IFITMs mediate viral evasion in acute and chronic hepatitis C virus infection

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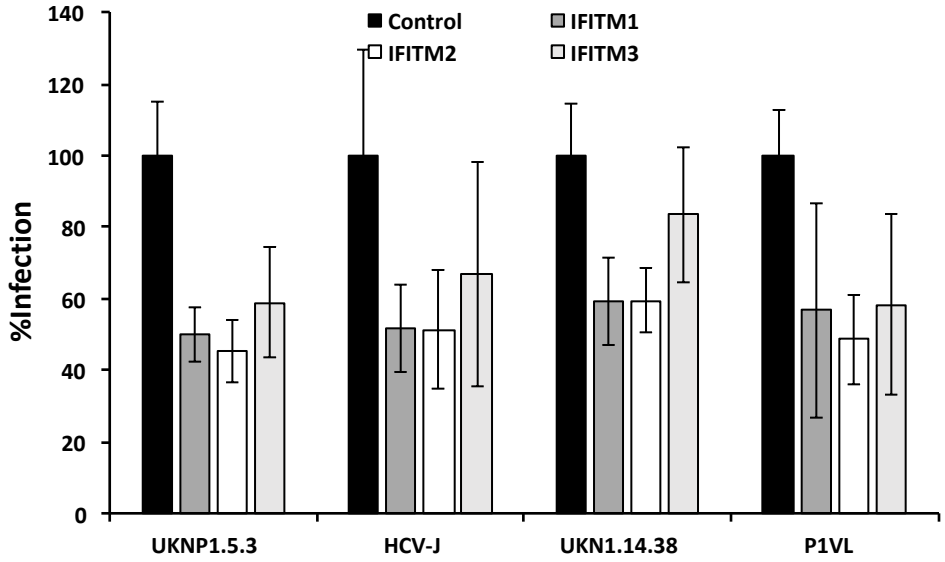
Supplementary Information

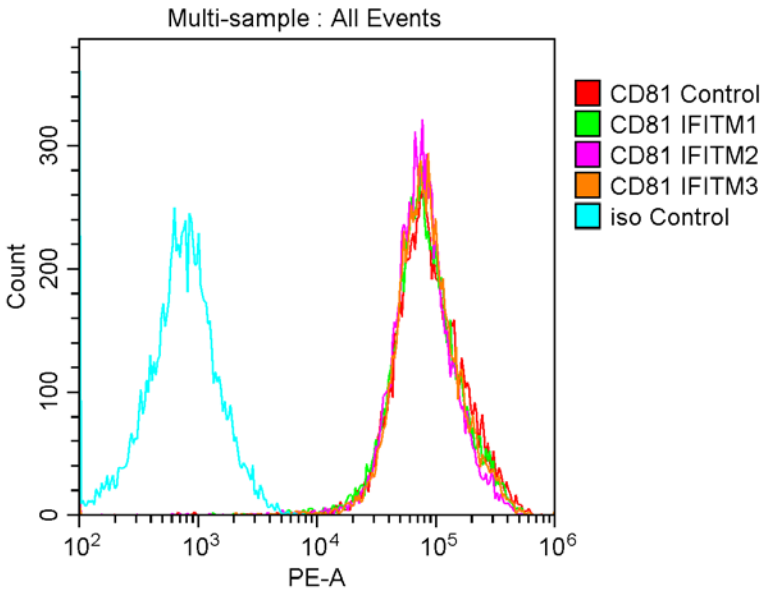
Supplementary Figures

Figure S1: Antiviral activity of IFITM proteins in polarized HepG2-CD81 cells.



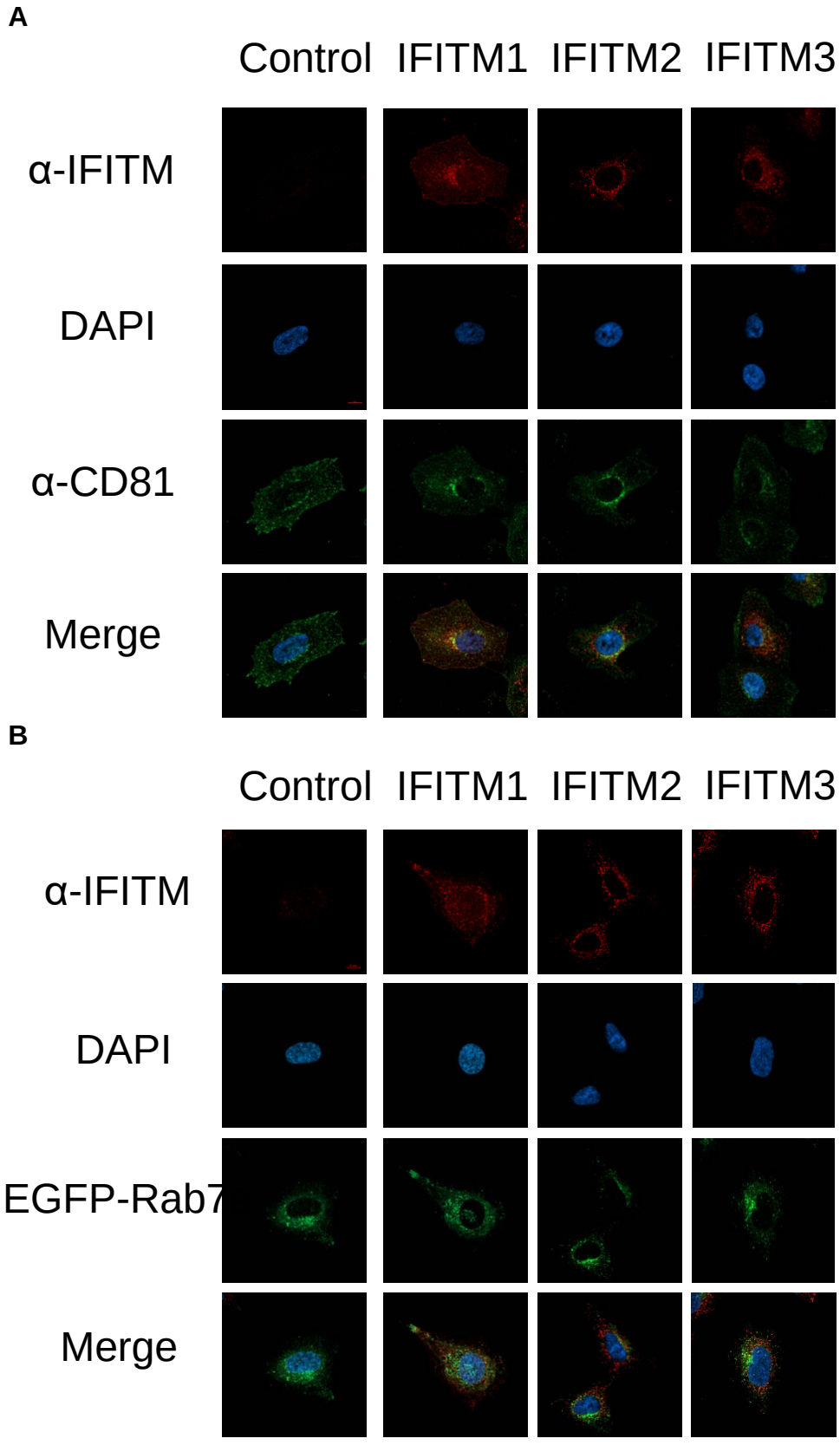
HepG2 cells stably expressing CD81 were transduced with to express IFITM1, 2 or 3, or empty vector as control for 2 days. Transduced cells were then infected for 3 days with HCVpp pseudotyped with HCV E1E2 patient variants isolated from chronic patients. Infection was assessed after 72 h by measuring luciferase activity. Results are displayed as % Infection compared to the empty vector control. Shown are the means of three experiments performed in triplicates.

Figure S2. Unchanged CD81 expression in IFITM-expressing cells.

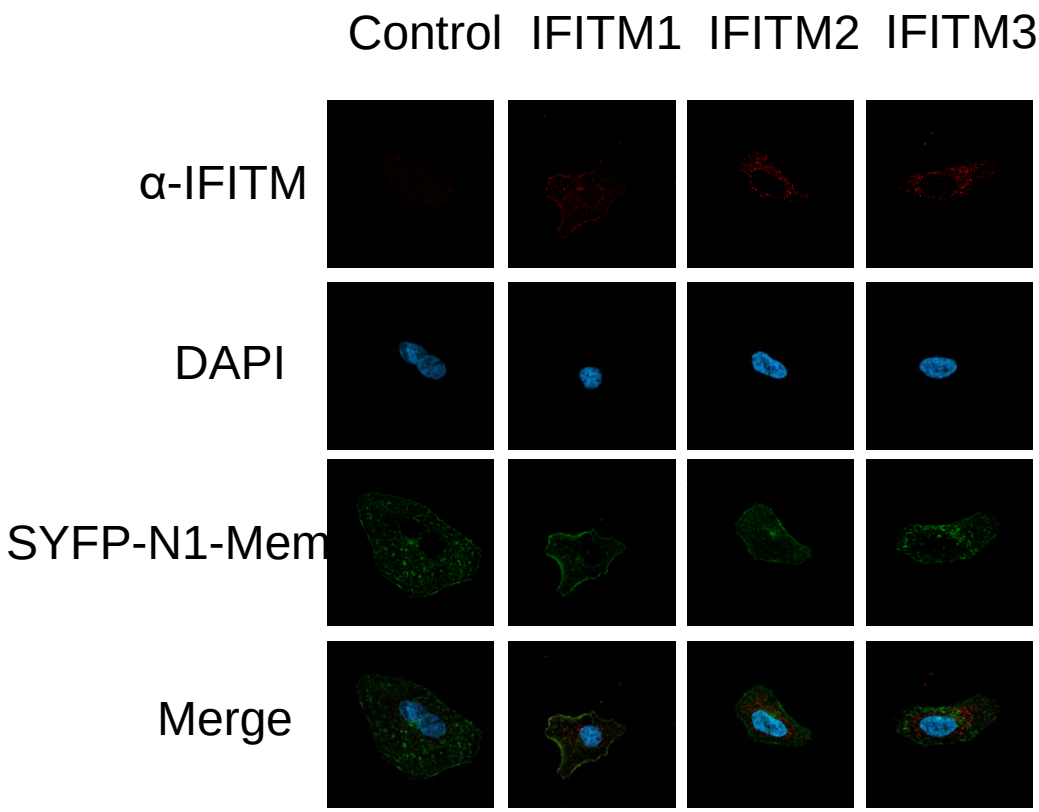


Huh7.5.1 cells were transduced to express IFITM proteins or with an empty plasmid as control. Cells were harvested 48 h after transduction, washed with PBS and CD81 expression was detected with a mouse anti-CD81 antibody (BD Pharmingen) at 5 µg/ml for 45 min. Staining was done with a secondary goat anti mouse-PE antibody.

Figure S3: Intracellular localization of IFITM proteins.



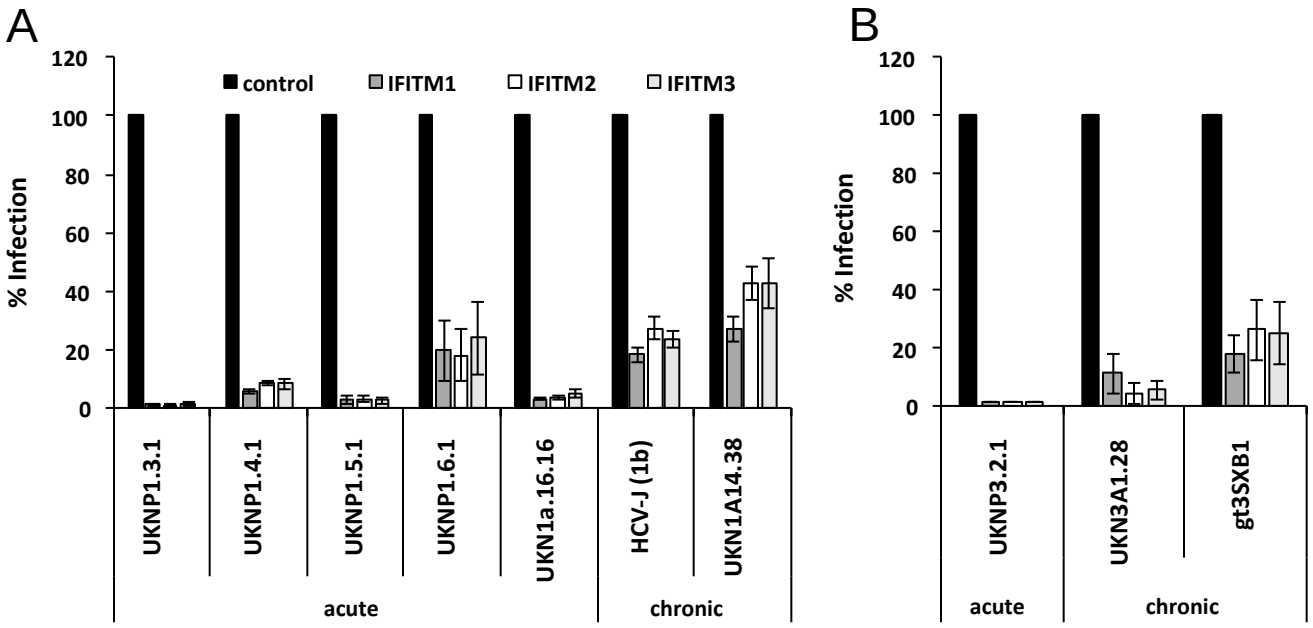
c



Huh7.5.1 Cells were seeded in 24 well plates at 50.000 cells per well and transduced to express IFITM proteins or a control vector on the next day. Cells in (B) and (C) were cotransduced with vectors coding for EGFP-Rab7a or SYFP-N1-Mem (SYFP with a membrane targeting signal) respectively. After 3 days, cells were detached and seeded into 24-well plates equipped with coverslips (12 mm diameter) at 10-fold dilution. After overnight growth incubation, cells were fixed in 4% paraformaldehyde for 10 min and subsequently permeabilized in PBS/0.2 % triton for 5 min followed by washing in PBS/0.1% tween. To block unspecific binding, cells were incubated for 1 h at room temperature in PBS containing 1% bovine serum albumine and 10% human or bovine serum. The cells were thereafter incubated for 1 h at room temperature with primary antibodies diluted in blocking solution followed by three wash steps in PBS/0.1% tween. As primary antibody we used mouse anti-IFITM1 (1:200 dilution), rabbit anti-IFITM2 (1:200 dilution) (both from Proteintech) or rat anti-CD81. Subsequently, cells were incubated 1 h at room temperature with secondary antibodies diluted in blocking solution

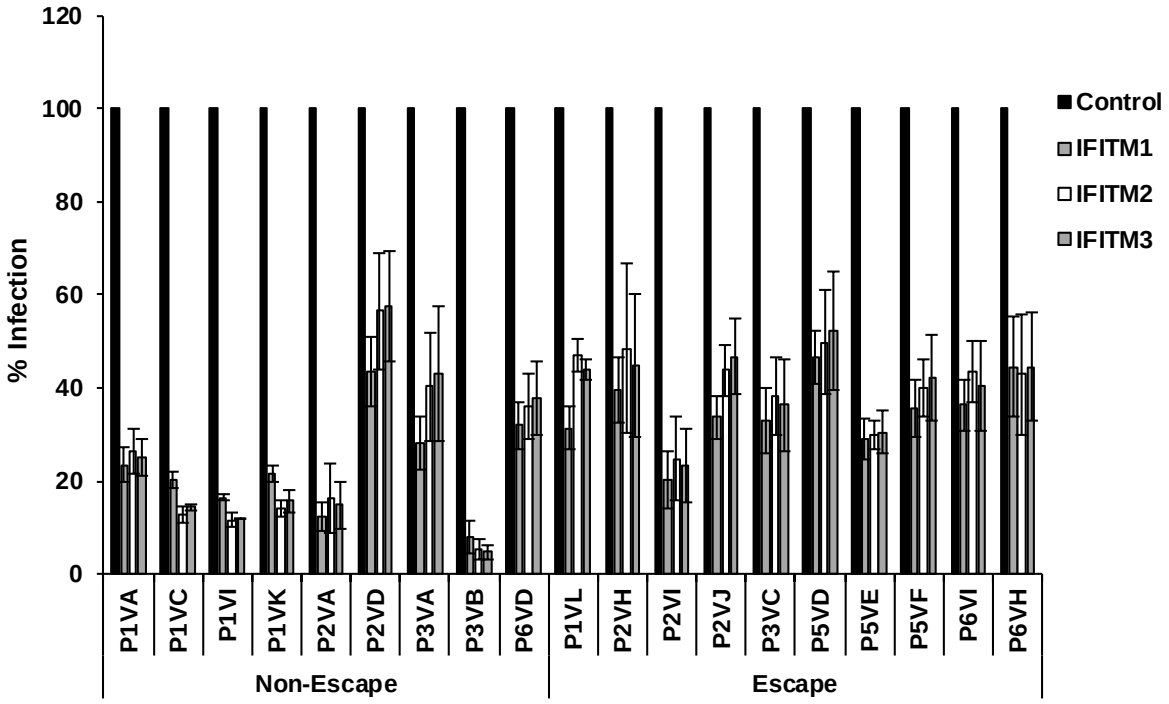
followed by three wash steps in PBS/0.1% tween. As secondary antibodies we used highly cross-adsorbed anti-mouse AlexaFluor488 (1:2000 dilution), anti-rat AlexaFluor488 (1:2000 dilution), anti-mouse AlexaFluor546 (1:500 dilution) or anti-rabbit AlexaFluor546 (1:500 dilution) (all from ThermoFisher). DAPI or Hoechst 33342 were added in the last wash step before coverslips were mounted in Mowiol/DABCO onto glass slides. Imaging was performed on a LSM800 Airyscan (Zeiss) equipped with solid state 405 nm, 488 nm and 561 nm laser lines, GaAsP detectors and a 63x/1.4 NA oil immersion objective. Filter settings were set with the ZEN 2.3 software (Zeiss) to minimize cross talk. Images were taken at the same laser power and gain for a whole image series and were only adjusted to avoid overexposure. Images were recorded at 2048x2048 px with 4-fold averaging. Processing of images for publication was done with ZEN lite.

Figure S4: Differential sensitivity of acute and chronic HCV variants of Gt1a and Gt3 to inhibition by IFITM proteins.



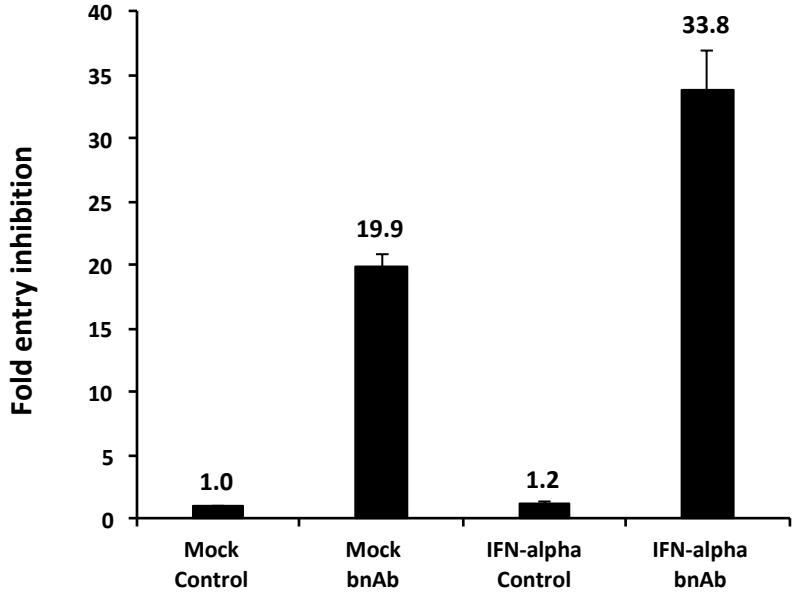
Huh7.5.1 cells were transduced to express the IFITM proteins or an empty control vector and subsequently infected with HCVpp expressing the envelope of variants isolated during acute or chronic HCV infection of genotype 1 (A) or genotype 3 (B). Entry of HCVpp was assessed 72 h post infection by measuring luciferase activity. Results are shown as % infection compared to empty vector control

Figure S5: IFITMs differentially restrict HCV patient variants isolated from patients undergoing liver transplantation.



Huh7.5.1 cells were transduced with an empty vector, pQCXIP-hIFITM1, pQCXIP-hIFITM2 or pQCXIP-hIFITM3 for 2 days. Transduced cells were then infected for 3 days with HCVpp pseudotyped with HCV E1E2 patient variants isolated from patients undergoing liver transplantation. Infection was assessed after 72 h by measuring luciferase activity. Results are displayed as % Infection compared to the empty vector control.

Figure S6: Interferon-alpha enhances neutralization by broadly neutralizing antibodies in Huh7 cells.



Huh7 cells were preatreated with 1000 U/mL of Interferon-alpha for 2 h. Huh7 cells were infected HCVpp bearing an envelope variant associated with viral escape. Before infection, the particles were coincubated with 5 µg/ml of the bnAb HC84.26.WH.5DL or with control antibody R04 at 37 °C for 1 h. Entry of HCVpp was assessed 72 h post infection by measuring luciferase activity. Results are shown as fold inhibition of virus entry. Inhibition of entry by control vector in combination with the control antibody R04 was set to 1. The graph represents the means of two experiments that were performed in triplicates.