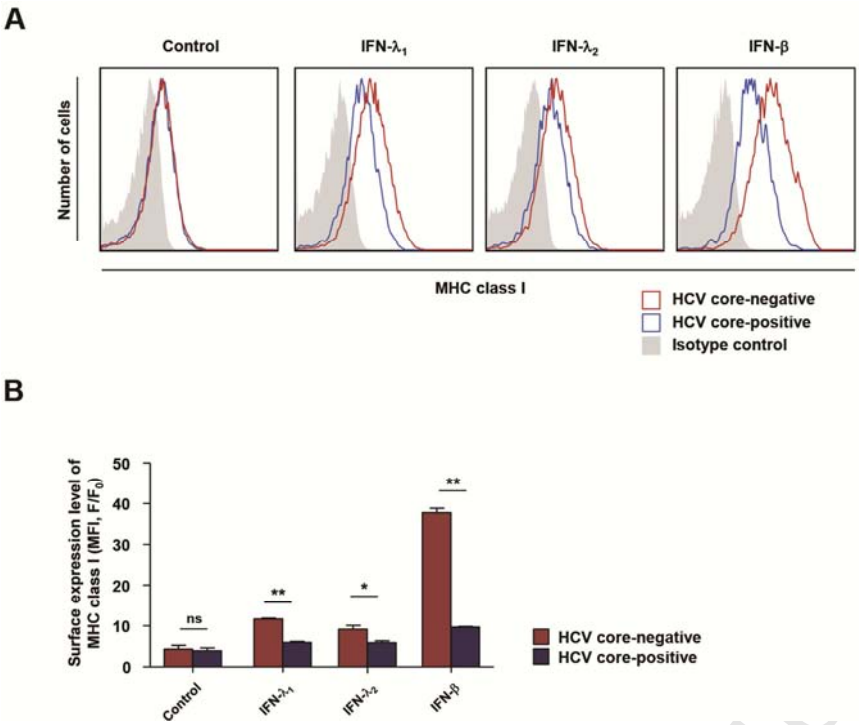

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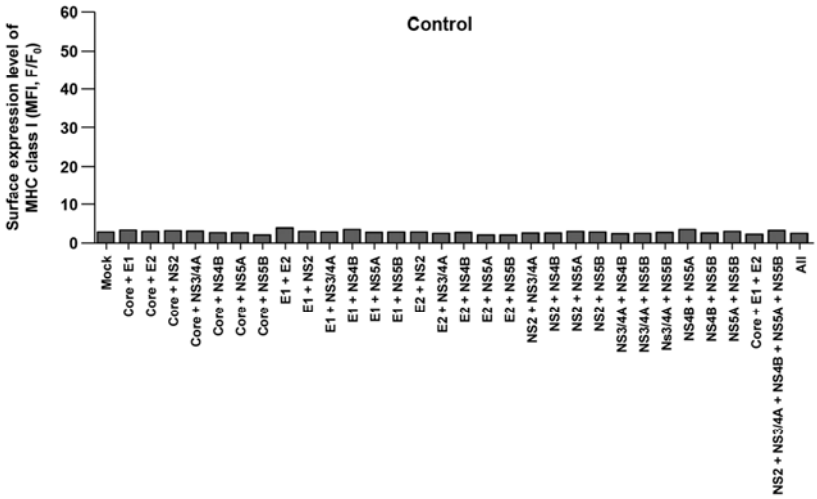

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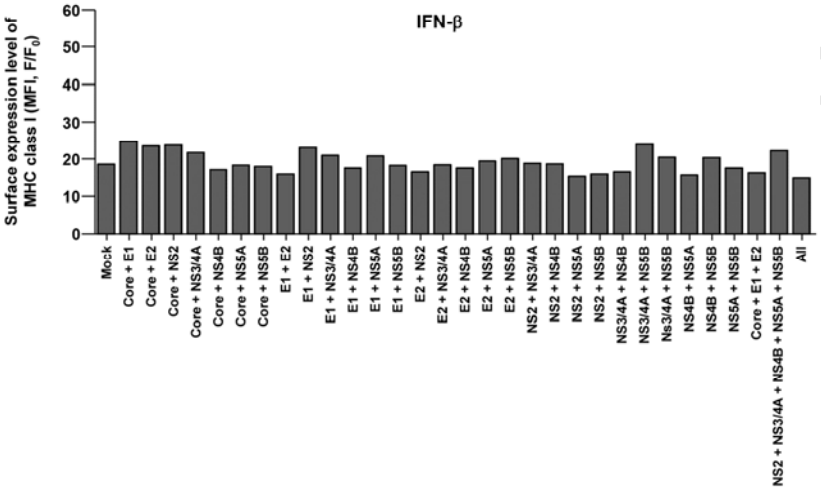

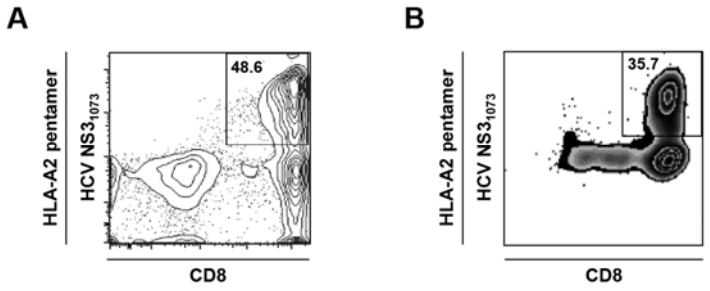

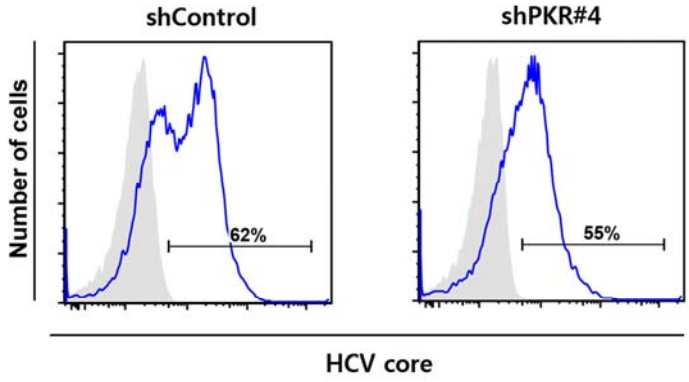

Supplementary Materials and Methods

Cell culture

Huh-7.5 cells were cultured at 37°C with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM; Welgene) supplemented with 10% fetal bovine serum (FBS; Welgene), 4.5 g/L glucose, L-glutamine, and 1% penicillin/streptomycin (Invitrogen). Stable HLA-A2-transfectants were maintained in complete medium supplemented with 1 mg/mL G418 (A.G. Scientific, San Diego, CA). PKR-silenced cell lines were maintained in complete medium supplemented with 1 μg/mL puromycin (Sigma-Aldrich). HCV-specific $CD8^+$ T cells were expanded in RPMI 1640 (Welgene) supplemented with 5% FBS, 1% penicillin/streptomycin in the presence of relevant cytokines as described below. For HCV RNA transfection studies, Huh-7.5 cells were transfected with *in vitro*-transcribed, replication-competent JFH-1 using DMRIE-C reagent (Invitrogen,).

Confocal microscopy

The antibodies used for confocal imaging included mouse monoclonal anti-HCV core IgG_1 (Clone C7-50), rabbit monoclonal anti-MHC class I (Clone EP1395Y), AlexaFluor 594-conjugated donkey anti-mouse IgG (Invitrogen), and AlexaFluor 488-conjugated donkey anti-rabbit IgG (Invitrogen). HCV-infected or uninfected Huh-7.5 cells were plated on 4-well Lab-Tek Chamber slides (Nunc, Naperville, IL) and treated with IFN-β for 24 h. Cells were fixed with 4% paraformaldehyde in PBS, permeabilized with 0.2% (v/v) Triton-X100, and incubated with primary antibodies (1:300 dilution). After washing with PBS, the slides were incubated with secondary antibody conjugates (1:500 dilution). After nuclear staining with DAPI and mounting, images were acquired on a LSM-710 confocal laser scanning microscope (Carl Zeiss, Jena, Germany).

PKR shRNA clones

Five validated clones of MISSION® shRNA bacterial glycerol stocks with lentiviral constructs expressing PKR shRNA were purchased from Sigma-Aldrich: NM_002759.x-2288s1c1 (TRCN0000001379), NM_002759.x-1409s1c1 (TRCN0000001381), NM_002759.x-2010s1c1 (TRCN0000001382), NM_002759.1-1870s1c1 (TRCN0000196400), and NM_002759.1-1580s1c1 (TRCN0000197012).

TaqMan real-time PCR

Total RNA isolation, cDNA synthesis, and TaqMan real-time PCR were performed as previously described [22]. In brief, total RNA was isolated with the RNeasy Mini kit (Qiagen, Valencia, CA), and cDNA was synthesized using the First-Strand cDNA Synthesis kit (Origene/Marligen Biosciences, Ijamsville, MD). TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA) were used to determine mRNA levels of HLA-A. Target mRNA levels were normalized to an endogenous reference gene (β-actin).

Statistical analysis

Data are presented as mean + standard error of mean. Two-tailed Mann-Whitney U test or unpaired t-test was performed where appropriate. Statistical analyses were performed with GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA). A *p* value of less than 0.05 was considered statistically significant.

Supplementary Figure legends

Supplementary Figure 1. IFN-λ-induced MHC class I expression is attenuated by HCV infection. Huh-7.5 cells were inoculated with HCVcc at 0.01 MOI and cultured for 3 days, with an infection rate of 30-45%. Cells were then treated with 100 ng/mL IFN-λ_1, 100 ng/mL IFN-λ_2 or 3 ng/mL IFN-β or were left untreated (control). (A) The expression level of MHC class I in HCV-infected (blue) or uninfected (red) cells was analyzed by co-staining for HCV core and MHC class I and flow cytometry. (B) Data from three independent experiments are presented as a bar graph (mean + SEM). *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

Supplementary Figure 2. Effect of HCV gene transfection on IFN-induced MHC class I expression. Huh-7.5 cells were transfected with pairs of HCV gene plasmids in 28 combinations. In addition, transfection was performed with all structural protein genes (core, E1 and E2), all non-structural protein genes (NS2, NS3/4A, NS4B, NS5A and NS5B) or all HCV genes (core, E1, E2, NS2, NS3/4A, NS4B, NS5A and NS5B). The transfected cells were treated with 3 ng/mL IFN-β for 24 h. The expression level of MHC class I was analyzed by flow cytometry (A), and the MFI of MHC class I expression is presented (B). F, fluorescence intensity of stained sample; F_0, fluorescence intensity of isotype control.

Supplementary Figure 3. HCV-specific CD8+ T cell lines used for co-culture experiments. NS31073 (CINGVCWTV)-specific CD8+ T cells and core35 (YLLPRRGPRL)-specific CD8+ T cells were expanded from PBMC of HLA-A2+ patients with acute HCV infection (genotype 1a) as described in Materials and Methods. The frequency of NS31073-specific CD8+ T cells (A) and core35-specific CD8+ T cells (B) was determined with MHC class I pentamers.

Supplementary Figure 4. HCV infection rates in PKR-silenced and unsilenced cells. shControl-transduced Huh-7.5/$P_{(HLA-A2)}$-(HLA-A2) (left) or shPKR#4-transduced Huh-7.5/$P_{(HLA-A2)}$-(HLA-A2) (right) cells were infected with HCVcc at 0.1 MOI, cultured for 3 days, and treated with 3 ng/mL IFN-β for 24 h. Then, the infection rate was determined with anti-HCV core immunostaining and flow cytometry. Representative FACS histograms are presented.