on BW reporter cell line stably expressing a construct of extracellular human CEACAM1 fused intracellular mouse zeta-chain (BW\(^{\text{CC1}}\), black empty histogram) and parental cell line (BW\(^{\text{Par}}\), empty gray histogram). (K) IL-2 secretion by BW\(^{\text{CC1}}\) cells when co-incubated with parental 721.221 (221\(^{\text{Par}}\)) cells or 721.221 cells stably expressing CEACAM1 (221\(^{\text{CC1}}\)) administered with CEACAM-Ig (CC1-Ig) or control Ig fusion protein (Ctrl-Ig). Figures are a representative of three independent experiments performed with similar results and are an average±SD of six replicates (B, D, F, H, K). ***P<0.001
Supplementary Experimental Procedures

Cells, organ culture, antibodies, antibody array, FACS, and IF

Primary HFF cells were cultured from human foreskin samples obtained in accordance to institutional guidelines and ethics approvals. Other cell lines used included ARPE-19, A549, B12, MEF, MDCK, NIH-3T3, SVEC, 293T, PD1.6, BW, and 721.221 (ATCC). All cells were maintained in fully supplemented DMEM medium except for ARPE-19 cells that were cultured in a DMEM medium additionally supplemented with F12 at a 1:1 ratio and PD1.6, BW and 721.221 cells, which were maintained with RPMI medium. MEF cells were harvested by standard procedure from BALB/c mouse embryos, and used at passage one or two for MCMV infections. Preparation and infection of decidua organ culture was performed as previously described (Weisblum et al., 2011). Briefly tissues were obtained from women undergoing first-trimester elective pregnancy that gave written informed consent. The procedure was performed according to the Declaration of Helsinki, good clinical practice guidelines, and the human experimentation guidelines of the Israeli ministry of Health. Tissues were washed with PBS, sectioned for approximately 10 cell thickness, and maintained in standard F12 and amphotericin supplemented DMEM medium. Infections were then immediately performed in 48-well plates, with 12h adsorption period of, wash cycles, and medium replacement every 2 days. Staining for human and mouse CEACAM1 was performed with anti-CD66 (Biolegend) and 5F4 clone (kindly provided by R.S. Blumberg), which demonstrate similar staining. Mouse IgG2b and IgG1 (Biolegend) were used as an isotype matched control antibodies, respectively. For staining of human HLAs W6/32 clone was used for HLA-A/B/C (MHC-I) as well as anti-HLA-G (Biolegend). Anti-mouse Dylight649
(Jackson) was used as a secondary antibody for the FACS staining. FACS staining was according to standard procedure with adherence to instructions from antibody manufacturer. For intracellular FACS staining, briefly, cells were perforated in methanol at -20°C, rehydrated in PBS for half an hour, and then stained with antibodies in 5% BSA in PBS at room temperature. Antibodies used were anti-IFI16 and SHP2 (Santa Cruz). Data acquisition was performed on a BD FACScalibur and analyzed using the FCS express software (De Novo). For western blots anti-human IRF3, RIG-I, and GAPDH (Santa Cruz) were used. Antibodies used in the Pathscan antibody array (Cell signaling), were (presented in the same order as depicted in figure 6a): Stat1 (pY701), Stat3 (pY705), Akt (T308), Akt (S473), mTOR (S2448), HSP27 (S78), p70 S6K (T389), SAPK/JNK (T183/Y185), PARP (Asp214 cleavage site), and Caspase-3 (Asp175 cleavage site). The Pathscan antibody array assay was performed according to the manufacturer’s recommendations, briefly, following equilibrated lysate incubation on antibody array a biotinylated detection antibody mix was applied followed by application of DyLight 680 conjugated streptavidin. Acquisition and quantification of the fluorescence was then performed with the LI-COR Odyssey scanner and imaging software. The control KIR2DS4-Ig and the CEACAM1-Ig fusion proteins were produced and purified as previously described (Katz et al., 2004; Markel et al., 2002) and were applied to the decidua culture at 5 µg/ml. Immunofluorescence staining was performed according to standard procedure. Briefly, frozen tissues were cryosectioned onto slides, fixed in 4% PFA and perforated with 0.1% - Triton in PBS solution. Sections were then blocked with CAS-Block (Invitrogen) and then stained with primary and secondary antibody in CAS-block solution for desired antigen or with DAPI for nuclear staining.
Tissues were then visualized with the FV1000 confocal microscope (Olympus).

**Viruses, infections, titrations, and transfections**

HCMV virus strains used were the TB40/E, TB40/F, TB40/E-GFP, AD169, and AD169Δpp65 (all from ATCC), were all propagated in HFF cells as previously described (Wolf et al., 2001). Other viruses used included MCMV (C3X strain; ATCC) and the influenza virus that was used was the influenza A/Puerto Rico/8/34/H1N1 (PR8) strain which was grown in eggs as previously described (Achdout et al., 2003). All viruses were titrated and infection at the indicated MOI was performed with 2h viral adsorption for HCMV infections and 1h adsorption with influenza infections followed by two wash cycles with phosphate-buffered saline (PBS) and fresh medium reconstitution. For all HCMV infections, cells were plated 3 days prior to infection at cell numbers that would reach confluency on the day of infection. Infection with clinical HCMV isolates was performed by preparing an infective mixture by direct administration of urine samples from patients with ongoing viremia into cell medium. UV inactivation prior to viral adsorption or viral DNA transfection, was performed with the UV Stratalinker 2400 (StrataGene) at 0.99 Joule. Viral inactivation was confirmed at >99.99% with plaque assays. Titrations of HCMV viruses were performed by plaque formation assay on confluent HFF grown in soft agar. Prior to all titrations in supernatant of virus-infected cells, cells were counted and no differences were observed in numbers. For infections with PR8, cells were plated one day prior to infection to reach 95% confluence and neutralize differences in proliferation, washed with PBS and infected with virus resuspended in supplement-free DMEM with 0.5% trypsin. Following infection, cells were maintained in DMEM with standard supplements, 2% fetal calf serum and 0.5%
trypsin. For influenza virus ELISA based titrations, MDCK cells were plated at in 96F plates overnight and then infected with supernatants with PR8 virus to be titrated as described above. Following 72h of incubation, cells are washed with PBS, fixed with 4% paraform-aldehyde and permeabilized with triton x 0.2%. This was followed by incubation with primary anti-influenza A antibody, incubation with HRP-conjugated secondary antibody, and development with substrate. For preparation of shRNA (bacterial glycerol stock from Sigma), IFI16, and SHP2 lentivirus and CEACAM1 retrovirus vectors, 293T cells were co-transfected with the lentiviral vector, a plasmid encoding the lentiviral Gag/Pol, and a plasmid encoding the VSV-G at a 10:6.5:3.5 ratio respectively. Supernatants with the viral particles were collected after 48 hours. Cells were selected for shRNA vector expression with puromycin at 5µg/ml for HFF and A549 cells as determined by puromycin sensitivity death curves, for a period of 6 days. Viral DNA and polyI:C transfection were performed on cells that were plated in 24 well plates at 50k cells/well, which were subsequently transfected with 1µg/ml of DNA with 2µl/µg of LT-1 (MirusBio) transfection reagent per DNA or polyI:C, according to manufacturer's recommendations. Viral DNA was isolated from cell free purified viral stocks with whole blood DNA purification kit (Qiagen).

**Realtime-PCR, Cloning, and shRNAs**

For mRNA quantification, total RNA was isolated from cells using the Total RNA isolation kit (Zymoresearch). RNA was reverse transcribed with Moloney murine leukemia virus reverse transcriptase (Invitrogen) and with polyT primer (Sigma). Quantitative amplification was conducted on an ABI PRISM 7900 real-time PCR system (Applied Biosystems) with gene specific primers and Platinum SYBR Green qPCR
SuperMix-UDG with ROX (Invitrogen). Briefly, labeling of nascent RNA was performed with 4-thiouridine (4sU) in cells for 60 min. Cellular RNA was purified and – SH groups were biotinylated (Pierce). RNA was precipitated with isopropanol and biotinylated RNA was purified with strepavidin beads column (Miltenyi). RNA was reverse transcribed and analyzed by real-time PCR. Primers used for the real-time PCR and cloning: Amplification of CEACAM1 fwd

5’TGCTGAACGTAAACTATAATGCTCT, rev 5’ GGAGACTGAGGGTTTGTGCT;
IFN-β fwd 5’AAGCAATTGTCAGTCCCCAG, rev 5’CCTGAAGGCCAAGGAGTACA;
IL-1β fwd 5’GGAGATTCGTAGCTGGATGC, rev
5’GAGCTCGCCAGTGAAATGT; IFI16 fwd 5’CATGAACGTCCTTGAAAT, rev
5’ACTCCTCAGATGCACACCAAC; IRF3 fwd 5’GATGCACAGCAGGAGGATTT, rev
5’ TAAACGCAACCCTTCTTGC; RIG-I fwd 5’ATCCCAGTGATAGAACAAGCAG;
rev 5’GCCTGTAAGCTCTATAACCCATGTC; HPRT and GAPDH used as normalizers for qPCR analyses. HPRT fwd 5’TGACACTGGCAAAACAATGCA, rev
5’GGTCCTTTCTACCAGCAAGCT; GAPDH fwd 5’TGCACCACCAACTGCTTA, rev
5’GGATGCAGGGATGTGTTCC; Following ChIP: CEACAM1 promoter fwd
5’ACCCAAATGTAGACAGAAAGGAA; rev 5’AGCTCTGTTTCTCTCTCTCT;
Control sequence upstream to CEACAM1 promoter fwd
5’CGGGAGGCTCTGGATTTTTA; rev 5’CATCTCCAGCAACAACCTCCA;
Cloning into pHAGE-DsRED(-)-eGFP(+) lentiviral vector (cloned with kozak priming and with NotI and XhoI) of IFI16 isoforms
fwd 5’CCCGCGGCCGCGCCGCCACCATGGGAAAAAAATACAAGAAC; rev
5’GGGCTCGAGCTAAGCGTAATCTGGAACATCGTATGGGTAGAAGAAAAAGT
CTGGTGA; and of SHP2
fwd 5’CCCGCGGCCGCGCCGCCACCATGACATCGCGGAGATGGTT;
rev 5’CCCCTCGAGTCATCTGAAACTTTTCTGCT
Cloned sequences were annotated and aligned to UniProt database for isoform
identification. Primers used for cloning and mutating CEACAM1 promoter (cloned with
XhoI and HindIII) in pGL4.14 firefly luciferase expression vector (Promega) fwd
5’CGCCTCGAGCCTGGACTTGGGTCTCTGTC; Mutation rev 5’
TCCTACCTTTTGCTCTTACCGCTTTGCCCCTTCTGTCTCATTTT; Mutation fwd
5’AAAATGTAGACAGAAAGGGCGAAAGCGGTAAGGACAAAGTAGGA;
CEACAM1 promoter rev 5’CGCAAGCTTTCACCTGTGGAGGAGAGCTT.
CEACAM1-3L (3 immunoglobulin extracellular domain long variant) was transduced
using the pQCXIP-Puromycin(+) retroviral vector.
shRNA sequences were based on the pLKO.1 lentiviral vector backbone with puromycin
selection marker (Sigma). All experiments were controlled to a scrambled sequence
encoded in the same vector (Sigma). Sequences of shRNA clones with most efficient
knockdown are included.
IFI16
5’CCGGGTCAGGTAACTCCCAGAAGAAGCGGTAAGGACAAAGTAGGA
IRF3
5’CCGGGATCTGATTACCTTCACGGAACTCGAGTTCCGTGAAGGTAATCAGATC
TTTTTT;
RIG-I
5’CCGGCCATGTGAAGTACAAGACATTCTCGAGAATGTCTTGTACTTCACATGGTTTTTTTG;
CEACAM1
5’CCGGCCACCTAACAAGATGAATGAACTCGAGTTCATTCATCTTGTTAGGTGTTTTTTTG;
SHP2
5’CCGGGCAGTTAAATTGTGCGCTGTACTCGAGTACAGCGCACAATTTAACTGCTTTTT;

Luciferase assay
The cells were grown to 50%–60% confluence in 24-well plates. Cells were then transfected using LT-1 (MirusBio), with 60 ng of the respective firefly luciferase reporter vector pGL4.14 and 5 ng of control vector, encoding Renilla luciferase, pRL-CMV (Promega), in a final volume of 0.5 ml. Firefly and Renilla luciferase activities were then measured consecutively using the Dual-luciferase assays (Promega), 48 hr after transfection.

ChIP and Co-IP
20 million HFF and 25 million A549 cells underwent 12.5 min fixation with 1.4% formaldehyde solution, quenching with 125mM glycine, lysis (10mM Tris pH 7.4, 0.5M
EDTA, 1% triton, 0.5% SDS, 0.1% NaDOC, 0.5M NaCl based solution) sonication with the M220 ultrasonicator (Covaris). Some lysate was set aside for input and chromatin was then precipitated from lysates with anti-human IRF3 antibody (Santa Cruz) conjugated protein G dynabeads (Invitrogen) for ON at 4°C. Beads were then extensively washed with lysis buffer at increasing concentrations of NaCl (up to 0.5M) and TE solution. DNA was then eluted and de-crosslinked with elution buffer (10mM Tris pH 8, NaCl 0.3M, 5mM EDTA, 0.5% SDS) at 65°C for 15h and was then purified with the Qiaquick kit (Qiagen). Co-IP was performed by digitonin based lysis and pull-down with anti-CEACAM1 5F4 antibody (provided by R.S.B) or IgG1 control conjugated to protein G sepharose beads (Santa Cruz) according to manufacturers instructions followed by probing with anti-SHP2 antibody (Santa Cruz).

**Protein Translation assay**

Cells grown in 24-well replicates were counted, washed in PBS and resuspended in methionine-free DMEM based (Sigma) medium. Cells were then replated back to 24-well plates at 100k cells/well HFF cells or 150k cells/well A549 cells and incubated with the methionine-free medium for one hour with HFF cells or half hour with A549 cells. Following methionine starvation, labeled $^{35}$S Methionine was supplemented to the medium to a final labeling concentration of 0.05µCi/µL for 5 hours of incubation for HFF cells and 0.025µCi/µL for 2 hours of incubation for A549 cells. Cells were then washed with PBS, lysed in 1ml of NaOH 1M, supplemented with 1:3 ration of scintillation liquid and analyzed by β-Counter. Experiments in which blocking of mTOR
activity was conducted, rapamycin (Sigma) was used at the indicated concentrations of either 1 or 5µM.

**BW reporter assay**

BW cells were seeded were seeded at 20k cells/well in a 96F plate at a ration of 1:3 with target 721.221 cells. Where applicable, Ig fusion proteins were administered at a final concentration of 5 µg/ml. After 3 days of incubation IL-2 levels in the supernatant were measured with an ELISA kit for IL-2 (Biolegend).

**References**

Achdout, H., Arnon, T.I., Markel, G., Gonen-Gross, T., Katz, G., Lieberman, N., Gazit, R., Joseph, A., Kedar, E., and Mandelboim, O. (2003). Enhanced recognition of human NK receptors after influenza virus infection. J Immunol 171, 915-923.

Katz, G., Gazit, R., Arnon, T.I., Gonen-Gross, T., Tarcic, G., Markel, G., Gruda, R., Achdout, H., Drize, O., Merims, S., et al. (2004). MHC class I-independent recognition of NK-activating receptor KIR2DS4. J Immunol 173, 1819-1825.

Markel, G., Wolf, D., Hanna, J., Gazit, R., Goldman-Wohl, D., Lavy, Y., Yagel, S., and Mandelboim, O. (2002). Pivotal role of CEACAM1 protein in the inhibition of activated decidual lymphocyte functions. The Journal of clinical investigation 110, 943-953.

Weisblum, Y., Panet, A., Zakay-Rones, Z., Haimov-Kochman, R., Goldman-Wohl, D., Ariel, I., Falk, H., Natanson-Yaron, S., Goldberg, M.D., Gilad, R., et al. (2011). Modeling of human cytomegalovirus maternal-fetal transmission in a novel decidual organ culture. J Virol 85, 13204-13213.
Wolf, D.G., Courcelle, C.T., Prichard, M.N., and Mocarski, E.S. (2001). Distinct and separate roles for herpesvirus-conserved UL97 kinase in cytomegalovirus DNA synthesis and encapsidation. Proc Natl Acad Sci U S A 98, 1895-1900.