SUPPORTING INFORMATION
FOR
Hepatitis C Virus Infects Rhesus Macaque Hepatocytes and Simianized Mice

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Supplementary materials and methods

Cells

Huh-7.5 (1) (generated in Charles Rice's laboratory) and Huh-7.5.1 (2) (kindly provided by Frank Chisari, The Scripps Research Institute) were maintained in Dulbecco's modified Eagle medium (DMEM; Gibco, Life Technologies) supplemented with 10% fetal bovine serum (FBS: HyClone, Thermo Scientific, Waltham, MA) and 0.1mM nonessential amino acids (NEAA; Gibco, Life Technologies, Carlsbad, CA) and Huh-7.5-CD81 knockdown cells in the same medium supplemented with 6μg/ml blasticidin (3). Huh-7.5—SCARB1 knockdown cells (4) were maintained in DMEM supplemented with 10% FBS and 0.1mM NEAA. The medium was further supplemented with 100ng/ml doxycycline (Sigma-Aldrich Corp.) to induce expression of the SCARB1-targeting shRNA. 293T (kindly provided by Paul Bieniasz, Aaron Diamond AIDS Research Center) and HEK293 as well as CMMT (ATCC #CRL-6299) and FRhk4 (ATCC #CRL-1688, Lot #58078579) rhesus macaque cell lines were maintained in DMEM supplemented with 10% FBS and 0.1mM NEAA. LLC-MK2 (ATCC #CCL-7) cells were maintained in Media 199 with 20% FBS. 786-O (ATCC #CRL-1932, Lot #3231792) cells were maintained in RPMI-1640 with 10% FBS. All cells were maintained at 37^{o}C with humidified 5% CO_2.

Generation of HCV NS3-4A-expressing cell lines utilized for MAVS cleavage analysis were done as follows: Huh-7.5 and CMMT cells were transduced with the TRIP-HCV(JFH1)-NS3/4A-TagRFP/Puro vector(5) and sorted for RFP

expression to ensure homogenous NS3-4A expression within each sample and across cell backgrounds; cells transfected with sub-genomic replicon RNA were selected via G418 antibiotic treatment and miR122 was expressed in the CMMT line (which is not of liver origin) via lentivirus-mediated transduction and blasticidin selection to boost replication as has been shown to be effective in other cell backgrounds(5).

Rhesus macaque and human adult primary hepatocytes used for engraftment into immunodeficient xenorecipients were purchased from Bioreclamation IVT (Baltimore, MD). De-identified human fetal livers cells (16-24 weeks gestation) were procured through Advanced Bioscience Resources (ABR; Alameda, CA) and prepared as described previously (6). Rhesus macaque hepatocytes used for in vitro experiments were isolated from a female rhesus macaque sacrificed for other IACUC-approved research in the Gilbert Laboratory (The Rockefeller University). Liver tissue - otherwise classified as refuse -- was obtained post-mortem and hepatocytes were isolated following standard perfusion and collagenase digestions methods and subsequently cryopreserved. For plating, rhesus macaque hepatocytes were thawed immediately after removal from storage in liquid nitrogen, enriched for viable cells using a BD Gentest High Viability CryoHepatocyte Recovery Kit (BD Biosciences) and plated on collagen coated plates. Hepatocytes were subsequently maintained in Williams' medium E (Gibco, Life Technologies) supplemented with 2mM L-glutamine, 1X ITS plus, 1X penicillin-streptomycin, 50

Pseudoparticles

Constructs:

TRIP-HCV(JFH1)-NS3/4A-TagRFP/puro (5), TRIP-miRNA122 (5), TRIP-Venus/YFP-human OCLN (6), TRIP–Cerulean/CFP-human CLDN1 (6) and TRIP-TagRFPnlsMAVS (7) were described previously. TRIP-miRNA122 was modified in this study to express the blasticidin resistance gene driven by an RSV promoter sequence inserted between miRNA122 and the EMCV IRES. cDNAs of CD81, SCARB1, CLDN1 and OCLN from human, mouse or rhesus macaque and rhesus macaque SCARB1 were PCR amplified using gene specific primers including attB sites allowing insertion of the resulting product into pDONOR221 (Invitrogen, containing attP sites) using BP clonase II and Gateway cloning technology. The respective cDNAs were then shuttled into the destination vector pTRIP.CMV.IVSb.ires.TagRFP-DEST (8) using LR clonase II (Gateway cloning technology, Life Technologies). N-terminal human or mouse SCARB1-mKate fusions (described in (9)) were cloned into pTRIP.

Production of HAV stocks and HAV infection of CMMT cells

In vitro transcribed RNA of HM175/18F (10), a cell culture-adapted variant of the HM175 strain of HAV was electroporated into Huh-7.5.1 cells. Virus was harvested by freeze-thaw of the cell pellet and infectivity of the resulting stock determined by limiting dilution assay (1.26x 10^6 TCID50/ml). CMMT cells were inoculated in 6 well plates with 0.5ml HAV diluted 1:2.5, 1:5 or 1:10 in medium. Plates were rocked periodically and infection proceeded for 6 hours before media

was exchanged. Cells were harvested at 48, 96 and 144hpi and infection frequency determine by flow cytometric analysis on a BD FACSCalibur following staining with mouse-anti HAV (K2-4F2; 1:500, kindly provided by Sue Emerson, NIH) and goat anti-mouse Alexa Fluor 647 (Molecular Probes, Life Technologies). Parallel wells were harvested in RIPA buffer supplemented with protease inhibitors for analysis by western blot as described below. CMMT cells infected with 1:2.5 dilution of HAV and harvested 144hpi were utilized in our final western blot analysis.

References

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Supplementary figure legends

Figure S1: HCV entry factor expression in rhesus macaque liver tissue.
(A) mRNA expression of HCV entry factors in human fetal or adult liver tissue or adult rhesus macaque liver tissue determined by RT-PCR. SD is shown across independent cDNA synthesis reactions (n=2 for human liver samples, n=3 for rhesus macaque liver samples). **(B)** Western blot of Huh-7.5, human fetal liver culture (HFLC), primary adult human hepatocyte (PHH) or primary rhesus macaque hepatocyte (PRMH) lysates detecting the minimal set of HCV entry factors (SCARB1, OCLN, CLDN and CD81) and beta actin.

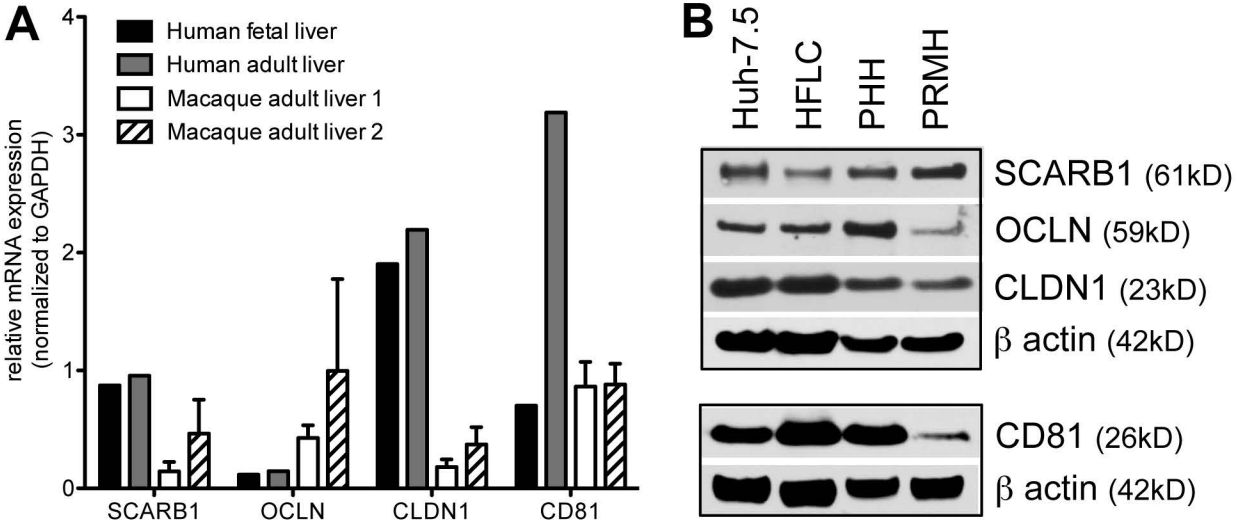
Figure S2: HCV entry factor amino acid sequence alignments across species.
Amino acid alignment of human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), rhesus macaque (*Macaca mulatta*) and mouse (*Mus musculus*) **(A)** CLDN1, **(B)** OCLN, **(C)** CD81 and **(D)** SCARB1. Residues relevant for HCV entry are highlighted in red.

Figure S3: Rhesus macaque SCARB1 and CD81 support HCV (gt2a) uptake.
(A) Jc1 infection of Huh-7.5 cells knocked down for endogenous SCARB1 or **(B)** CD81 and transduced with mouse, human or rhesus macaque SCARB1 or CD81, respectively. Entry efficiency is defined by the percentage of NS5A antigen positive cells in total live cells, normalized to cells containing control shRNA. **(C)** Western blot analysis of lysates from cells used in panel A detecting SCARB1 and beta actin. Note that human and mouse SCARB1 are expressed here as mKate-SRB1 fusion proteins. **(D)** CD81 transgene (tagRFP) expression in cells used in panel B. **(E)** Mean fluorescence intensity of CD81 transgene (tagRFP) expression and **(F)** percent of live cells that are tagRFP-positive.

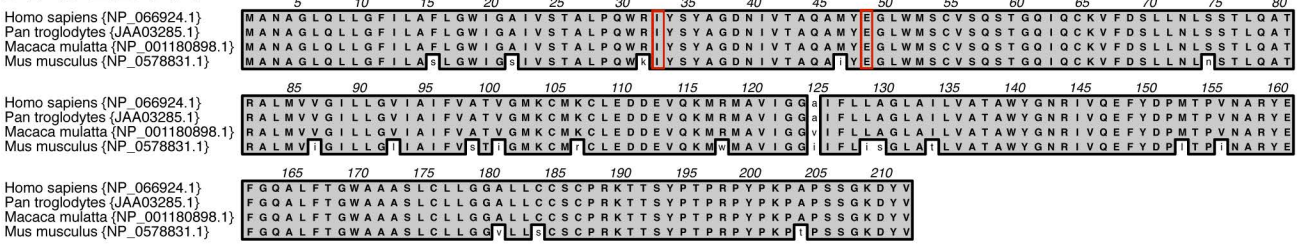
Figure S4: HAV infection of CMMT cells.
Flow cytometic analysis of HAV-infected CMMT cells over time.

Figure S5: HCV infection in Huh-7.5 cells and primary rhesus macaque heptocytes.
(A) Luciferase secreted into the culture medium as a measure of HCV replication in Huh-7.5 human hepatoma cells ($4x10^4$ cells per well in a 24-well plate) and PRMH cultures (also plated in 24-well plate format) inoculated with Jc1[p7nsGluc2a]. Following inoculation, cells were washed extensively and fresh medium containing DMSO (vehicle control; also provided to mock-inoculated cultures), 5mM 2'CMA or 2mM Jak inhibitor (INCB018424) was supplied. After collection of the supernatant two days post-inoculation, cultures were again washed and fresh medium containing DMSO or inhibitors replenished. **(B)** Infectious virus in supernatants from Huh-7.5 cells and PRMH cultures described in panel (A). Titers were determined by limited dilution assay on naïve Huh-7.5 cells. Data shown in all panels is the mean and standard deviation (SD) of biological triplicates from a single experiment. Lower limit of quantification is

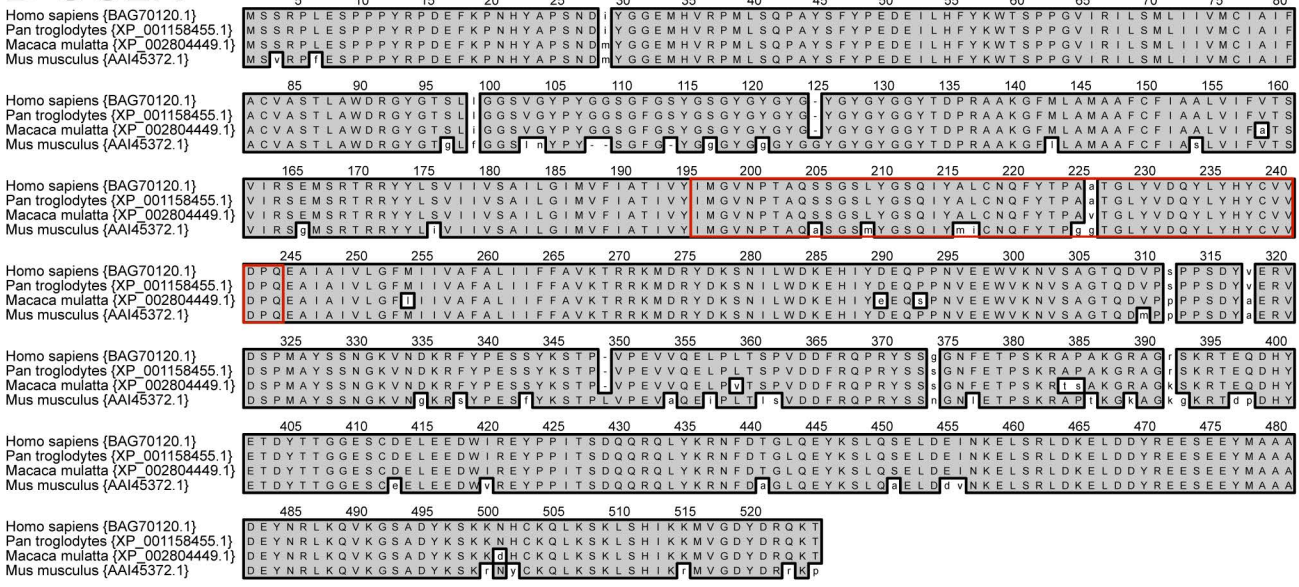
denoted as LOQ.


A
Human fetal liver
Human adult liver
Macaque adult liver 1
Macaque adult liver 2
relative mRNA expression
(normalized to GAPDH)
0
1
2
3
4
SCARB1
OCLN
CLDN1
CD81
B
Huh-7.5
HFLC
PHH
PRMH
SCARB1 (61kD)
OCLN (59kD)
CLDN1 (23kD)
β actin (42kD)
CD81 (26kD)
β actin (42kD)

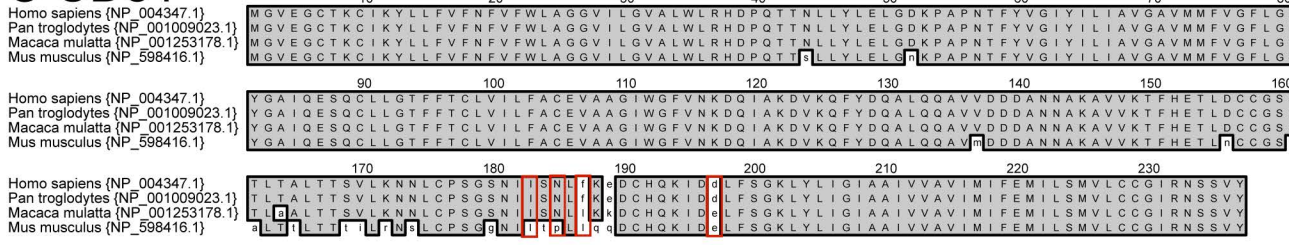
A CLDN1



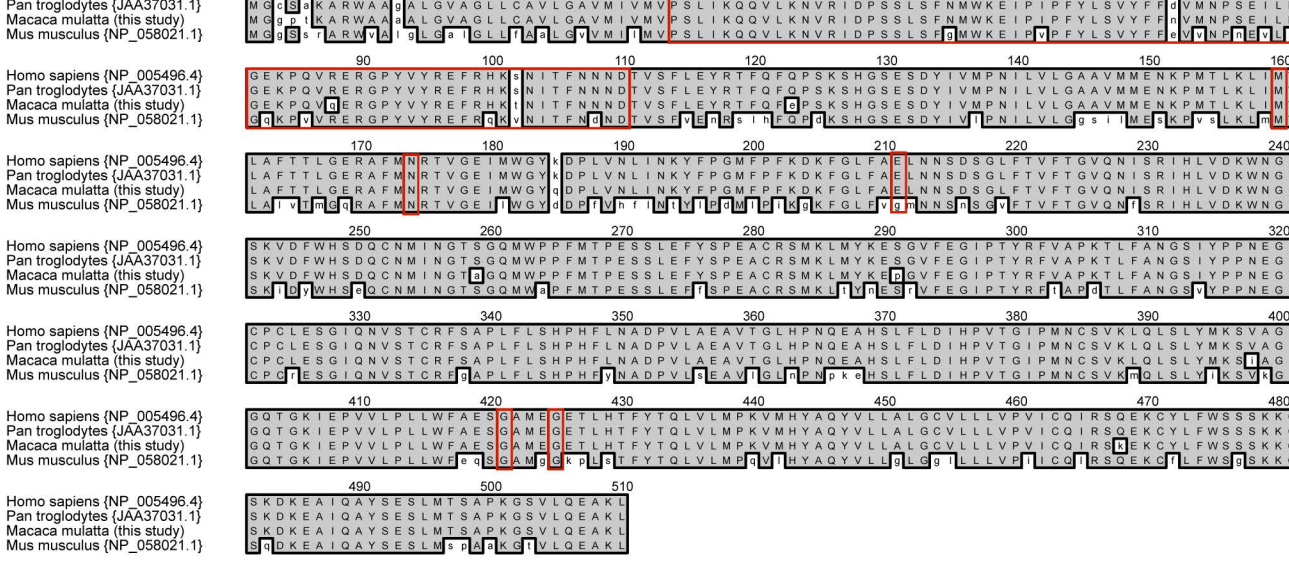
B OCLN

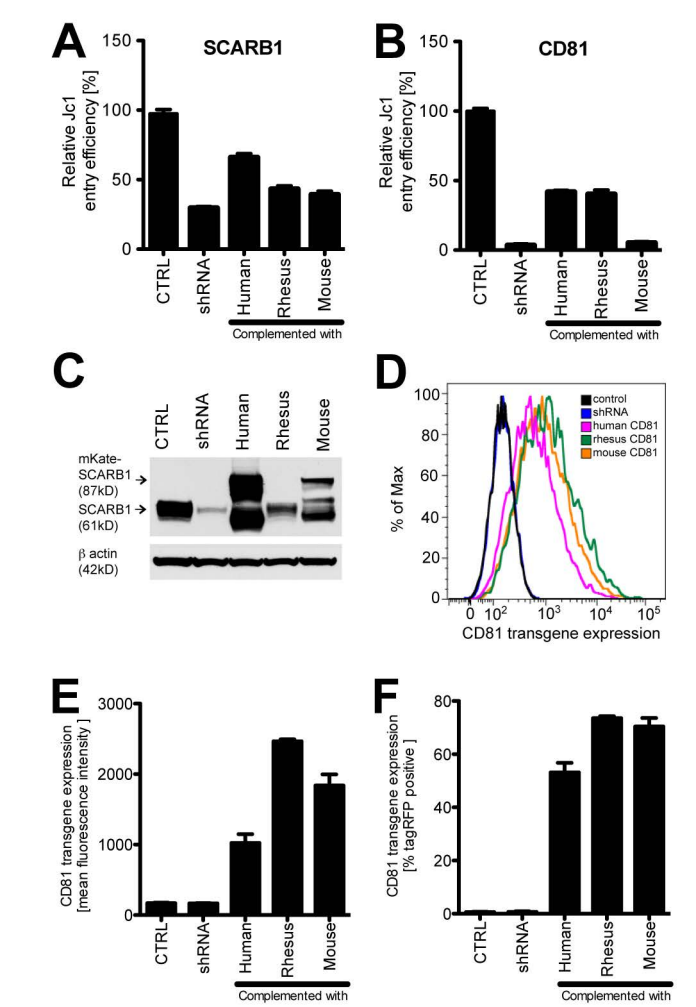


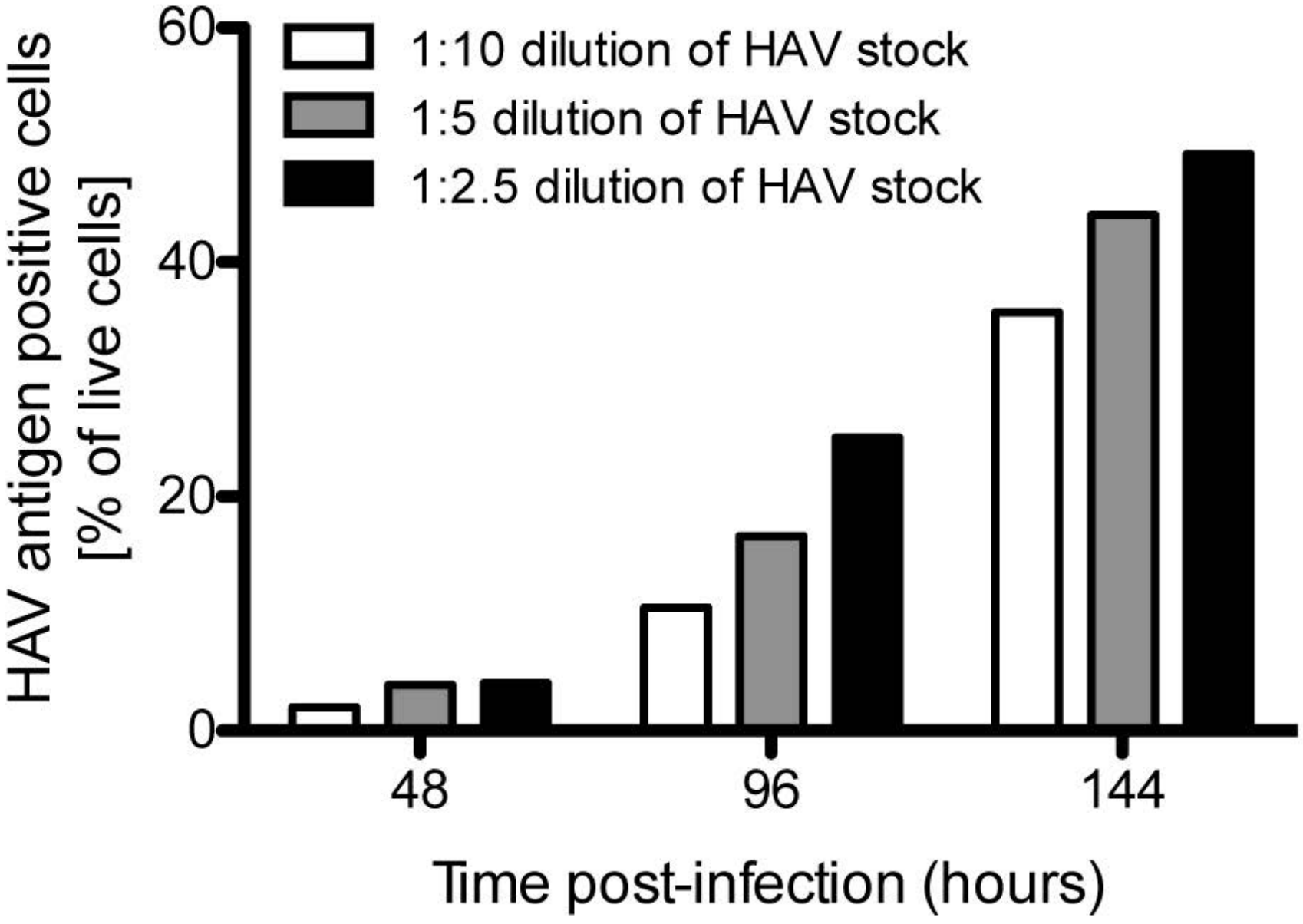
C CD81

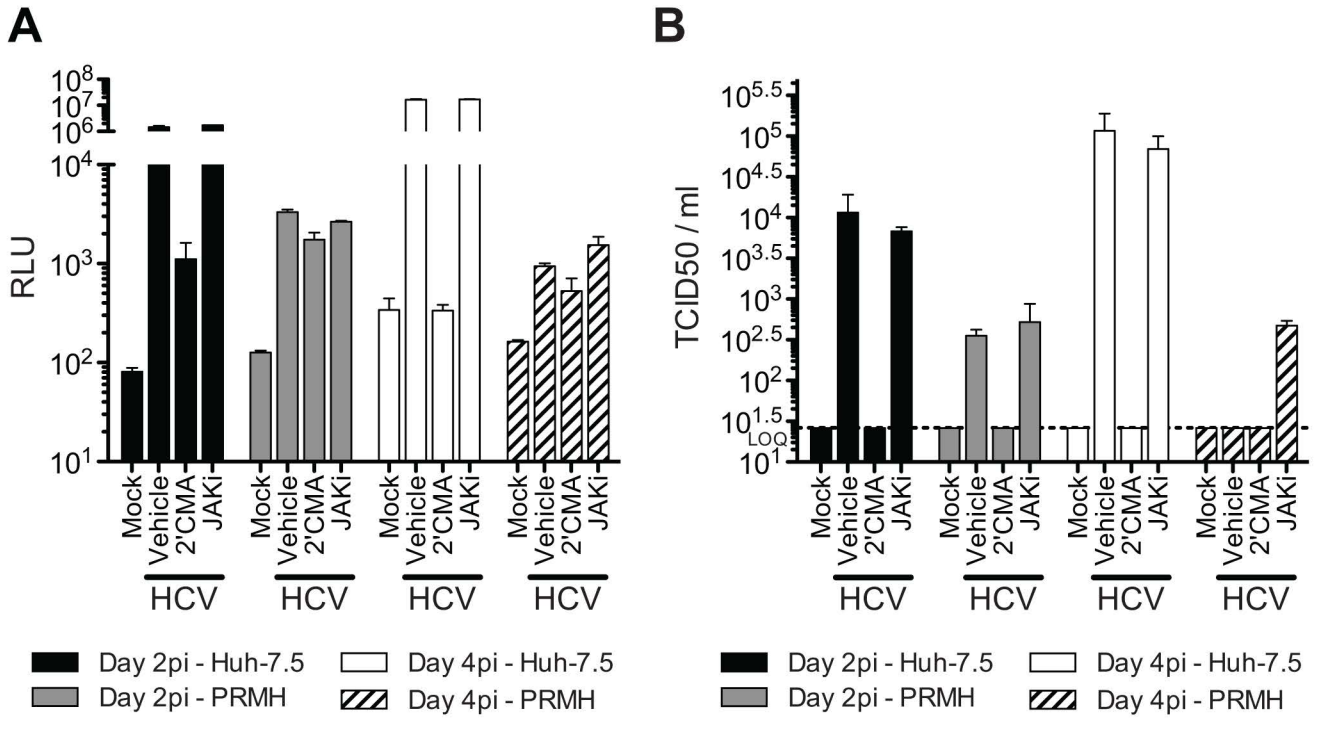


D SCARB1




A
SCARB1
Relative Jc1 entry efficiency [%]
150
100
50
0
CTRL
shRNA
Human
Rhesus
Mouse
Complemented with
B
CD81
Relative Jc1 entry efficiency [%]
150
100
50
0
CTRL
shRNA
Human
Rhesus
Mouse
Complemented with
C
CTRL
shRNA
Human
Rhesus
Mouse
mKate-SCARB1 (87kD)
SCARB1 (61kD)
β actin (42kD)
D
control
shRNA
human CD81
rhesus CD81
mouse CD81
% of Max
100
80
60
40
20
0
0
10^2
10^3
10^4
10^5
CD81 transgene expression
E
CD81 transgene expression [mean fluorescence intensity]
3000
2000
1000
0
CTRL
shRNA
Human
Rhesus
Mouse
Complemented with
F
CD81 transgene expression [% tagRFP positive]
80
60
40
20
0
CTRL
shRNA
Human
Rhesus
Mouse
Complemented with


1:10 dilution of HAV stock
1:5 dilution of HAV stock
1:2.5 dilution of HAV stock
HAV antigen positive cells
[% of live cells]
60
40
20
0
48
96
144
Time post-infection (hours)


A
RLU
10^8
10^7
10^6
10^4
10^3
10^2
10^1
Mock
Vehicle
2'CMA
JAKi
HCV
B
TCID50 / ml
$10^{5.5}$
10^5
$10^{4.5}$
10^4
$10^{3.5}$
10^3
$10^{2.5}$
10^2
$10^{1.5}$
LOQ
10^1
Day 2pi - Huh-7.5
Day 2pi - PRMH
Day 4pi - Huh-7.5
Day 4pi - PRMH