### 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 supplemented with 6µg/ml blasticidin (3). Huh-7.5—SCARB1 medium 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 macague 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°C with humidified 5% CO<sub>2</sub>.

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

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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 postmortem 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 □ = B 糖 CIIB メC 個C S 種 糎

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### **Pseudoparticles**

### Constructs:

TRIP-HCV(JFH1)-NS3/4A-TagRFP/puro (5). TRIP-miRNA122 TRIP-(5). Venus/YFP-human OCLN (6), TRIP-Cerulean/CFP-human CLDN1 (6) and TRIP-TagRFPnIsMAVS (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<sup>6</sup> 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<sup>4</sup> 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.



# 5 PRMH Ŧ

SCARB1 (61kD) OCLN (59kD) CLDN1 (23kD) β actin (42kD)



CD81 (26kD) β actin (42kD)

# A CLDN1

Homo sapiens {NP\_066924.1} Pan troglodytes {JAA03285.1} Macaca mulatta {NP\_001180898.1} Mus musculus {NP\_0578831.1}

Homo sapiens {NP\_066924.1} Pan troglodytes {JAA03285.1} Macaca mulatta {NP\_001180898.1} Mus musculus {NP\_0578831.1}

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# **B** OCLN

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### **C** CD81

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### **D** SCARB1 MG MG MG

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1} .1}	405 E T D Y T T E T D Y T T E T D Y T T E T D Y T T	410 GGESCD GGESCD GGESCD GGESC	415 ELEED ELEED ELEED ELEED	420 WIREY WIREY WIREY WVREY	425 PPITSC PPITSC PPITSC PPITSC	430 0 Q Q R Q 0 Q Q R Q 0 Q Q R Q	435 L Y K R N   L Y K R N   L Y K R N   L Y K R N	440 FDTGL FDTGL FDTGL FD <mark>aGL</mark>	445 Q E Y K S Q E Y K S Q E Y K S Q E Y K S	450 SLQSEL SLQSEL SLQSEL SLQSEL	455 DEIN DEIN DEIN DEIN	460 KELSR KELSR KELSR KELSR	465 L D K E L L D K E L L D K E L L D K E L		475 ESEEYM ESEEYM ESEEYM	480 vi a a a vi a a a vi a a a m a a a
1} .1}	485 DEYNRL DEYNRL DEYNRL DEYNRL	490 K Q V K G S K Q V K G S K Q V K G S K Q V K G S	495 A D Y K S A D Y K S A D Y K S A D Y K S	500 ккинс ккинс кканс кгиус	505 KQLKSK KQLKSK KQLKSK	510 (LSHI (LSHI (LSHI (LSHI	515 ккм v G I ккм v G I ккм v G I к г м v G I	520 DYDRQ DYDRQ DYDRQ DYDRQ	КТ КТ КР							

1} 0023.1} 3178.1} 1}	M M M	G V G V G V G V	E ( E ( E (	T T T	к ( к ( к (		<u>к к к к</u>	Y I Y I Y I Y I	. L . L . L	F F F		= N = N = N	20 F \ F \ F \ F \	/ F / F / F / F	W W W	- A - A - A	GGGG	G \ G \ G \ G \	/ 1 / 1 / 1	30 L L L L	G V G V G V	/ A / A / A		N L N L N L	RRRR	4 0 P 0 P 0 P	00000	TT TT TT TT	N N S	L L L L L L	Y Y Y	L E L E L E	50 L L L	G C G C G C	K K K	P A P A P A	A P A P A P A P	N 1 N 1 N 1 N 1	F	60 Y V Y V Y V Y V	GGGG	Y   Y   Y   Y			7 / G / G / G		/ M / M / M / M	M F M F M F	G F G F G F G F	8 LG LG LG	00000
1} 0023.1} 3178.1} 1}	Y Y Y Y	G A G A G A G A		S S S S		90 2 L 2 L 2 L 2 L	L L L	G 1 G 1 G 1 G 1	F F F F F F	F F F	T ( T ( T ( T (		100 V V V V		F. F. F.		E E E	V A V A V A		11( G G G			F F F	V N V N V N	<b>кккк</b>	12 ו ב ו ב ו ב	A A A A	K D K D K D		KC	F	Y C Y C Y C Y C	130 0 Q 0 Q	) A L A L A L	0000					D A D A D A D A	N N N	NANANA	KAKA	A V A V A V A V	15 ( T ( T ( T ( T	F I F I F I	H E H E H E H E	T L T L T L T L		16 G S G S G S G S	0 S S S n
1} 0023.1} 3178.1} 1}	T T T	L T L T L a L T	A I A I I I	T T T	1 S \ S \ S \ t	70 / L / L L	K K K		V L V L V L	0000	P S P S P S	G G G G G G G	180 S I S I 9 I	N I N I N I	     	6 N 6 N 6 N	LLL	f H f H I H I C	e e k	190 D D D		1010	кккк		d d e e	20 FS FS FS	00000	K L K L K L	Y Y Y Y	L   L   L   L	GGGG	A   A   A   A	210 A A A A	)   \   \   \   \		A \ A \ A \ A \	/   /   /	M I M I M I M I	FFF	220 E N E N E N		L S L S L S L S	M N M N M N	V L V L V L	230 3 G 3 G 3 G 5 G	)         	R N R N R N R N	S S S S S S S S	Y Y Y		

	20	30	40	50	00	70	00
M G c S a K A R W A A q A L	GVAGLLCAVLG	AVMIVMVPSL	IKQQVLKNVF	RIDPSSLSFNM	AWKEIPIPFYL	SVYFFdVMNF	SEILK
MGCSAKARWAADAL	GVAGLICAVIG	AVMIVMVPSI	IKOOVIKNVE		AWKEIDIDEVI	SVYEEdVMNE	SELLK
M O C O a K A K W A A G A C	OVAGEECAVEO.		TROQUERIE	(1010020114)			O L I L K
MGgptKARWAAaAL	G V A G L L C A V L G	AVMIVMVPSL	IKQQVLKNVH	RIDPSSLSF <u>N</u> M	MWKEIP <u>I</u> PFYL	SVYFFNVMNP	SEILK
MGqSsrARWyAlqL	GalGLLIAALG	VMIIMVPSL	IKQQVLKNVF	RIDPSSLSFOM	AWKEIPVPFYL	SVYFFEVVNF	PnEvLn
				5			
00	100	110	120	120	140	150	160
90	100	110	120	130	140	150	100
GEKPQVRERGPYVY	REFRHKSNITF	NNNDTVSFLE	YRTFQFQPSK	(SHGSESDYI)	/ M P N I L V L G A A	VMMENKPMTL	. K L I M T
GEKPOVRERGPYVY	REERHKSNITE	NNNDTVSELE	YRTEOFOPSK	SHGSESDYIN	MPNILVIGAA	VMMENKPMTI	KIIMT
GERPUVQERGPTVT	REFRANTINI	NNNDIVSFLE	TRIFUEPSP	SHGSESUTIV	MPNILVLGAA	VMMENKPMIL	
GqKPVVRERGPYVY	REFRQKVNITF	NdNDTVSFVE	n R s I h F Q P d K	(SHGSESDYI)	/ I P N I L V L G g s	i I M E s K P v s L	K L m M T
170	180	190	200	210	220	230	240
170	100	150	200	210	220	200	240
LAFTTLGERAFMNR	TVGEIMWGYKD	PLVNLINKYF	PGMFPFKDKF	GLFAELNNSI	DSGLFTVFTGV	QNISRIHLVC	OKWNGL
LAFTTLGERAFMNR	TVGEIMWGYKD	PLVNLINKYF	PGMFPFKDKF	GLFAELNNSE	DSGLFTVFTGV	QNISRIHLVC	KWNGL
LAETTICEDAEMND	TYCELMMCYCD	DIVNIINKYE	DOMEDEKDKE		ROLETVETOW	ONLEDIHIVE	KIMNCL
				OLI ALL NINGL			NUNCL
LAIVTMGQRAFMNR	TVGEIIWGYdD	PfVhfINtYI	PdMIPiKgKF	- G L F v g m N N S r	n S G v F T V F T G V	QNISRIHLVD	OKWNGL
250	260	270	280	290	300	310	320
SKVDEWHSDOCNML	NCTECOMMERE	ATRECCIEEV	SPEACREMKI	MYKEROVEE	LETVEEVABL	TIEANCELVE	DNECE
			OT LACKOMKL		S I I I I K I V A I K	TETANOOTTI	TNEOT
SKVDFWHSDQCNMI	NGISGQMWPPFI	WIPESSLEFY	SPEACRSMKL	. MYKE <u>S</u> GVFEC	3 I P I Y R F V A P K	ILFANGSIYP	PNEGF
SKVDFWHSDQCNMI	NGTAGQMWPPF	WTPESSLEFY	SPEACRSMKL	MYKEPGVFEC	G I P T Y R F V A P K	TLFANGSIYP	PNEGF
S KUDWH SOO CNM L	NOTSCOMMADE	AT DECCIEE	S D E A C D S M K I	VYDE SUVEE		TIEANCENYE	DNECE
3 K I D Y W H 3 C Q C N M I	NGI 3 GQ MW AFFI	WIFE33LEFI	SFEACKSMAL	I THEST VEED	STETIKELAFU	I L FANGSVI F	FNEOF
000	2.10	250	200	070	000	200	100
330	340	350	360	370	380	390	400
CPCLESGIQNVSTC	RFSAPLFLSHP	HFLNADPVLA	EAVTGLHPNC	EAHSLFLDI	+ P V T G I P M N C S	VKLQLSLYMK	(SVAGI
CRCLESGLONVSTC	RESARIEISHR	HELNADRVIA	EAVIGIHENC	EAHSLELDIN	+ P V T G I P M N C S	VKLOISLYMK	SVAGI
0102200101010	RTOATETEONT	IT ENADIVEA	EAVIOL III NG	EANOLIEDII		VICEGEOLIMI	
CPCLESGIQNVSIC	RFSAPLFLSHP	HFLNADPVLA	EAVIGLHPNC	<u>IEA</u> HSLFLDII	HPVIGIPMNCS	VKLQLSLYMK	(STAGI
CPCIESGIQNVSTC	RFQAPLFLSHP	HFYNADPVLS	EAVIGLNPN	keHSLFLDII	H P V T G I P M N C S	VKmQLSLYiK	(SV k G I
410	420	430	440	450	460	470	480
		400		400	400	470	400
GQIGKIEPVVLPLLI	NFAESGAMEGE	ILHIFYIQLV	LMPKVMHYAG	A V L L A L G C V I	LLLVPVICQIR	SQEKCYLFWS	SSKKG
GQTGKIEPVVLPLLV	NFAESGAMEGE	TLHTFYTQLV	LMPKVMHYAC	YVLLALGCVI	LLLVPVICQIR	SQEKCYLFWS	SSKKG
COTCKIERVVIRII	NEAESCAMECE	TINTENTOIV	IMPKVMHVAC	XVIIALGOVI	LIVEVICOIE	SPEKCYLEWS	CCVVC
GQIGKILFVVLFLL	AT ALSOAMLOL					SALKOTLIWS	
GQTGKIEPVVLPLLV	N Feq SGA MgGk	PLSTFYTQLV	LMPqVIHYAC	2 Y V L L g L G g I I	LLVPIICQIR	SQEKCILFWS	SGSKKG
	and the second se						
490	500	510					
SKDKEALOAVSESI	UTSABKCSVIO	EAKI					
SKOKEATQATSESET	VII SAFKOSVLQ	LAKL					
SKUKEAIQAYSESLI	WISAPKGSVLQ	EAKL					
SKDKEAIQAYSESLI	MTSAPKGSVLQ	EAKL					
SODKEALOAYSESLI	MS DAAK GUVIO	EAKI					
d d b k c k i Q K i O C O C i							

50

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