

Supplementary material

The post-binding activity of scavenger receptor BI mediates initiation of hepatitis C virus infection and viral dissemination

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Supplementary Material and Methods

Cell culture-derived HCV (HCVcc) and HCV pseudoparticles (HCVpp). Luciferase reporter chimeric HCVcc of genotypes 1-4 (H77/1a/R2a, Con1/1b/R2a, J8/2b/R2a, S52/3a/R2a and ED43/4a/R2a) and HCVpp of genotypes 1-6 (H77, HCV-J, JFH1, UKN3A1.28, UKN4.21.16, UKN5.14.4 and UKN6.5.340) have been described.¹⁻³ Patient-derived HCVpp (P02VJ) from a patient (P02) undergoing liver transplantation have been as described.^{2,4} HCVpp bearing the envelope glycoproteins of strain H77, H77 deleted of hypervariable region 1 (HVR1) within E2 (Δ G384-N411) or H77 containing a point mutation within HVR1 (L399R) have been described.⁵ Luc-Jc1 HCVcc lacking HVR1 (Δ HVR1) have been described.⁶

Investigation of the antiviral effects of antibodies and antibody combinations on HCV entry and infection. HCVcc and pseudoparticle infection and kinetic assays have been described.^{2,7-10} Briefly, for infection experiments, Huh7.5.1 cells were pre-incubated in the presence or absence of antibodies for 1h at 37°C and infected at 37°C for 4h with HCVcc or pseudoparticles. 72h later infection was analyzed in cell lysates by quantification of luciferase activity using a Promega kit. For kinetic entry experiments, Huh7.5.1 were inoculated with

1 HCVcc (10^4 - 10^5 TCID₅₀/mL) for 1h at 4°C in the presence or absence of compounds.
2 Subsequently, cells were washed three times with ice cold PBS, supplied with fresh culture
3 fluid pre-warmed to 37°C and supplemented with the respective compounds and shifted to
4 37°C. The compounds were removed after 4h and cells were supplied with fresh culture fluid
5 without compounds and cultured an additional 48h at 37°C before quantification of luciferase
6 activity in cell lysates. For combination experiments, each antibody was tested individually or
7 in combination with the second antibody. Huh7.5.1 cells were pre-incubated with anti-SR-BI
8 or control antibody for 1 h and then incubated for 4h at 37°C with HCVcc (Luc-Jc1) or HCVpp
9 (P02VJ) (pre-incubated for 1h with or without anti-envelope antibodies).

10

11 **Assessment of synergy.** Synergy was assessed by two independent methods comprising
12 the Combination Index and the method of Prichard and Shipman. The Combination Index
13 (CI) was calculated as described.^{11,12} A CI less than, equal to, and more than 1 indicates
14 synergy, additivity, and antagonism, respectively¹¹. The method of Prichard and Shipman
15 was applied as described.¹³ In brief, the theoretical additive effect is calculated from the
16 dose-response curves of individual compounds by the equation $Z=X+Y(1-X)$ where X and Y
17 represent the inhibition produced by the individual compounds and Z represents the effect
18 produced by the combination of compounds. The theoretical additive surface is subtracted
19 from the actual experimental surface, resulting in a horizontal surface that equals the zero
20 plane when the combination is additive. A surface raising more than 20% above the zero
21 plane indicates a synergistic effect of the combination and a surface dropping lower than
22 20% below the zero plane indicates antagonism. The antiviral assay was performed as
23 described above except that the compound dilutions were added in a checkerboard format.
24 Combination studies for each pair of compounds were performed in triplicate.

25

26 **Chimeric human/mouse SR-BI and human SR-BI mutants.** Retroviral vectors expressing
27 human SR-BI (Z22555) or human SR-BI point mutants Q402R, E418R, Q402R-E418R and
28 G420H-G424H as well as mouse SR-BI (NM_016741) or human/mouse chimeric SR-BI were

1 described previously.^{14,15} Briefly, mouse SR-BI (NM_016741) or human/mouse chimeric SR-
2 BI cDNAs were inserted in CNC MLV (murine leukemia virus) vector backbones (kind gift of
3 M. Collins) harboring selectable marker genes for puromycin and G418, respectively. Using
4 SR-BI sequence comparisons as well as structural features predictions, three regions in the
5 SR-BI ectodomain were delineated between amino acid (aa) positions 38-215, 216-398 and
6 399-432. cDNAs encoding three human/mouse SR-BI chimeras were generated by PCR by
7 swapping these three SR-BI regions. While the HHH and MMM SR-BI constructs refer to the
8 wild-type human (H) and mouse (M) SR-BI molecules, respectively, the human/mouse SR-BI
9 chimeras were denominated according to the origin of either SR-BI region, e.g., HMM bears
10 region 1 from human SR-BI and regions 2 and 3 from murine SR-BI (Supplementary Figure
11 5).¹⁵ All mutants were sequenced to ensure that the clones possessed only the expected
12 mutation.¹⁵ Retroviral vectors containing these cDNAs were produced from 293T cells as
13 VSVG-pseudoparticles as described previously. Stable expression of either receptor in target
14 cells was obtained by transduction with vector particle-containing supernatants of 293T
15 producer cells, followed by antibiotic selection. CHO and BRL3A cells expressing human,
16 mouse, human/mouse chimeric as well as mutant SR-BI were produced as described.^{8,14,15}

17
18 **Epitope mapping.** BRL3A or CHO cells were transduced with retroviral vectors expressing
19 human, mouse or human-mouse chimeric SR-BI or previously described human SR-BI
20 mutants.^{14,15} Transduced cells were selected using antibiotics and proper SR-BI expression
21 was studied using flow cytometry and commercial anti-SR-BI antibodies. Anti-SR-BI mAb
22 binding was assessed using flow cytometry.¹⁴

23
24 **Immunoblotting.** Huh7.5.1 cells were lysed with Glo lysis buffer (Promega) and 50 µg of
25 protein of each sample were separated by 12% SDS-PAGE, transferred to HyBond-P
26 nitrocellulose membranes (GE Healthcare) and then incubated with anti-SR-BI mAbs QQ-
27 4A3-A1, QQ-2A10-A5, QQ-4G9-A6, PS-6A7-C4, NK-8H5-E3, NK-6B10-E6 and NK-6G8-B5
28 (5 µg/mL) or EP1556Y (Abcam, 1:100) and AP-labelled secondary antibodies.⁸

1 **Supplementary Results**

2 **Anti-SR-BI antibodies do not interfere with sE2 binding to target cells.** As HCV E2
3 directly binds hSR-BI, we assessed their ability to interfere with E2-SR-BI binding using
4 recombinant soluble E2 (sE2) as a surrogate model for HCV and Huh7.5.1 cells as target
5 cells. In contrast to a polyclonal anti-SR-BI rat serum⁸ and an anti-CD81 mAb, none of the
6 anti-SR-BI mAbs inhibited sE2-SR-BI binding (Supplementary Figure 1B-C, statistically not
7 significant). Given that Huh7.5.1 cells express all known HCV receptors that may also
8 contribute to sE2 binding, we also used rat BRL cells lacking SR-BI¹⁴ to assess sE2 binding
9 to exogenously expressed hSR-BI in the absence of other HCV receptors. Although one
10 antibody (NK-8H5-E3) appeared to have a minor inhibitory effect in some experiments,
11 inhibition of sE2 binding was not statistically significant and not robust compared to the
12 polyclonal anti-SRBI rat serum (Supplementary Figure 1D). Surprisingly, rat anti-SR-BI mAbs
13 increased sE2-SR-BI binding. It is conceivable that binding of the rat mAb to SR-BI results in
14 a different interaction of sE2 with other HCV attachment factors on BRL cells such as
15 heparan sulfate which subsequently enhances HCV attachment. Taken together, these data
16 confirm the findings obtained for cellular attachment of HCVcc (Figure 2) and suggest that
17 interference with E2 binding to target cells does not play a major role for the antiviral action
18 of SR-BI-specific mAbs described in this study.

19

20 **Functional impact of HCV HVR1 and SR-BI during post-binding steps of the viral entry**
21 **process.** The 27 amino acid long hypervariable region 1 (HVR1) at the N-terminus of HCV
22 E2 has been shown to mediate E2 binding to SR-BI and also to contribute to HDL-mediated
23 enhancement of HCV entry that is dependent on the lipid transfer function of SR-BI but
24 independent of HDL binding.¹⁴⁻¹⁶ Given this complex role of HVR1 in SR-BI-dependent HCV
25 entry steps, we investigated the effect of anti-SR-BI mAbs inhibiting HCV post-binding steps
26 on HCVcc and HCVpp deleted in HVR1 (Δ HVR1). Interestingly, HCVcc and HCVpp lacking
27 HVR1 were less sensitive to inhibition by anti-SR-BI mAbs (Supplementary Figure 4A-B,
28 $p < 0.01$) although requiring SR-BI for cell entry as cells lacking SR-BI are not permissive for

1 Δ HVR1 HCV (data not shown). This was also confirmed using HCVpp L399R containing a
2 point mutation within HVR1 (Supplementary Figure 4C, $p < 0.01$). Taken together, these data
3 suggest that HVR1 may play a role during SR-BI-mediated post-binding steps of the HCV
4 entry process. Since anti-SR-BI antibodies did not interfere with cellular binding of sE2, it is
5 conceivable that the functional role of HVR during SR-BI mediated post-binding steps may be
6 beyond direct E2-HVR1-SR-BI interactions.

7

8 **Protein determinants relevant for HCV post-binding steps lie within the N-terminal half**
9 **of the human SR-BI ectodomain.** To map the protein determinants important for SR-BI
10 post-binding function during HCV entry, we first performed cross-competition studies in order
11 to determine whether these antibodies recognize overlapping or distinct epitopes. Labelled
12 anti-SR-BI mAb NK-8H5-E3 was incubated with Huh7.5.1 cells in the presence of increasing
13 concentrations of unlabelled anti-SR-BI mAbs. Cross-competition experiments with labelled
14 versions of QQ-4A3-A1, QQ-2A10-A5 and QQ-4G9-A6 demonstrated that each of these
15 mAbs reduced binding of unlabelled rat mAbs but not mouse mAb (Supplementary Figure
16 6A-C). Moreover, in contrast to unlabelled mouse NK-8H5-E3, none of the three unlabelled
17 rat mAbs (QQ-4A3-A1, QQ-2A10-A5 and QQ-4G9-A6) reduced binding of NK-8H5-E3 to
18 Huh7.5.1 cells, comparable to control isotype mAb (Supplementary Figure 6D). The mutual
19 cross competition between the three rat mAbs suggests that they recognize overlapping or
20 closely related epitopes on SR-BI while the mouse mAb recognizes a distinct epitope. To
21 further define the epitopes targeted by these antibodies, we investigated their ability to bind
22 to human-mouse SR-BI chimeras, where part of the mouse SR-BI ectodomain was replaced
23 by the corresponding human sequence (Supplementary Figure 5A-C).¹⁵ While the HHH and
24 MMM SR-BI constructs refer to the wild-type human (H) and mouse (M) SR-BI molecules,
25 respectively, the human/mouse SR-BI chimeras were denominated according to the origin of
26 either SR-BI region, e.g., HMM bears region 1 from human SR-BI and regions 2 and 3 from
27 murine SR-BI (Supplementary Figure 5B-C). The overall homology between human and
28 mouse SR-BI is 80% (54 aa difference) (Supplementary Figure 5A). There are a total of 31,

1 14 and 9 different aa within the first, second and third region of the SR-BI human/mouse
2 chimeras, respectively (Supplementary Figure 5A). The three rat anti-SR-BI mAbs QQ-4A3-
3 A1, QQ-2A10-A5, QQ-4G9-A6 bind to HMM SR-BI, i. e. aa 38-215, with high affinity and also
4 to MHM, i. e. 216-398, to a lesser extent while the mouse mAb NK-8H5-E3 only recognizes
5 HMM SR-BI with high affinity (Supplementary Figure 5D). These data suggest that the
6 epitope targeted by NK-8H5-E3 lies in the N-terminal half of the human SR-BI ectodomain,
7 between aa 38 and aa 215, while the epitope(s) targeted by QQ-4A3-A1, QQ-2A10-A5, QQ-
8 4G9-A6 probably lie more downstream within the SR-BI ectodomain. To further map residues
9 within SR-BI contributing to antibody binding we used previously described SR-BI point
10 mutants.¹⁴ Interestingly, point mutation G420H and double mutations Q402R-E418R and
11 G420H-G424H within human SR-BI markedly reduced binding of the four anti-SR-BI mAbs
12 inhibiting HCV infection (Supplementary Figure 5E). These data suggest that aa 402, 418,
13 420 and 424 may be part of the epitopes of these antibodies or that these mutations may
14 induce conformational changes within the epitopes. Finally, to further characterize the nature
15 of the epitopes targeted by our panel of anti-SR-BI mAbs, we assessed the ability of the anti-
16 SR-BI mAbs to bind to human SR-BI using SDS-PAGE and Western blot. Immunostaining of
17 SR-BI by anti-SR-BI mAbs PS-6A7-C4, NK-6B10-E6 and NK-6G8-B5 suggest that the
18 epitopes interacting with these antibodies, that do not inhibit HCV infection, probably include
19 linear domains (data not shown). In contrast, none of the antibodies inhibiting HCV infection
20 interacted with linear SR-BI in Western blot experiments suggesting that the antibodies
21 inhibiting HCV infection likely recognize predominantly conformational epitopes (data not
22 shown). Taken together, these data indicate that anti-SR-BI mAbs inhibiting HCVcc infection
23 recognize conformational epitopes within the N-terminal half of the SR-BI ectodomain.
24 Moreover, these data suggest that the N-terminal ectodomain of SR-BI contains protein
25 determinants relevant for the SR-BI post-binding function in HCV entry.

26

1 **Supplementary Figure legends**

2 **Supplementary Figure 1. Monoclonal antibodies specific for human SR-BI do not block**
3 **HCV E2 binding.** (A) BRL3A cells engineered to express mouse (m) or human (h) SR-BI
4 were first incubated with monoclonal anti-SR-BI antibodies (20 $\mu\text{g}/\text{mL}$) for 1h at RT before
5 bound antibodies were detected using PE-labelled secondary antibodies and flow cytometry.
6 Results are expressed as net mean fluorescence intensity (ΔMFI). (B-C) Huh7.5.1 cells were
7 pre-incubated with anti-CD81 (5 $\mu\text{g}/\text{mL}$), anti-SR-BI or control serum (1:100), anti-SR-BI (20
8 $\mu\text{g}/\text{mL}$) or control antibodies for 1h at room temperature (RT) before incubation with sE2 for
9 1h at RT. (B) sE2 binding was detected using mouse anti-His antibody followed by PE-
10 labelled anti-mouse antibody and flow cytometry. (C) sE2 binding was detected using FITC-
11 labelled mouse anti-His antibody and flow cytometry. Results are expressed as means \pm SD
12 % sE2 binding in the absence of antibody of three independent experiments performed in
13 duplicate. (D) BRL cells engineered to express human SR-BI were pre-incubated with
14 polyclonal anti-SR-BI or control (CTRL) serum (1:50), anti-SR-BI (20 $\mu\text{g}/\text{mL}$) or control
15 (CTRL) antibodies for 1h at room temperature (RT) before incubation with sE2 for 1h at RT.
16 sE2 binding was detected using FITC-labelled mouse anti-His antibody and flow cytometry.
17 Results are expressed as means \pm SD % sE2 binding in the absence of antibody of four
18 independent experiments. * $P < 0.01$

19

20 **Supplementary Figure 2. Monoclonal anti-SR-BI antibodies block HCV cell-to-cell**
21 **transmission and spread.** (A-B) Quantification of HCV-infected target cells (T_i) after co-
22 cultivation with HCV producer cells (P_i) during incubation with (A) control or anti-SR-BI mAb
23 QQ-4G9-A6 (10 $\mu\text{g}/\text{mL}$) or (B) control or anti-SR-BI mAb QQ-2A10-A5 (10 $\mu\text{g}/\text{mL}$) in the
24 presence of E2-neutralizing antibody AP33 (25 $\mu\text{g}/\text{mL}$) by flow cytometry. (C) Cell viability
25 after long-term exposure to anti-SR-BI mAbs QQ-4G9-A6 and NK-8H5-E3. Cell viability was
26 assessed using MTT assay after incubation of Huh7.5.1 cells for 14 days in the presence or
27 absence of control or anti-SR-BI mAbs at 1, 10, or 100 $\mu\text{g}/\text{mL}$. Control medium and medium
28 containing antibodies were replenished every 4 days. Data are expressed as % cell viability

1 relative to cells incubated in the absence of mAb and represent means \pm SD from one
2 experiment.

3

4 **Supplementary Figure 3. Genotype-independent inhibition of HCVpp infection by**
5 **monoclonal anti-SR-BI antibodies.** Inhibition of entry into Huh7.5.1 cells of HCVpp bearing
6 envelope glycoproteins from genotypes 5 and 6. Huh7.5.1 cells were pre-incubated with
7 control or anti-SR-BI mAbs (50 μ g/mL) for 1h at 37°C before infection with HCVpp bearing
8 envelope glycoproteins of strains UKN5.14.4 (5) or UKN6.5.340 (6) and VSV-Gpp. HCVpp
9 entry was analyzed by luciferase reporter gene expression. Results are expressed as %
10 HCVpp entry and represent means \pm SD from 3 independent experiments performed in
11 triplicate. * P<0.01

12

13 **Supplementary Figure 4. Inhibition of HVR1-deleted HCVcc and HCVpp by monoclonal**
14 **anti-SR-BI antibodies.** Huh7.5.1 cells were pre-incubated with control or anti-SR-BI mAbs
15 (20 μ g/mL) for 1h at 37°C before infection with (A) Luc-Jc1 HCVcc deleted of HVR1 (Δ HVR1)
16 or (B-C) HCVpp bearing the envelope glycoproteins of strain H77, (B) H77 deleted of HVR1
17 (Δ G384-N411) or (C) H77 containing a point mutation within HVR1 (L399R). HCVpp and
18 HCVcc infection was analyzed by luciferase reporter gene expression. Results are
19 expressed as % HCVpp entry or HCVcc infection and represent means \pm SD from (A) one
20 experiment performed in triplicate and (B-C) 3 independent experiments performed in
21 triplicate. * P<0.01

22

23 **Supplementary Figure 5. Binding of monoclonal anti-SR-BI antibodies to human,**
24 **mouse or chimeric mouse and human SR-BI as well as human SR-BI lipid transfer**
25 **mutants.** (A) Alignment of amino acid sequences of mouse and human SR-BI. (B-C)
26 Schematic representations of three human/murine SR-BI chimeras that were generated
27 through PCR by swapping three SR-BI domains between amino-acid positions 38-215
28 (region 1), 216-398 (region 2) and 399-432 (region 3), respectively. While the HHH and

1 MMM SR-BI constructs refer to the wild-type human (H) and murine (M) SR-BI molecules,
2 respectively, the human/mouse SR-BI chimeras were denominated according to the origin of
3 either SR-BI domain, e.g., HMM bears the region 1 from human SR-BI and the regions 2 and
4 3 from murine SR-BI.¹⁵ (D) BRL3A cells engineered to express human (HHH), mouse (MMM)
5 or chimeric mouse and human (HMM, MHM, MMH) SR-BI were first incubated with
6 monoclonal anti-SR-BI antibodies (20 µg/mL) for 1h at room temperature before bound
7 antibodies were detected using PE-labelled secondary antibodies. Results are expressed as
8 means ± SD net mean fluorescence intensity (ΔMFI). (E) BRL3A cells engineered to express
9 wild-type human SR-BI (SR-BI wt) or human SR-BI point mutants (G420H, Q402R, E418R,
10 Q402R-E418R and G420H-G424H) were first incubated with monoclonal anti-SR-BI
11 antibodies (20 µg/mL) for 1h at RT before bound antibodies were detected using PE-labelled
12 secondary antibodies. Results are expressed as % binding of antibodies as compared to SR-
13 BI wt and represent means ± SD from one out of 2 independent experiments.

14

15 **Supplementary Figure 6. Competition of monoclonal anti-SR-BI antibodies for cellular**
16 **binding.** Huh7.5.1 cells were incubated with 0.1 µg/mL of biotinylated anti-SR-BI mAb (A)
17 QQ-4A3-A1, (B) QQ-2A10-A5, (C) QQ-4G9-A6 or (D) NK-8H5-E3, together with increasing
18 concentrations of unlabeled control or anti-SR-BI mAb (QQ-4A3-A1, QQ-2A10-A5, QQ-4G9-
19 A6, NK-8H5-E3) as competitors. Following washing of cells with PBS, binding of labelled
20 mAbs was determined by flow cytometry and is shown % binding relative to biotinylated mAb
21 incubated in the absence of antibody.

22

23 **Supplementary Figure 7. Combination of anti-SR-BI and neutralizing antibodies results**
24 **in a synergistic activity in inhibiting HCV infection.** Patient derived HCVpp P02VJ (A, C,
25 E) or HCVcc (Luc-Jc1) (B, D, F) were pre-incubated with (A-B) anti-E1 (IGH526) or (C-D)
26 anti-E2 (IGH461) mAbs or (E-F) purified heterologous anti-HCV IgG obtained from an
27 unrelated chronically infected subject or isotype control IgG at the indicated concentrations
28 for 1h at 37°C and added to Huh7.5.1 cells pre-incubated with increasing concentrations of

1 control or anti-SR-BI mAbs (NK-8H5-E3). HCVpp and HCVcc infection was analyzed by
2 luciferase reporter gene expression. Results are expressed as mean % HCVpp entry or
3 HCVcc infection from a representative experiment. Synergy was assessed by the
4 Combination Index calculated as described.^{11, 12} Combination of anti-E1 or anti-E2 or patient-
5 derived anti-HCV IgG with a sub-IC₅₀ concentration of anti-SR-BI mAb - which exerts only
6 minimal inhibitory effect on HCV infection - resulted in a synergistic activity in inhibition of
7 HCVcc infection (CIs of 0.06 to 0.67). These combinations reduced the IC₅₀ of anti-SR-BI
8 mAb by up to 100-fold. (A, C, E) CI NK-8H5-E3 + anti-E1 (1 µg/mL): 0.30; CI NK-8H5-E3 +
9 anti-E2 (1 µg/mL): 0.51; CI NK-8H5-E3 + anti-HCV IgG (1 µg/mL): 0.67 (B, D, F) CI NK-8H5-
10 E3 + anti-E1 (0.01 µg/mL): 0.06; CI NK-8H5-E3 + anti-E2 (0.01 µg/mL): 0.25; CI NK-8H5-E3
11 + anti-HCV IgG (0.1 µg/mL): 0.14.

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1 **Supplementary References**

- 2 1. Haid S, Novodomska A, Gentzsch J, Grethe C, Geuenich S, Bankwitz D, Chhatwal P,
3 et al. A plant-derived flavonoid inhibits entry of all HCV genotypes into human hepatocytes.
4 *Gastroenterology* 2012;143:213-222 e215.
- 5 2. Fofana I, Krieger SE, Grunert F, Glauben S, Xiao F, Fafi-Kremer S, Soulier E, et al.
6 Monoclonal anti-claudin 1 antibodies prevent hepatitis C virus infection of primary human
7 hepatocytes. *Gastroenterology* 2010;39:953-964.
- 8 3. Lupberger J, Zeisel MB, Xiao F, Thumann C, Fofana I, Zona L, Davis C, et al. EGFR
9 and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral
10 therapy. *Nature Medicine* 2011;17:589-595.
- 11 4. Fafi-Kremer S, Fofana I, Soulier E, Carolla P, Meuleman P, Leroux-Roels G, Patel
12 AH, et al. Viral entry and escape from antibody-mediated neutralization influence hepatitis C
13 virus reinfection in liver transplantation. *J Exp Med* 2010;207:2019-2031.
- 14 5. Bartosch B, Verney G, Dreux M, Donot P, Morice Y, Penin F, Pawlotsky JM, et al. An
15 interplay between hypervariable region 1 of the hepatitis C virus E2 glycoprotein, the
16 scavenger receptor BI, and high-density lipoprotein promotes both enhancement of infection
17 and protection against neutralizing antibodies. *J Virol* 2005;79:8217-8229.
- 18 6. Bankwitz D, Steinmann E, Bitzegeio J, Ciesek S, Friesland M, Herrmann E, Zeisel
19 MB, et al. Hepatitis C virus hypervariable region 1 modulates receptor interactions, conceals
20 the CD81 binding site, and protects conserved neutralizing epitopes. *J Virol* 2010;84:5751-
21 5763.
- 22 7. Pestka JM, Zeisel MB, Blaser E, Schurmann P, Bartosch B, Cosset FL, Patel AH, et
23 al. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source
24 outbreak of hepatitis C. *Proc Natl Acad Sci U S A* 2007;104:6025-6030.
- 25 8. Zeisel MB, Koutsoudakis G, Schnober EK, Haberstroh A, Blum HE, Cosset F-L,
26 Wakita T, et al. Scavenger receptor BI is a key host factor for Hepatitis C virus infection
27 required for an entry step closely linked to CD81. *Hepatology* 2007; 46:1722-1731.

- 1 9. Haberstroh A, Schnober EK, Zeisel MB, Carolla P, Barth H, Blum HE, Cosset FL, et
2 al. Neutralizing host responses in hepatitis C virus infection target viral entry at postbinding
3 steps and membrane fusion. *Gastroenterology* 2008;135:1719-1728 e1711.
- 4 10. Krieger SE, Zeisel MB, Davis C, Thumann C, Harris HJ, Schnober EK, Mee C, et al.
5 Inhibition of hepatitis C virus infection by anti-claudin-1 antibodies is mediated by
6 neutralization of E2-CD81-claudin-1 associations. *Hepatology* 2010;51:1144-1157.
- 7 11. Zhao L, Wientjes MG, Au JL. Evaluation of combination chemotherapy: integration of
8 nonlinear regression, curve shift, isobologram, and combination index analyses. *Clin Cancer*
9 *Res* 2004;10:7994-8004.
- 10 12. Zhu H, Wong-Staal F, Lee H, Syder A, McKelvy J, Schooley RT, Wyles DL.
11 Evaluation of ITX 5061, a scavenger receptor B1 antagonist: resistance selection and activity
12 in combination with other hepatitis C virus antivirals. *J Infect Dis* 2012;205:656-662.
- 13 13. Prichard MN, Shipman C, Jr. A three-dimensional model to analyze drug-drug
14 interactions. *Antiviral Res* 1990;14:181-205.
- 15 14. Dreux M, Dao Thi VL, Fresquet J, Guerin M, Julia Z, Verney G, Durantel D, et al.
16 Receptor complementation and mutagenesis reveal SR-BI as an essential HCV entry factor
17 and functionally imply its intra- and extra-cellular domains. *PLoS Pathog* 2009;5:e1000310.
- 18 15. Dao Thi VL, Granier C, Zeisel MB, Guerin M, Mancip J, Granio O, Penin F, et al.
19 Characterization of hepatitis C virus particle sub-populations reveals multiple usage of the
20 scavenger receptor BI for entry steps. *J Biol Chem* 2012. Jul 6 [Epub ahead of print]
- 21 16. Bartosch B, Vitelli A, Granier C, Goujon C, Dubuisson J, Pascale S, Scarselli E, et al.
22 Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin
23 and the SR-B1 scavenger receptor. *J Biol Chem* 2003;278:41624-41630.

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