Clearance of persistent hepatitis C virus infection in humanized mice using a claudin-1-targeting monoclonal antibody

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Hepatitis C virus (HCV) infection is a leading cause of liver cirrhosis and cancer1. Cell entry of HCV2 and other pathogens3–5 is mediated by tight junction (TJ) proteins, but successful therapeutic targeting of TJ proteins has not been reported yet. Using a human liver–chimeric mouse model6, we show that a monoclonal antibody specific for the TJ protein claudin-1 (ref. 7) eliminates chronic HCV infection without detectable toxicity. This antibody inhibits HCV entry, cell-cell transmission and virus-induced signaling events. Antibody treatment reduces the number of HCV-infected hepatocytes in vivo, highlighting the need for de novo infection by means of host entry factors to maintain chronic infection. In summary, we demonstrate that an antibody targeting a virus receptor can cure chronic viral infection and uncover TJ proteins as targets for antiviral therapy.

The TJ protein claudin-1 (CLDN1) mediates hepatitis C virus (HCV) entry into host cells2. TJs are also implicated in the entry of other pathogens including dengue virus3, adenovirus3, coxsackievirus3 and shigella4. However, the role of TJ proteins in viral pathogenesis and as antiviral targets is unknown. To investigate this we used a recently developed inhibitory CLDN1-specific monoclonal antibody (mAb)7 and the human chimeric uPA-SCID (urokinase-type plasminogen activator-transgenic severe combined immunodeficiency) mouse model6. Owing to discontinuation of research in chimpanzees for ethical reasons, this mouse model is the only available model that supports robust long-term chronic HCV infection. Although these mice lack a functional immune system, precluding the study of immune-mediated events, they have substantially contributed to our understanding of viral pathogenesis and the development of antivirals8–11.

First, we analyzed CLDN1 expression in the chimeric liver using a commercially available mAb that recognizes human and mouse CLDN1 (Supplementary Fig. 1). Confocal imaging demonstrated that the majority of CLDN1 on human hepatocytes co-localized with apical marker CD10, demonstrating the formation of bile canalicul structures (Fig. 1a). A minor pool of protein was detected at the basolateral membrane as identified with cytokeratin-8 staining12 (Fig. 1a and data not shown). Comparative staining of normal human liver tissue demonstrated comparable subcellular localization (Fig. 1a). Transmission electron microscopic (TEM) analysis confirmed that hepatocytes in the chimeric mouse liver form TJs that were structurally indistinguishable from those in human liver tissue (Supplementary Fig. 2a,b). The similar localization of hepatocellular CLDN1 and hepatocyte architecture suggests that the uPA-SCID chimeric mouse is a relevant model to evaluate CLDN1 as a therapeutic target.

Next, we characterized the subcellular localization of CLDN1 recognized by the inhibitory CLDN1-specific mAb (OM-7D3-B3) when administered intraperitoneally in vivo. TEM and immunogold labeling of the CLDN1 mAb showed reactivity with basolateral and...
sinusoidal hepatocyte membranes in vivo (Fig. 1b), whereas we failed to detect staining of TJs (Fig. 1b). Basolateral membrane staining was confirmed using live-cell imaging of polarized hepatoma HepG2 cells (Fig. 1c)\(^1\). Collectively, these data suggest that the CLDN1-specific mAb predominantly binds to nonjunctional pools of CLDN1 on the hepatocyte basolateral membrane in vivo.

To investigate the antiviral effect of this CLDN1-specific mAb, we administered to chimeric uPA-SCID mice CLDN1-specific (\(n = 5\)) or control isotype-matched irrelevant mAb (\(n = 4\)) 1 day before inoculation, and 1 and 5 days after infection with a primary HCV genotype 1b serum. The CLDN1-specific mAb provided complete and sustained protection from infection as evidenced by undetectable HCV RNA in the sera for up to 6 weeks after infection (Fig. 2a and Supplementary Table 1). In contrast, all control-mAb–treated mice became infected (Fig. 2a). Similar results were observed following infection with an HCV of the difficult-to-treat genotype 4 (isolate ED43 (ref. 14)), Fig. 2b and Supplementary Table 1), demonstrating a significant level of anti-viral activity (\(P < 0.0002\), pooled data from experiments of both genotypes, Mann-Whitney test). Pharmacokinetic studies showed that CLDN1-specific mAb levels declined rapidly and were undetectable 2 weeks after administration (Fig. 2f,g). Collectively, these data show that short-term administration of a CLDN1-specific mAb prevents de novo HCV infection and suggests that it might be used to prevent HCV re-infection during liver transplantation.

To investigate the effect of the CLDN1-specific mAb on chronic HCV infection, we used mice persistently infected with the cell culture–derived HCV (HCVcc) strain Jc1 (genotype 2a/2a chimera)\(^{15}\), a JFH1-derived\(^{16}\) prototype strain. Persistently infected mice (24–50 days after infection) were given four doses total (one in each week of the study period) of CLDN1-specific (\(n = 5\)) or control mAbs (\(n = 4\)). All CLDN1-specific mAb–treated mice had undetectable HCV RNA levels in the blood after 2 to 4 injections (Fig. 2c and Supplementary Table 1) and, except for one case, animals remained free of virus until the end of the study period. The relapsed mouse showed a low antibody serum concentration during the early treatment period and declined rapidly following discontinuation of mAb injection (Fig. 2h). Similar results were observed in four additional HCV Jc1-infected and CLDN1-specific mAb–treated mice with follow-up of 3 weeks (data not shown).

CLDN1 mAb also induced viral clearance from mice chronically infected with HCVcc VL-JFH1 (genotype 1b/2a chimera) (\(n = 5\)), a well-characterized strain\(^{17}\) encoding the structural proteins from a highly infectious HCV neutralization escape variant isolated from a patient undergoing liver transplantation (Fig. 2d and Supplementary Table 1). One mouse showing a partial viral response (Fig. 2d and Supplementary Table 1) was characterized by undetectable mAb concentration after the second injection (Fig. 2i). The therapeutic effect was robust (HCV RNA decline > 2 logs), sustained (until the end of the study period corresponding to 11 weeks) and significant (\(P < 0.0007\), pooled data for experiments of both genotypes, Mann-Whitney test). Furthermore, a similar effect was observed in mice chronically infected with HCV genotype 2a serum (Fig. 2e and Supplementary Table 1). Mice infected with primary isolates of HCV genotype 4 (\(n = 4\)) also showed a marked and time-dependent decrease in HCV RNA levels (Supplementary Fig. 5a) with no viral rebound and stable function of the engrafted human hepatocytes as shown by serum albumin levels (Supplementary Fig. 5b). In summary, these data show that a CLDN1-specific mAb can clear chronic HCV infection in vivo and exhibits antiviral activity against different viral genotypes. These findings suggest CLDN1 as a therapeutic target in a clinically relevant animal model.

To assess antibody safety, we analyzed the histopathology of chimeric uPA-SCID mouse livers. Human hepatocyte-specific staining
in vivo and in vitro studies. Using protein association studies and a Förster resonance energy transfer (FRET)-based assay, we found that the CLDN1-specific mAb interfered with CD81-CLDN1 co-receptor complex formation (Supplementary Fig. 11a)—a key step required for HCV entry. Co-receptor

Figure 2 Prevention and clearance of chronic HCV infection using a CLDN1-specific mAb in vivo. (a,b) Prevention studies. Chimeric uPA-SCID mice received 500 µg CLDN1-specific (n = 5) or control mAb (n = 4) on days −1, 1 and 5 (arrows) of inoculation (asterisk) with genotype 1b (a) or genotype 4 (b) HCV-containing serum. One infected mouse injected with control mAb died on day 26 (a, cross). (c–e) Treatment studies. Chimeric uPA-SCID mice were chronically infected with HCVcc Jc1 (ref. 15) (genotype 2a/2a) (cstudies (c)

Twenty-four to 50 days following inoculation, the animals received 500 µg control (c,d,e; n = 4, 3 and 4, respectively) or CLDN1-specific mAb (c,d,e; n = 5, 5 and 6, respectively) each week (arrows) for 4 weeks. Two CLDN1-specific mAb-treated mice were euthanized following viral clearance for FISH studies (e). (f–j) Serum concentration of CLDN1-specific mAb from (a–e, respectively) was determined by ELISA. Serum viral load (a–e) was quantified by the clinically licensed Abbott RealTime HCV assay. The horizontal dashed line indicates the limit of quantification (LOQ). (c,d,h,i) The viral load and antibody concentration of the mice exhibiting a relapse is indicated by a dashed line. Values of individual animals are shown.

demonstrated similar repopulation and structure of human hepatocytes in HCV Jc1-infected mice treated with the control or CLDN1-specific mAb (Supplementary Fig. 6a). TEM analysis of chimeric-infected livers of treated mice showed no detectable alteration in hepatocyte morphology or TJ ultrastructure (data not shown). Human albumin, transaminases (ALT, AST) and total bilirubin levels remained stable following antibody administration and were similar in control and CLDN1-specific mAb–treated mice at all time points tested (Supplementary Fig. 6b–e).

To assess the functional integrity of CLDN1–mAb–treated human hepatocytes in mice, we challenged the mice with HCV of a different strain and genotype. CLDN1-specific mAb–treated animals previously protected from HCV infection (Fig. 2a) supported viral infection following mAb elimination (Supplementary Fig. 6f). These functional data corroborate the presence of fully viable and functional hepatocytes following anti-CLDN1 mAb treatment and exclude adverse effects on hepatocyte function in vivo.

To address potential side effects of the CLDN1-specific mAb in other organs and tissues, we assessed antibody binding to human and murine CLDN1 (Supplementary Fig. 7a–g). The antibody-bound primary mouse hepatocytes and 293T cells expressing murine CLDN1 with an apparent Kd of 83.6 ± 14.1 nM compared to the apparent Kd for human CLDN1 expressed in 293T cells (17.05 ± 1.14 nM) (Supplementary Fig. 7g). Moreover, the latter was in a similar range as the apparent Kd of CLDN1-specific mAb binding to human hepatocytes. Next we measured in vivo biodistribution in BALB/c mice and observed enrichment in skin, kidneys, lungs, intestines and liver (Supplementary Fig. 8a,b). Toxicity studies in BALB/c mice including clinical (data not shown), biochemical and hematological parameters as well as histopathological analyses did not reveal any toxicity (Supplementary Fig. 9 and Supplementary Tables 2 and 3). Because TJs play a key role in intestinal paracellular permeability, we investigated the effect of the CLDN1-specific mAb on intestinal barrier function in vivo. The CLDN1-specific mAb had no effect on intestinal paracellular permeability (Supplementary Fig. 8c) or total intestinal transit time (Supplementary Fig. 8d), in contrast to irradiation, which was used as a positive control for intestinal injury. Additional ex vivo studies showed that the CLDN1-specific antibody had no detectable effects on paracellular permeability (Supplementary Fig. 8e) or tissue conductance in the small intestine or colon (Supplementary Fig. 8f). Moreover, the mAb had no effect on the transepithelial electrical resistance of intestinal Caco-2 cells (Supplementary Fig. 8g), which express abundant CLDN1 levels (Supplementary Fig. 8h), or on TJ function in hepatoma HepG2 cells (Supplementary Fig. 10a,b), the CLDN1-specific mAb had no impact (Supplementary Fig. 10c). Furthermore, flow cytometry experiments measuring CLDN1 cell surface expression suggested that the CLDN1-specific mAb did not promote cellular CLDN1 internalization (Supplementary Fig. 10d).

Finally, a detailed analysis of cytokine mRNA expression in antibody-treated mice did not reveal evidence for antibody-induced inflammatory changes in the mouse jejunum (data not shown). Collectively, these data exclude major toxicity or side effects induced by the CLDN1-specific mAb in mice. Because the rat antibody did not interact with the mouse Fc receptor, immune-mediated adverse effects, such as antibody-dependent cell-mediated cytotoxicity (ADCC), cannot be assessed in this model. Humanization of the antibody using an IgG4 backbone—a well-established strategy to minimize ADCC—is underway for its clinical development.

To explore the antiviral mechanism of the CLDN1-specific mAb, we performed both in vitro and in vivo studies. Using protein association studies and a Förster resonance energy transfer (FRET)-based assay, we found that the CLDN1-specific mAb interfered with CD81-CLDN1 co-receptor complex formation (Supplementary Fig. 11a)—a key step required for HCV entry. Co-receptor
perturbation most likely occurs at the hepatocyte basolateral membrane as shown by immunofluorescence studies of polarized HepG2 cells or immunogold affinity labeling of hepatocytes (Fig. 1b,c). Furthermore, we demonstrate that the CLDN1-specific mAb significantly inhibits cell-free and cell-cell routes (P < 0.01, t-test) of transmission (Supplementary Fig. 1b-e). Of note, inhibition of cell-cell transmission led to a marked decrease of virus spread in hepatoma cells when the CLDN1-specific mAb was added 48 h after infection (Supplementary Fig. 11c). The ability of the antibody to inhibit cell-free and cell-cell infection may explain, at least partly, the antiviral effects of the CLDN1-specific mAb to cure persistent HCV infection in vivo. As this mAb impaired neither HCV replication nor assembly (Fig. 1a), we conclude that the mAb acts predominantly by interfering with HCV entry.

Because claudins can modulate signaling pathways, we investigated whether the antibody modulated the microRNA (miRNA) and kinome profiles of persistent HCV-infected Huh7.5.1 cells. Consistent with previous observations, our deep sequencing studies confirmed that HCV infection modulates miRNA profiles in Huh7.5.1 cells. However, the CLDN1-specific mAb had no detectable effect on miRNA expression (Supplementary Fig. 12).

HCV binding to hepatocellular CD81 and associated receptors activates EGFR and mitogen-activated protein kinase (MAPK) signaling. HCV Jc1 infection increased ERK1/2 phosphorylation, and this was reversed by the CLDN1-specific mAb (Fig. 3a,b). The significance of HCV-induced MAPK signaling was supported by analyzing liver biopsies from HCV-infected patients. Liver biopsies from HCV-infected patients showed a significant (P < 0.01, Mann-Whitney test) 2.45-fold increase in p-ERK1/2 levels over that observed in the control (Fig. 3c, Supplementary Table 5).

We observed an antiviral effect of small-molecule inhibitors targeting MAPK signaling (Fig. 3e) at nontoxic concentrations. To confirm the effect of the CLDN1-specific mAb on HCV-induced signaling in liver tissue from chronically HCV-infected patients (Supplementary Table 4), freshly isolated liver biopsies were treated ex vivo with CLDN1-specific or control mAb, and we observed a reduction of ERK1/2 phosphorylation compared to control-treated biopsies (Fig. 3f and Supplementary Fig. 13b).
Figure 4: CLDN1-specific mAb leads to elimination of HCV-infected cells from the livers of human chimeric mice in a dose- and time-dependent manner.

(a) Detection of human hepatocytes and HCV RNA with probes specific for human GAPDH mRNA (green) or HCV Jc1 RNA (red) using FISH. HCV Jc1 RNA probe specifically stained HCV-infected human hepatocytes but not uninfected human liver (left top panel) or BALB/c mouse liver (right top panel). FISH analyses were done in livers of control mAb (left bottom panel) or CLDN1-specific mAb (right bottom panel)-treated chimeric uPA-SCID mice. Red arrowheads indicate HCV+ cells. Scale bar, 50 μm. (b) Quantification of the percentage of infected cells in the liver of uninfected chimeric mice (black circles) or HCV Jc1-infected mice treated either four times with control mAb (gray triangles), or once (blue diamonds) or four times (blue circles) with CLDN1-specific mAb. Livers were collected 1 week after the last antibody injection. The quantification was performed after confocal microscopy acquisition. ns, not significant. * P < 0.05, ** P < 0.01, **** P < 0.0001 (Kruskal-Wallis test).

These studies show that HCV infection activated MAPK signaling and CLDN1-specific mAb partially reversed this virus-dependent signaling event and did so without inhibiting ERK activation by the physiological EGFR ligand EGF (Fig. 3g,h and Supplementary Fig. 13c). These data suggest a model in which the anti-CLDN1 mAb impairs HCV-induced cross-activation of EGFR and ERK by interfering with CD81-CLDN1 association (Fig. 3i). Because MAPK signaling has been shown to be relevant for the HCV life cycle, including viral entry, it is likely that this interference contributes to the ability of the antibody to clear an established infection.

Finally, we performed mechanistic studies in chimeric mice to study the consequences of the observed mechanistic findings for viral clearance in vivo. Using fluorescence in situ hybridization (FISH) with HCV- and human GAPDH-RNA-specific probes (Fig. 4a), we demonstrate that the CLDN1-specific mAb results in a dose- and time-dependent decrease in the number of HCV-infected cells leading to their final elimination (Fig. 4b). The decline of HCV-infected hepatocytes paralleled the decline in peripheral HCV RNA levels, showing a monophasic decline (Fig. 4b and Supplementary Fig. 14). Notably, the baseline frequency of HCV-infected hepatocytes (Fig. 4b) in the chimeric mouse liver were comparable to those reported in chronically infected human liver.

Assuming an inhibition of entry (Supplementary Fig. 11) and a negligible effect on viral replication or assembly (Supplementary Fig. 11f,g), mathemetic modeling predicts a time-dependent elimination of virus-infected hepatocytes with concomitant lack of de novo infection. Applying this model, we estimated the half-life of infected cells to be ~1.3 days for HCV genotype 2a and 5.4 days for HCV genotype 4, which is in the range of hepatocyte half-lives reported in patients. Indeed, HCV has been reported to promote apoptosis by both FISH analyses of liver tissue sections (Fig. 4) and cell culture studies where the CLDN1-specific mAb eliminates HCV-infected cells (Supplementary Fig. 15c,d). The different half-lives of HCV-infected cells and the magnitude of virus-induced cell death may explain the varying magnitude of the decline in viral load for diverse HCV strains (Supplementary Figs. 5a and 14).

Collectively, we demonstrate that a host-targeting entry inhibitor clears chronic viral infection (Fig. 2c–e). Our results show that viral entry and spread are required for persistent infection in vivo and that CLDN1 is a valid target to prevent and treat chronic HCV infection. The interaction of the mAb with nonjunctional pools of CLDN1 (Fig. 1b,c) that complex with the viral co-receptor CD81 (Supplementary Fig. 11a) may explain the non-detectable toxicity (Supplementary Figs. 6a–f, 8c–g and 9 and Supplementary Tables 2 and 3).

In summary, therapeutic administration of a CLDN1-specific mAb demonstrates activity against several HCV genotypes (Fig. 2 and Supplementary Figs. 5 and 11b) without detectable toxicity (Supplementary Figs. 6, 8, 9 and Supplementary Tables 2 and 3). The absence of detectable viral resistance (Supplementary Fig. 3) suggests that CLDN1 is an antiviral target with a high genetic barrier for resistance. Although strain-dependent use of CLDN1 and CLDN6 has been described in hepatoma cells, our data suggest that CLDN1 is essential for HCV infection in vivo and low expression levels of CLDN6 and CLDN9 in human hepatocytes (Supplementary Fig. 4) appear to preclude a relevant entry function of these molecules in vivo in the human chimeric mouse model.

In contrast to clinically licensed direct-acting antivirals, such as HCV protease, NS5A or polymerase inhibitors the CLDN1-specific mAb eliminates viral infection in monotherapy in the state-of-the-art animal model (Fig. 2 and Supplementary Fig. 5a) without detectable resistance (Supplementary Fig. 3). These features are likely due to its unique mechanism of action targeting a host factor (Supplementary Fig. 11a) that is essential for viral persistence in vivo as well as for modulating signaling of virus-infected cells (Fig. 3), likely contributing to clearance. Our data suggest that antibodies or small molecules targeting CLDN1 are promising candidates to prevent liver graft infection and to eliminate chronic HCV infection. Finally, similar antimicrobial strategies may be designed for a broad range of pathogens that utilize TJ proteins as host factors including dengue virus, coxsackievirus, adenovirus and shigella.

METHODS
Methods and any associated references are available in the online version of the paper.
ONLINE METHODS

Research experiments on live vertebrates. In vivo experiments were performed in the Inserm Unit 1110 animal facility according to local laws and ethical committee approval (CREMAEA, project numbers AL/02/19/08/12 and AL/01/18/08/12).

Human subjects. Human material including liver biopsies and liver tissue from patients undergoing surgical resection was obtained with informed consent from all patients. The respective protocols were approved by the Ethics Committee of the University Hospital of Basel, Switzerland (EKBB 13 December 2004), University of Strasbourg Hospital, France (CPP 10–17), and the University of Birmingham, UK (LREC, Birmingham, West Midlands, project 04/Q2708/40).

Antibodies. The human CLDN1-specific (OM-7D3-B3, IgG2b, rat	extsuperscript{7}), CD81-specific (WU-9E1-G2, IgG2b, rat), CLDN6-specific (WU-9E1-G2, IgG2b, rat	extsuperscript{70}), CLDN9-specific (YD-4E9-A2, IgG2b, rat	extsuperscript{70}) mAbs have been described previously and were produced by Aldevron Freiburg. HCV E2-specific AP33 (mouse)	extsuperscript{17}, E1-specific IGH433 (human)	extsuperscript{7}, E2-specific CHB-23 (human) mAbs and patient-derived HCV-specific IgG have been described. Control mAb (rat IgG2b clone LTF-2, Bio X Cell), human CLDN1-specific (Life Technologies, rabbit; R&D, rat polyclonal), human CD10-specific (clone 56C6, Vector Laboratories, mouse), human CD81-specific (clone 2s131, mouse), human CK18-specific (clone VP-C414, Vector Laboratories, mouse), Alexa-fluor 488–conjugated IgG rabbit-specific, Alexa-fluor 488–conjugated IgG mouse-specific, Alexa-fluor 488–conjugated IgG rat-specific and Alexa-fluor 594–conjugated IgG mouse-specific Abs (Invitrogen, goat) were used for fluorescent imaging as described	extsuperscript{31}. ERK1/2 (rabbit, Cell Signaling), PERK1/2 (Thr202, Tyr204, mouse, clone E10, Cell Signaling) and beta-actin (clone AC-15, mouse, Sigma)-specific antibodies and alkaline–phosphatase (AP)–labeled goat, (GE Healthcare) as well as IRDye 800CW-conjugated goat anti-rabbit and IRDye 680-conjugated goat anti-mouse (Li-Cor Biosciences) secondary antibodies were used for immunoglobin analyses. Rabbit anti-rat bridging antibody (Cappel #55704) was used for immunogold labeling.

Cell lines. Huh7.5.1, Huh7.5-GFP, Huh7.5 shCD81.1, 293T, HepG2 DsRed-CD81 and Caco-2 cells were cultured as described	extsuperscript{9,32–35}.

Imaging of CLDN1 in human and chimeric livers. Chimeric mouse livers were formalin fixed and paraffin embedded. Human liver tissue was obtained from patients with HCV undergoing liver transplantsations or from normal livers taken from surplus donor tissue used for reduced size transplantation. Informed consent from each patient was obtained with regional ethics committee approval (project number 04/Q2708/40). Sections were subjected to low-temperature antigen-retention pretreatment, followed by fluorescent detection of CLDN1, CD10 or CK18, as previously described	extsuperscript{31}. Labeled sections were visualized using laser scanning confocal microscopy (Zeiss LSM 780, 100 x Plan Achromat 1.4NA oil immersion objective). Background and autofluorescence of tissue samples were corrected throughout and 3D composite images were generated using the Zeiss Zen analysis software.

TEM and immunogold labeling of CLDN1 in vivo. Human liver–chimeric mice were injected with 500 µg CLDN1-specific or control mAb at day −1 before and days 1 and 5 after inoculation of a genotype 1b or 4 HCV-infected serum. HCV infection was performed as previously described	extsuperscript{6}. For treatment studies, chimeric uPA-SCID mice were chronically infected with HCVcc of prototype strain Jc1 (ref. 15), a genotype 1b/2a chimeric VL-JFH1 (ref. 17) or genotype 2a and 4 serum. Chronically infected mice received 500 µg CLDN1-specific or control mAb once a week for 4 weeks, except for genotype 4 where mice received 500 µg twice a week for 3 weeks followed by 200 µg twice a week up to the end of the study period. Blood was harvested by retro-orbital puncture under general anesthesia. Mice were randomly assigned to the different experimental groups by a blinded technician. Experiments were performed in the Inserm Unit 1110 animal facility according to local laws and ethical committee approval (AL/02/19/08/12 and AL/01/18/08/12).

Viral load. Viral load was quantified by a blinded technician using Abbott RealTime HCV assay (Abbott). The linear range of the assay is 12 IU/ml to 10⁸ IU/ml, the limit of quantification (LOQ) of HCV RNA is 12 IU/ml. Given a mouse serum dilution of 1:100 in PBS, LOQ is 1,200 UI/ml, i.e., 5,160 copies/ml.

Analysis of CLDN1-specific mAb serum concentrations. Serum antibody concentrations were quantified using a rat IgG2b antibody-specific enzyme-linked immunosorbent assay (ELISA) (ref. E110-111, Bethyl Laboratories, Euromedex) following the recommendations of the supplier.

Liver biopsies. Eligible patients were identified by a systematic review of patient charts at the Hepatology outpatient clinic of the University Hospital of Basel, Switzerland. The protocol was approved by the Ethics Committee of the University Hospital of Basel, Switzerland. Written informed consent was obtained from all patients. Histopathological grading and staging of the HCV liver biopsies according to the Metavir classification system was performed at the Pathology Institute of the University Hospital Basel and are summarized in Supplementary Table 3. All patients that donated liver tissue were male between 27 and 61 years old and female between 39 and 62 years old (Supplementary Table 4). Liver biopsy tissues from patients were analyzed as described	extsuperscript{37}. In brief, liver tissue was lysed in 100 mM NaCl, 50 mM Tris pH 7.5, 1 mM EDTA, 0.1% Triton X-100, 10 mM NaF, 1 mM PMSE, and 1 mM sodium ortho–vanadate during mechanical homogenization followed by 20 min incubation on ice. Lysates were cleared by centrifugation at 4 °C and 21,000g for 5 min before immunoblot analysis of 5 µg protein per sample.
For ex vivo stimulation, fresh liver biopsies were divided in two equal pieces and incubated for 4 h at 37 °C in DMEM medium supplemented with 10% FCS and 100 µg/ml control or CLDN1-specific mAb, respectively.

**Analysis of protein expression and phosphorylation.** Immunoblots of cell lysates using ERK1/2-specific antibodies were performed using Hybond–P membranes and visualized using ECF substrate (GE Healthcare), according to the manufacturer’s protocol. Fluorescence emission was detected using Typhoon Trio high-performance fluorescence scanner (GE Healthcare) and quantified using Image Quant TL software (GE Healthcare). Phospho-array analysis was performed using Proteome Profiler Human Phospho-kinase Array (R&D Systems) as described by the manufacturer. For imaging, blots were incubated with ECL (GE Healthcare) and exposed to ECL Hyperfilm (GE Healthcare). Phospho-kinase array results were quantified by integrating the dot blot densities using ImageJ software (NIH). Immunoblots from biopsies were analyzed as described24.

**Inhibition of HCV infection by protein kinase inhibitors.** Huh7.5.1 cells were plated in 96-well plates (2 × 10^3 cells/well) and infected 24 h later for 3 days with HCVcc Luc-Jc1. Medium was then removed and replaced with medium containing 10 µM erlotinib (LC Laboratories) or UO126 (Calbiochem) and final concentration of 1% DMSO. Control medium contains only 1% DMSO. Three days later, HCV replication was quantified by luciferase activity measurement.

**EGF-induced MAPK activation.** Huh7.5.1 cells were serum starved for 4 h before 1 h incubation with 100 µg/ml control or CLDN1-specific mAb and 15-min incubation with increasing concentrations (1, 10 and 100 ng/ml) of EGF. p-EGFR, p-ERK1/2 and total ERK were detected by western blot analysis. Quantification was performed using a Typhoon Trio laser scanner and ImageQuant Software (GE Healthcare).

**FISH analyses of human chimeric livers.** Liver samples from mice were collected during necropsy, immediately embedded in optimal cutting temperature compound (OCT) and frozen in liquid nitrogen chilled 2-methylbutane. Tissues were then stored at −80 °C until use. Sections (10 µm) were cut at cryostat (Leica), fixed overnight in 4% formaldehyde at 4 °C and hybridized, as previously described26, with the following modifications. Briefly, tissue sections were pretreated by boiling (90–95 °C) in pretreatment solution (Affymetrix – Panomics) for 1 min, followed by a protease QF (Affymetrix – Panomics) digestion for 10 min at 40 °C. Hybridization was performed using probe sets against JFH-1 HCV RNA (target region 4513–6253) and against human GAPDH mRNA (VA6-12786-06, Affymetrix–Panomics). Pre-amplification, amplification and detection were performed according to provider’s protocol. Images were acquired with a LSCM (LSM710, Carl Zeiss Microscopy) and Zen2 software, using the same settings for all the tissues analyzed. Five to seven random fields were selected from each section, based only on the presence of human GAPDH signal to ensure the presence of human hepatocytes. Images were then acquired also including the channel for the HCV detection. Image analysis was performed using ImageJ and CellProfiler software, with a customized pipeline. Total number of cells, number of human hepatocytes (HH), number of HCV+ HH and HCV signal average intensity (and s.d.) were evaluated.

**Statistical analyses.** For in vivo and ex vivo data, the one-way ANOVA followed by Tukey’s post hoc test or the Kruskal-Wallis followed by Dunn’s post hoc test were used after determination of distribution by the Shapiro-Wilk normality test. The Wilcoxon rank test as well as the two-tailed Mann-Whitney test were also used. The in vitro data are presented as the mean ± s.d. except where mean ± s.e.m. is indicated, and were analyzed by the unpaired Student’s t-test or the two-tailed Mann-Whitney test as indicated. P ≤ 0.05 was considered significant. Statistical analyses were performed with GraphPad Prism 6 software.

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