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1 A monoclonal antibody targeting non-junctional Claudin-1 inhibits fibrosis in
2 patient-derived models by modulating cell plasticity

3

4 **Authors:** Natascha Roehlen^{1†}, Antonio Saviano^{1,2†}, Houssein El Saghire¹, Emilie Crouchet¹,
5 Zeina Nehme¹, Fabio Del Zompo¹, Frank Jühling¹, Marine A. Oudot¹, Sarah C. Durand¹, François
6 H.T. Duong¹, Sara Cherradi¹, Victor Gonzalez Motos¹, Nuno Almeida¹, Clara Ponsolles¹, Laura
7 Heydmann¹, Tessa Ostyn³, Antonin Lallement^{1,4}, Patrick Pessaux^{1,2}, Emanuele Felli^{1,2}, Andrea
8 Cavalli⁵, Jacopo Sgrignani⁵, Christine Thumann¹, Olga Koutsopoulos¹, Bryan C. Fuchs^{6#}, Yujin
9 Hoshida⁷, Maike Hofmann⁸, Mogens Vyberg⁹, Birgitte Martine Viuff¹⁰, Elisabeth D. Galsgaard¹⁰,
10 Greg Elson¹¹, Alberto Toso¹¹, Markus Meyer¹¹, Roberto Iacone¹¹, Tamas Schweighoffer¹¹,
11 Geoffrey Teixeira¹¹, Solange Moll¹², Claudio De Vito¹², Tania Roskams³, Irwin Davidson⁴,
12 Danijela Heide¹³, Mathias Heikenwälder¹³, Mirjam B. Zeisel^{1§}, Joachim Lupberger¹, Laurent
13 Maily¹, Catherine Schuster¹, Thomas F. Baumert^{1,2,15*}

14

15 **Affiliations:**

16 ¹Université de Strasbourg, Inserm, Institut de Recherche sur les Maladies Virales et Hépatiques
17 UMR-S1110, 67000 Strasbourg, France.

18 ²Service d'hépatogastroentérologie, Hôpitaux Universitaires de Strasbourg, 67000 Strasbourg,
19 France.

20 ³Department of Imaging and Pathology, University of Leuven, 3000 Leuven, Belgium.

21 ⁴Department of Functional Genomics and Cancer, Institut de Génétique et de Biologie Moléculaire
22 et Cellulaire, CNRS/INSERM/UNISTRA, 67400 Illkirch Cedex, France.

23 ⁵Institute for Research in Biomedicine, Università della Svizzera Italiana, 6500 Bellinzona,
24 Switzerland.

25 ⁶Division of Gastrointestinal and Oncologic Surgery, Massachusetts General Hospital Cancer
26 Center, Harvard Medical School, Boston, MA 02215, USA.

27 ⁷Liver Tumor Translational Research Program, Harold C. Simmons Comprehensive Cancer
28 Center, Division of Digestive and Liver Diseases, University of Texas Southwestern Medical
29 Center, Dallas, TX 75390, USA.

30 ⁸Department of Medicine II (Gastroenterology, Hepatology, Endocrinology and Infectious
31 Diseases), Freiburg University Medical Center, Faculty of Medicine, University of Freiburg,
32 79106 Freiburg, Germany.

33 ⁹Center of RNA Medicine, Department of Clinical Medicine, Aalborg University Copenhagen,
34 2450 København, Denmark, and Department of Pathology, Copenhagen University Hospital
35 Hvidovre, 2650 Hvidovre, Denmark.

36 ¹⁰Novo Nordisk A/S, 2760 Måløv, Denmark.

37 ¹¹Alentis Therapeutics, 4123 Allschwil, Switzerland.

38 ¹²Department of Pathology, University Hospital of Geneva, 1205 Geneva, Switzerland.

39 ¹³Division of Chronic Inflammation and Cancer, German Cancer Research Center, 69120
40 Heidelberg, Germany.

41 ¹⁴Institut Universitaire de France, 75006 Paris, France.

42 † These authors contributed equally to this work and share first authorship.

43

44 #Present address: Ferring Research Institute 4245 Sorrento Valley Boulevard San Diego, CA
45 92121, USA.

46 §Present address: Cancer Research Center of Lyon (CRCL), UMR Inserm 1052 CNRS 5286 Mixte
47 CLB, Université de Lyon 1 (UCBL1), 69008 Lyon, France.

48

49 *Corresponding author. E-mail: thomas.baumert@unistra.fr.

50

51 Overline: FIBROSIS

52 **One Sentence Summary:** Claudin-1 is a mediator of and therapeutic target for organ
53 fibrosis.

54

55

56 **Abstract:** Tissue fibrosis is a key driver of end-stage organ failure and cancer, overall accounting
57 for up to 45% of deaths in developed countries. There is a large unmet medical need for anti-
58 fibrotic therapies. Claudin-1 (CLDN1) is a member of the tight junction protein family. Although
59 the role of CLDN1 incorporated in tight junctions is well established, the function of non-
60 junctional CLDN1 (njCLDN1) is largely unknown. Using highly specific monoclonal antibodies
61 targeting a conformation-dependent epitope of exposed njCLDN1, we show in patient-derived
62 liver 3D fibrosis and human liver chimeric mouse models that CLDN1 is a previously unknown
63 mediator and target for liver fibrosis. Targeting CLDN1 reverted inflammation-induced hepatocyte
64 pro-fibrogenic signaling and cell fate and suppressed the myofibroblast differentiation of hepatic
65 stellate cells. Safety studies of a fully humanized antibody in non-human primates did not reveal
66 any serious adverse events even at high steady-state concentrations. Our results provide preclinical
67 proof-of-concept for CLDN1-specific mAbs for treatment of advanced liver fibrosis and cancer
68 prevention. Antifibrotic effects in lung and kidney fibrosis models further indicate a role of
69 CLDN1 as a therapeutic target for tissue fibrosis across organs. In conclusion, our data pave the
70 way for further therapeutic exploration of CLDN1-targeting therapies for fibrotic diseases in
71 patients.

72

73

74 **INTRODUCTION**

75 Organ fibrosis is the result of excessive accumulation of extracellular matrix (ECM) that
76 results from a wound healing response to repeated and chronic tissue injury. Organ fibrosis
77 accounts for up to 45% of deaths in developed countries(1) and is a major risk factor for tumor
78 development. Yet, approved therapies that aim to treat fibrosis are either absent, as for the liver,
79 or show limited efficacy and safety as for the lung(2-4). Importantly, several key features and
80 cellular drivers appear to be similar across different organs suggesting common pathways in the
81 development of end-stage organ fibrosis(1).

82 The major causes of liver fibrosis are chronic hepatitis B (HBV) and C (HCV), alcoholic
83 liver disease (ALD) and non-alcoholic steatohepatitis (NASH). Hepatocellular carcinoma (HCC)
84 nearly always arises in the context of advanced liver fibrosis. Thus, direct anti-fibrotic agents are
85 urgently needed to improve patient survival and outcome in advanced fibrosis by preventing liver
86 disease progression, cancer risk and mortality(2).

87 Claudin-1 (CLDN1) is a member of the tight junction protein family(5) which is expressed
88 in a junctional and non-junctional (nj) form. In the liver, njCLDN1 serves as a cell entry factor of
89 HCV(5, 6), a major cause of liver fibrosis and cancer. Previous studies demonstrated that CLDN1
90 expression is upregulated in liver cirrhosis and HCC(7). Furthermore, CLDN1 overexpression
91 induces epithelial-mesenchymal transition in liver cells(8), a pathogenic mechanism implicated in
92 fibrosis and cancer.

93 Although its function within tight junctions for cell-cell-adhesion is well established, the
94 role of njCLDN1 in disease biology and as a therapeutic target is largely unknown. We have
95 previously developed monoclonal antibodies (mAbs) targeting the extracellular loop 1 of CLDN1
96 expressed on the hepatocyte basolateral membrane outside the tight junctions which potently

97 inhibit HCV cell entry into hepatocytes. Using a panel of mAbs targeting the ECL1 of CLDN1
98 combined with patient-derived models, we investigated the role of CLDN1 as a mediator and target
99 for liver fibrosis.

100

101 **RESULTS**

102 **CLDN1 expression is associated with liver fibrosis and disease progression**

103 We first analyzed *CLDN1* gene expression in liver tissues of patients with chronic liver
104 disease (HBV, HCV, or NASH) in several independent cohorts from Gene Expression Omnibus
105 (GEO) and in a NASH cohort from the University of Strasbourg (table S1). *CLDN1* is upregulated
106 in liver tissue of patients with liver disease of all major etiologies (**Fig. 1A**). *CLDN1* expression is
107 associated with fibrotic disease progression in patients with NASH and HCV-infected individuals
108 post transplantation(12) (**Fig. 1B**). Corroborating a pathogenetic role of CLDN1 for liver fibrosis,
109 *CLDN1* is the most highly expressed *CLDN* family member in fibrotic liver (fig. S1A) and the
110 only family member significantly upregulated in fibrosis (fig. S1B, $p < 0.0001$).

111 We next investigated *CLDN1* expression in the liver at the single-cell level. Comprehensive
112 expression studies including analysis of single cell (sc) and single nucleus (sn) RNA-seq data (**Fig.**
113 **1C-E**, fig. S2A-D) showed that *CLDN1* was highly expressed by hepatocytes and cholangiocytes
114 ($p < 0.0001$, U-test, respectively) with increasing expression towards liver bipotent progenitor
115 cells(17) (**Fig. 1D**).

116 Within the liver microenvironment, activated hepatic stellate cells (aHSCs)/human liver
117 myofibroblasts (HLMFs) robustly expressed *CLDN1* (**Fig. 1C**, fig. S2C). In contrast, *CDLNI*
118 abundance in liver endothelial cells, monocytes/macrophages and lymphocytes appeared low or
119 absent (**Fig. 1C, E**, fig. S2A-E). *CLDN1* expression in hepatocytes, cholangiocytes, progenitor
120 cells and fibroblasts was confirmed at the protein level by in situ hybridization (ISH),
121 immunohistochemistry (IHC) and double color immunofluorescence (**Fig. 1F-H** and fig. S2E).

122 In the fibrotic liver, scRNA analyses revealed that *CLDN1* was differentially expressed in
123 a subpopulation of diseased hepatocytes at the epithelial-stromal interface (**Fig. 1E-F**) and

124 correlated with grade of de-differentiation towards a progenitor or bile-duct like cell type(16)
125 ($p=3.75 \times 10^{-8}$, fig. S2F). Within the mesenchymal cell compartment in fibrotic liver, *CLDN1* was
126 highly expressed by mesothelial cells ($p<0.0001$, U-test, GSE136103, **Fig. 1E** and S2G) that have
127 previously been described as fibrosis-associated fibroblast progenitor cells(18, 19) (fig. S2H).
128 Taken together, our studies show that *CLDN1* is robustly expressed in hepatocytes, liver progenitor
129 cells, cholangiocytes, mesenchymal cells including aHSCs/HLMFs and mesothelial cells. The up-
130 regulation of *CLDN1* expression in fibrotic liver and its association with disease progression
131 among different etiologies suggests a functional role in liver fibrosis.

132 To study the expression of njCLDN1 protein in human healthy and fibrotic liver, we used
133 our well characterized panel of humanized CLDN1-specific antibodies specifically targeting a
134 conformation-dependent epitope in the EL1 of CLDN1(9-11). Structural modeling revealed that
135 this epitope is only accessible outside tight junctions (**Fig. 1I**). A subsequent genome-wide protein
136 array demonstrated that these antibodies selectively bound CLDN1 without any cross-reactivity
137 (fig. S3). Non-fibrotic or healthy liver tissues showed low or non-detectable njCLDN1 expression.
138 In contrast, njCLDN1 was specifically upregulated in advanced fibrosis compared to total CLDN1
139 detected by an antibody targeting the intracellular CLDN1 C-terminal domain (**Fig. 1J**). Consistent
140 with scRNASeq analyses (**Fig. 1E-F**), CLDN1 upregulation in cirrhosis was associated with
141 epithelial cellular adhesion molecule (EPCAM) expression, corroborating a role of non-junctional
142 CLDN1 for hepatocyte-cholangiocyte plasticity that is frequently observable as ductular
143 reaction(20) (**Fig. 1K**). Immunofluorescence and confocal microscopy of aHSCs/HLMFs isolated
144 from patients with non-alcoholic fatty liver disease (NAFLD) validated the presence of
145 homogenous membranous CLDN1 expression in these stromal cells (fig. S4A-B).

146 We next aimed to elucidate drivers of *CLDN1* upregulation in chronic liver disease. TNF-
147 α -NF κ B signaling is a key pathway upregulated in chronic inflammatory liver tissue, liver
148 fibrogenesis and carcinogenesis(2). Treatment of aHSCs with TNF- α significantly enhanced
149 *CLDN1* expression (**Fig. 1L**, $p < 0.0001$). A similar upregulation was observed in TNF- α -treated
150 primary human hepatocytes, albeit to a lower magnitude (**Fig. 1L**). TNF- α -mediated upregulation
151 was reduced after pharmacological inhibition of NF κ B signaling in both aHSCs and primary
152 human hepatocytes (**Fig. 1L**). To determine whether TNF- α treatment directly induced *CLDN1*
153 expression via recruitment of NF κ B to the *CLDN1* locus, we performed CUT&Tag and ChIP-seq
154 using antibodies against the p65 subunit of NF κ B in LX2 stellate cells with or without 24 hours
155 treatment with TNF- α . Two previously defined ENCODE cis-regulatory elements upstream and
156 downstream of *CLDN1* showed robust TNF- α -dependent p65 recruitment (**Fig. 1M**). These sites,
157 previously identified as bound by NF κ B in the ReMAP2022 data set, were validated by ChIP-seq
158 and CUT&Tag (**Fig. 1M**). These data show that *CLDN1* is a direct target gene of TNF- α -NF κ B
159 pathway. Collectively, these results suggest TNF- α -NF κ B signaling drives *CLDN1* upregulation
160 in chronic inflammatory liver disease.

161

162 **In vivo knockdown of CLDN1 using GalNAc siRNA technology reduces liver fibrosis**

163 We next assessed the effect of in vivo CLDN1 knockdown in a patient-derived human liver
164 chimeric mouse model that expresses human CLDN1 and closely recapitulates key features of
165 clinical liver fibrosis based on *Fah*^{-/-}/*Rag2*^{-/-}/*Il2rg*^{-/-} (*FRG*)-*NOD* mice robustly repopulated
166 with primary human hepatocytes (21). To model NASH-induced advanced fibrosis, we applied a
167 well-established long-term choline-deficient, L-amino acid-defined, high fat diet (CDA-HFD)(22)
168 (**Fig. 2A**). For hepatocyte-specific *in vivo* knockdown of human CLDN1 we designed *CLDN1*

169 specific siRNAs covalently linked to a ligand containing three *N*-acetylgalactosamine (GalNAc)
170 residues(23). After 12 weeks of diet, mice were randomized to 2 groups receiving either siRNA
171 targeting *CLDN1* or control siRNA at 3mg/kg/week for 8 weeks. GalNAc siRNAs reduced
172 *CLDN1* protein expression in Huh7 liver cells and in vivo (fig. S5A, fig. S2B, C). Mice treated
173 with *CLDN1*-targeting GalNAc siRNA showed significantly reduced fibrosis compared to mice
174 treated with GalNAc control siRNA (**Fig. 2B, C**, table S2, p=0.045). Moreover, mice in the *CLDN1*
175 knockdown group developed significantly fewer liver nodules (**Fig. 2B, C**, table S2, p=0.044).
176 Taken together, these data suggest that *CLDN1* plays a functional role in diet-induced liver
177 fibrosis.

178

179 **Targeting non-junctional *CLDN1* reduces fibrosis in a human liver chimeric mouse model**

180 We next evaluated the therapeutic effect of the humanized anti-human *CLDN1* mAb H3L3
181 in the humanized mouse model of NASH-associated liver fibrosis (21). After 16 weeks of CDA-
182 HFD diet, mice were randomized to 2 groups and received a weekly intraperitoneal (i.p.) injection
183 of either the humanized *CLDN1* mAb or an equivalent vehicle control for 8 weeks (**Fig. 2D**) while
184 the diet was continued. Sirius red staining and fibronectin-1 (FN1) staining analysis revealed
185 reduced liver fibrosis in independently performed studies (**Fig. 2E-H**, table S3). The anti-fibrotic
186 effects were independent from the degree of human engraftment as shown by an absent correlation
187 between human albumin (quantifying the humanization extent of the mice(21)) and fibrosis (fig.
188 S5B). Humanized mice treated with *CLDN1* mAb H3L3 showed downregulated hepatic gene
189 expression of fibrosis markers, including tissue metalloproteinase inhibitor 1 (*Timp1*) and collagen
190 type I alpha 1 chain (*Colla1*) (**Fig. 2H**). Last, *CLDN1* mAb-treated mice exhibited strongly

191 reduced plasma concentrations of C-reactive protein (CRP), a secreted inflammatory biomarker
192 (fig. S5C).

193 Macroscopic examination of humanized livers revealed significantly reduced liver nodules
194 in CLDN1 mAb-treated mice in both experiments (**Fig. 2G-H**, fig. S6 and table S3, $p < 0.05$ and
195 $p = 0.009$ respectively in experiments 1 and 2). Detailed histopathology analyses revealed that the
196 expansive nodules consisted of nodular growing mouse hepatocytes, reactive ductules, and human
197 hepatocytes (fig. S6). Improvement of liver disease was associated with a marked effect on the
198 expression of a clinical prognostic liver signature (PLS) predicting liver disease progression,
199 outcome, and survival in patients with chronic liver disease including NASH(25). mAb treatment
200 significantly reverted the poor-prognosis status of the PLS in the livers of humanized mice (fig.
201 S5D).

202

203 **A CLDN1-specific mAb reduces fibrosis and hepatocarcinogenesis in a mouse model of** 204 **NASH fibrosis and HCC**

205 To further validate anti-fibrotic and cancer-preventive effects of targeting CLDN1 in a fully
206 immunocompetent mouse model, we engineered a murinized version of our previously established
207 rat anti-human CLDN1 mAb(9). Despite lower affinity of these antibodies to mouse CLDN1
208 compared to the human one(10), the murinized CLDN1 mAb showed satisfactory target-
209 engagement (fig. S7A-G).

210 In addition to CDA-HFD to induce NASH and fibrosis, we injected one dose of
211 diethylnitrosamine (DEN) to accelerate hepatocarcinogenesis(22). At week 9, the mice were
212 randomized in 2 groups and received a weekly i.p. injection of either the murinized CLDN1 mAb
213 or an equivalent vehicle control for 16 weeks (**Fig. 3A**). Two mice in the control group died during

214 the experiment of unknown causes; no deaths occurred in the CLDN1 mAb-treated mice. Sirius
215 red staining and automated analysis of the collagen proportional area revealed significantly
216 reduced fibrosis in the CLDN1 mAb group with a relative median fibrosis improvement of 28.4%
217 (**Fig. 3B-C**, table S4, p=0.003). Furthermore, CLDN1 mAb-treated mice showed significantly
218 reduced abundance of FN1 (**Fig. 3B-C**, p=0.01) and *Colla1* (**Fig. 3D**, p=0.004), two major
219 components of the fibrotic ECM(2). Reduced expression of *Acta2* and α -smooth muscle actin (α -
220 SMA) in a subset of the treated mice (**Fig. 3D**, fig. S8A-B) suggested reduced prevalence or
221 activity of HLMFs.

222 We observed a significant improvement of liver steatosis and NAFLD activity score in
223 CLDN1 mAb-treated animals (**Fig. 3E**, fig. S8A, respectively p=0.047 and p=0.0098). Similarly,
224 administration of the mAb was accompanied by a significant reduction of ALT concentrations
225 (p=0.033) whereas total bilirubin and alkaline phosphatase concentrations remained unchanged
226 (table S5). Staining for immune cell subpopulations revealed a moderately reduced infiltration of
227 CD4⁺ T cells with no changes in the other main immune cell types in CLDN1 mAb-treated mice
228 (**Fig. 3F** and fig. S8C-D). In situ hybridization in paraffin-embedded tissues of inflammatory
229 cytokines demonstrated a reduction of *Ccl2* (fig. S8E), a cytokine involved in liver inflammation
230 and fibrosis and recruitment of circulating monocytes(24). In contrast, there was no effect on in
231 situ expression of *Ccl20* and *Cxcl10*.

232 Similarly to the humanized mouse model, we observed a robust reversion of the poor
233 prognosis status of the clinical PLS which predicts survival, fibrosis progression and HCC risk in
234 patients with chronic liver disease (fig. S8F). Macroscopic and microscopic examination of mouse
235 livers showed a marked difference in liver tumor development and burden (**Fig. 3G-I**, table S4).
236 Histopathological analyses revealed that the tumor nodules consisted of preneoplastic lesions or

237 well-differentiated HCC (fig. S9), confirming the validity of this model for HCC prevention
238 studies as well as a robust inhibition of hepatocarcinogenesis by the antibody. Extensive safety
239 studies including histopathology of major organs, complete serum chemistry, and renal and liver
240 function tests did not show any detectable adverse effects (fig. S10, table S5).

241

242 **Treatment with CLDN1-specific mAb inhibits fibrosis progression in a mouse model of** 243 **biliary fibrosis**

244 To validate the anti-fibrotic efficacy of the CLDN1 mAb in another mouse model of
245 fibrosis, we assessed the effects of CLDN1 mAb in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine
246 (DDC) model, a widely accepted animal model for biliary fibrosis modeling obstructive cholestasis
247 and fibrosis(27). Seven-week-old C57BL/6J were fed a 0.1% DDC-supplemented diet and after 1
248 week, when peri-biliary fibrosis was established, they were randomized into groups receiving
249 weekly i.p. injection of CLDN1 mAb or vehicle control for 3 weeks (**Fig. 3J**). Sirius red staining
250 and automated analysis of collagen proportional area revealed significantly reduced fibrosis and
251 total FN1 (**Fig. 3L-K**, table S6, respectively $p < 0.0001$ and $p = 0.001$) as well as reduced Ishak score
252 (**Fig. 3M**, table S6).

253

254 **Validation of the profibrogenic role of CLDN1 in patient-derived 3D liver fibrosis and NASH** 255 **models**

256 We next validated the antifibrotic effects of the CLDN1 mAb in patient-derived *ex vivo*
257 models. The 3D ExVivo Human Liver Tissue model (Organovo) mimics distinct features of NASH
258 and fibrosis and allows the assessment of liver disease therapeutics(28) (**Fig. 4A**). In this human
259 NASH model, mAb treatment reduced hepatocyte ballooning and macro- and micro-steatosis in

260 three out of four tissue preparations (**Fig. 4B**). Image-based quantification of the collagen
261 proportional area revealed that median fibrosis in CLDN1 mAb-treated ExVivo tissues was
262 significantly reduced compared to control mAb-treated tissues (2.69% vs. 6.14%, **Fig. 4C**,
263 $p < 0.0001$).

264 We studied effects of CLDN1 on fibrosis in patient-derived human liver spheroids, 3D
265 micro-tissues recapitulating the liver microenvironment(29). Liver tissues from patients with and
266 without chronic liver disease and fibrosis (table S7) were dissociated and cultured in ultra-low
267 attachment plates (**Fig. 4D**). This protocol allows the formation of patient-derived spheroids
268 harboring original liver cell populations (**Fig. 4E**). Treatment with a profibrogenic medium
269 containing transforming growth factor beta (TGF- β), free fatty acids (FFA) and
270 lipopolysaccharides (LPS) induced the expression of *COL1A1*, *COL3A1*, *COL4A1*, and *CTGF*
271 (**Fig. 4F**). Treatment of patient spheroids with CLDN1 mAb reduced the induction of these pro-
272 fibrogenic markers (**Fig. 4F**). Moreover, CLDN1 mAb treatment decreased collagen deposition
273 compared to resmetirom, a NASH compound having reached late-stage clinical development (30)
274 (**Fig. 4G**).

275 Last, we aimed to validate the effect of CLDN1 on the PLS predicting liver disease
276 progression, survival and HCC risk in liver tissues from patients with advanced fibrosis(31). Liver
277 slices of NASH patients with NASH with different stages of fibrosis (table S8) were incubated
278 with CLDN1 mAb or control and analyzed for expression of the clinical PLS (**Fig. 4H**). Treatment
279 with CLDN1 mAb significantly reverted the PLS from poor to good prognosis for samples of
280 patients #1-4 (NASH fibrosis #1-#5, poor prognosis genes: false discovery rate (FDR) = 0.16,
281 0.16, 0.09, 0.17 and 0.28, respectively; good prognosis genes: FDR= 0.50, 0.004, 0.09, 0.20, 0.80,
282 respectively, **Fig. 4I**).

283 **Targeting CLDN1 reverses inflammation-induced perturbation of cell plasticity in**
284 **hepatocytes, progenitor cells, and myofibroblasts**

285 We next aimed to evaluate the molecular mechanism of the antifibrotic effect of the
286 CLDN1-specific mAb. Given the association of CLDN1 expression with cell state, we assessed
287 scRNA-seq-derived cell lineage marker genes in human liver chimeric mice side-by-side with
288 transcriptomic data derived from a clinical cohort of NASH patients with mild or advanced fibrosis
289 (**Fig. 5A**). Gene sets encompassing marker genes of the EPCAM⁺ progenitor compartment were
290 significantly enriched in patients with NASH and advanced fibrosis (Cell cluster maker genes C4,
291 C39, C24, C7: NES=1.99, 2.05, 2.07, 2.19, FDR<0.001), whereas genes characterizing healthy
292 mature hepatocytes were strongly downregulated (C30, C17, C14, C11: NES=-1.39, -1.71, -2.21,
293 -2.23, FDR<0.02) (**Fig. 5B**). Similar results were obtained in fibrotic livers derived from two
294 NASH fibrosis mouse models compared to corresponding regular diet mice (fig. S11A-B).
295 Treatment with CLDN1 mAb considerably reverted the hepatocyte-progenitor cell plasticity in
296 both mouse models (**Fig. 5C**, fig. S11C, D) suggesting that CLDN1-specific mAb treatment
297 reverts the disease-induced immature progenitor phenotype of hepatocytes back to a mature
298 phenotype.

299 We next examined the effect of CLDN1 on cell plasticity of mesenchymal cells in the DEN-
300 CDAHFD mouse model. Expression of marker genes of PDGFRA⁺ scar-associated myofibroblasts
301 (table S9, markers from(16)) was induced both in livers of NASH patients with NASH with
302 advanced fibrosis as well as fibrotic mouse livers (**Fig. 5D**, fig. S11E). CLDN1 mAb treatment
303 significantly suppressed expression of these scar-associated myofibroblast gene signatures in the
304 immunocompetent NASH fibrosis mouse model (FDR<0.001, **Fig. 5E**). Similarly, examining cell
305 circuit modules driving cirrhosis in patients, we found reduced gene expression related to

306 myofibroblast differentiation and ECM production (module 1 and 24) as well as restoration of
307 physiological hepatocyte metabolism (module 9, 22 and 23) (**Fig. 5F**, upper panel) in mice treated
308 with CLDN1-specific mAb.

309 Collectively, our integrative analyses in patient liver tissues and patient-derived mouse
310 models suggest that treatment with CLDN1 mAb reverses fibrosis-associated cell fate and
311 plasticity in hepatocytes and fibroblasts.

312

313 **Targeting CLDN1 reverses perturbation of liver cell circuits and signaling mediating chronic** 314 **inflammation and fibrosis**

315 To unravel the specific cell circuits and signaling pathways involved in liver disease
316 progression in vivo and restored by mAb treatment, we analyzed transcriptional signatures of
317 fibrosis- and carcinogenesis-related signaling in the mouse models (fig. S12A). Fibrotic mouse
318 NASH livers exhibited upregulated fibrosis-associated pathways similar to NASH patients with
319 NASH with advanced fibrosis (**Fig. 5F**). Treatment with CLDN1 mAb reversed the induction of
320 these fibrogenic circuits, with the most pronounced effects on TNF- α -NF κ B signaling (**Fig. 5F**).
321 Similarly, carcinogenesis-associated pathways were upregulated in NASH patients with advanced
322 fibrosis but suppressed by CLDN1 mAb treatment in both animal models (**Fig. 5F**). Similar results
323 were obtained by unbiased GSEA of hallmark gene sets in both mouse models (fig. S12B). The
324 suppression of key target pathways such as NF κ B (RELA and RELB) and MAPK (p-p38) was
325 confirmed by immunohistochemistry in the DEN-CDAHFD NASH fibrosis mouse model (**Fig.**
326 **5G**, fig. S12C-E).

327

328 **CLDN1-specific mAb inhibits pro-fibrotic signaling and modulates cell-matrix interaction**
329 **and plasticity in hepatocytes**

330 Next, we investigated the specific effects of CLDN1 mAb on signaling and plasticity in the
331 cell types identified in the expression and binding studies. Using stable CLDN1 knockout (KO)
332 and pharmacological intervention, we found that CLDN1 is a driver of the poor prognosis status
333 of the PLS in cell-based models(25) (fig. S13A-C). Reversal of the poor prognosis status was
334 CLDN1 mAb dose-dependent (fig. S13C). RNA-seq and GSEA further confirmed mAb-mediated
335 suppression of hepatocyte-progenitor cell plasticity (fig. S13D) as well as pro-fibrogenic and
336 carcinogenic signaling (fig. S13E) in cell culture models of both viral and metabolic liver disease.

337 To study the molecular events after CLDN1 mAb target engagement, we identified the
338 molecular partners of CLDN1 at the membrane of epithelial cells using Huh7 cells with high
339 CLDN1 expression as a tractable model for immature liver epithelial cells. Co-
340 immunoprecipitation of CLDN1 identified more than 300 proteins as potential CLDN1 interactants
341 (data file S2). String analysis with clustering revealed 3 clusters of proteins interacting with
342 CLDN1 (**Fig. 6A**): cell adhesion proteins, integrins and ECM proteins as well as proteins related
343 to cell proliferation and fate. Epithelial growth factor receptor (EGFR), EPCAM, the ECM
344 receptor integrin alpha 5 (ITGA5), and ECM component laminin 5 (LAMA5) were confirmed as
345 molecular partners by Western blot analyses (**Fig. 6B**). Next, we investigated the consequences of
346 these cell membrane molecular interactions on pro-fibrotic and pro-carcinogenic signaling
347 mediated by these pathways. CLDN1 mAb treatment suppressed EGFR and extracellular signal-
348 regulated kinases (ERK) phosphorylation (**Fig. 6C-D**) and inhibited SRC proto-oncogene, non-
349 receptor tyrosine kinase (SRC) signaling, a key downstream pathway of cell-ECM
350 mechanoreceptors(32). Plasma membrane isolation showed a decrease of SRC recruitment at the

351 cell membrane after treatment (**Fig. 6E**). Moreover, CLDN1 mAb inhibited SRC activation by
352 phosphorylation in a 3D Huh7 spheroid model (**Fig. 6F**). Unbiased proteomic assessment of
353 signaling in a Huh7-LX2 co-culture model using phospho-specific antibody capture arrays
354 validated CLDN1 mAb-induced suppression of SRC family and MAPK kinase (p38) activation
355 and revealed downregulation of other known profibrotic and pro-carcinogenic downstream
356 effectors including CREB5 and TOR(33)(**Fig. 6G**). The effects of CLDN1 mAb on EGFR-MAPK
357 and ITGA5-SRC signaling were further validated in precision cut liver slices (PCLS) of patient-
358 derived tissues (fig. S14A) as well as in patient-derived multicellular spheroids exposed to
359 profibrogenic injury (**Fig. 6H**). Besides SRC and EGFR, we detected reduced kinase activity of
360 other kinases known to mediate fibrosis progression, such as IGF1R and IKK-E as well as reduced
361 activity of kinases involved in carcinogenesis, including ARG, ABL, AKT1/2, and HER2 (**Fig.**
362 **6H**). A comparative analysis with a liver fibrosis kinome atlas suggests that CLDN1 mAb
363 treatment in patient spheroids reverts the profibrogenic signaling cascade identified in patients
364 with advanced liver fibrosis (fig. S14B)(34).

365 Collectively, our data indicate that CLDN1 interacts with EPCAM, EGFR, ITGA5,
366 laminin, and other proteins at the cell membrane of liver epithelial cells. Target engagement of
367 CLDN1 mAb with cell surface CLDN1 inhibits downstream profibrogenic and pro-carcinogenic
368 signaling mediating cell plasticity, fibrogenesis, and carcinogenesis (fig. S14C).

369

370 **Treatment with CLDN1-specific mAb modulates liver progenitor cell phenotype and** 371 **plasticity**

372 Given the high abundance of CLDN1 expression in liver progenitor cells and
373 cholangiocytes, effects on liver cell plasticity might also be mediated via direct effects of CLDN1

374 mAb on ductal cells which play a role in fibrotic liver disease(35). The “ductular reaction”, a
375 regenerative cholangiocyte and liver progenitor cell proliferation, has been reported as a potential
376 anti-fibrotic target(35). We generated intrahepatic bile duct-derived organoids from human
377 cirrhotic liver tissue and treated these cells with CLDN1 mAb or control mAb for 4 days. These
378 organoids showed high expression of CLDN1 and progenitor markers (fig. S15A). CLDN1 mAb
379 treatment modulated the phenotype of the organoids with an altered morphology, such as thinner
380 walls and cystic deformation (**Fig. 7A, left panel**). Computational analysis of the organoid size
381 further confirmed functional effects on organoid growth (**Fig. 7A, right panel**). Transcriptomic
382 profiling of organoids by RNA-seq revealed pronounced effects of the antibody on liver organoid
383 gene expression (**Fig. 7B**). Computational assessment indicated enrichment of upregulated genes
384 into developmental pathways and downregulated genes into proliferative biological processes
385 (**Fig. 7C**, fig. S15B-C) suggesting functional effects on cell proliferation and plasticity of CLDN1
386 mAb on progenitor cells and cholangiocytes. Validation of significant downregulation of the
387 expression of liver progenitor cell marker TACSTD2/TROP2(*15*) in mAb-treated organoids
388 confirmed the functional effect of treatment on cell plasticity (p=0.04, **Fig. 7D**).

389

390 **CLDN1-specific mAb inhibits profibrogenic differentiation in aHSCs by interfering with** 391 **TNF- α -NF κ B signaling**

392 We evaluated direct effects of CLDN1 mAb on mesenchymal cells by RNA-seq analysis
393 of CLDN1 mAb-treated patient-derived aHSCs/HLMFs (**Fig. 7E**). Marker genes of scar-
394 associated myofibroblasts type A (table S10), the major phenotype of fibrotic
395 myofibroblasts(*16*), were significantly inhibited in CLDN1-mAb treated HLMFs (FDR<0.08, **Fig.**
396 **7E**). Moreover, CLDN1 mAb strongly suppressed TNF- α -NF κ B signaling in HLMFs as validated

397 by Western Blot analyses of the TNF- α canonical pathway (**Fig. 7F-H**). Assessment of TGF- β
398 signaling did not reveal major effects of the antibody on the TGF- β canonical SMAD2/3 signaling
399 pathway, but showed inhibition of TGF- β induced non-canonical pathways, such as pAKT, p-P38
400 and pERK signaling (**Fig. 7H, I**). Last, we confirmed direct downstream effects on myofibroblast
401 effector functions. Indeed, CLDN1 mAb treatment of HLMFs from different donors (table S12)
402 markedly inhibited key activation markers, including *ACTA2*, *COL1A1* and *FNI* (**Fig. 7J**).

403

404 **CLDN1 is a candidate target for treatment of lung and kidney fibrosis**

405 The mechanistic role of CLDN1 during fibrosis is not necessarily limited to the liver.
406 Several studies have suggested a role of CLDN1 in the pathogenesis of chronic kidney disease(37).
407 Upregulation of *CLDN1* expression in patients with glomerulonephritis as well as murine fibrotic
408 kidneys(38) (**Fig. 8A**) suggests the involvement of *CLDN1* in the pathogenesis of renal fibrotic
409 disease. Furthermore, *CLDN1* was significantly overexpressed in patients with idiopathic
410 pulmonary fibrosis (IPF) with increasing expression along disease progression (p=0.03, **Fig. 8B**).

411 To investigate the role of CLDN1 as a therapeutic target in other organs, we used two state-
412 of-the-art mouse models for kidney and lung fibrosis (**Fig. 8C**). Treatment with the murinized
413 CLDN1-specific mAb (fig. S2) resulted in robust anti-fibrotic effects in the unilateral ureteral
414 obstruction (UUO) mouse model of kidney fibrosis(40) (**Fig. 8D-E**, table S13). Moreover,
415 histological assessment of mouse kidneys revealed suppression of macrophage infiltration by
416 CLDN1 mAb (**Fig. 8F**).

417 We studied the effects of CLDN1 mAb in a bleomycin-induced pulmonary fibrosis mouse
418 model and compared to dexamethasone, an off-label used drug with protective effects in patients

419 with lung fibrosis(3) (**Fig. 8C**). Treatment with CLDN1 mAb suppressed lung fibrosis in these
420 animals (**Fig. 8G-H**, table S14). Similar to the liver, CLDN1 was expressed and regulated via
421 TNF- α -NF κ B signaling in both lung (**Fig. 8I-J**) and kidney fibroblasts (**Fig. 8J**). In line with the
422 role of CLDN1 in liver cell fate and differentiation, treatment of IPF patient-derived
423 myofibroblasts resulted in reversal of pro-fibrogenic lung fibroblast differentiation states(41)
424 (**Fig. 8K**). CLDN1 mAb strongly suppressed expression of marker genes of ACTA2⁺
425 myofibroblasts, PLIN2⁺ lipomyofibroblasts, and HAS1^{hi} fibroblasts (table S15-17). As observed
426 in HLMFs, CLDN1 mAb-treatment suppressed TNF α -NF κ B signaling in primary lung fibroblasts
427 (**Fig. 8L**). These findings suggest CLDN1 as a candidate target for kidney and lung fibrosis which
428 warrants further investigation.

429

430 **ALE.F02, an CLDN1 therapeutic candidate antibody for treating human fibrotic diseases, is**
431 **safe in cynomolgus monkeys**

432 To ensure the safety of CLDN1 mAbs in a species with full human target homology and
433 equivalent antibody affinity, we expanded toxicity studies to non-human primates (table S18). As
434 a candidate for future human therapeutic applications, we chose a fully humanized variant which
435 we designated ALE.F02. Differently from H3L3, the Fc region of the ALE.F02 molecule contains
436 three mutations (L234F, L235E, and P331S) which have been introduced to reduce binding to Fc
437 gamma receptors. In cynomolgus monkeys (*M. fascicularis*), the sequence of human CLDN1 and
438 the antibody's binding epitope is 100% conserved. A rapid escalation protocol achieved safe,
439 multiple weekly dosing up to the highest tested dose of 150 mg/kg. No major clinical changes were
440 observed. Notably, there was no indication of NISCH syndrome in the animals, a condition caused
441 by genetic CLDN1 deficiency in humans. These studies confirmed that CLDN1-targeting therapies

442 are safe *in vivo* and that ALE.F02 did not affect the integrity or barrier function of tight junctions.
443 Serum analysis indicated a dose-dependent, sustainable and effective antibody concentration in
444 macaques (fig. S16A). Simulations using the macaque data predicted that PK profiles in humans
445 with a single dose of ~3-10 mg/kg ALE-F02 would saturate CLDN1 for about 2 weeks (fig. S16B).
446
447

448 **DISCUSSION**

449 In this study we uncovered CLDN1 as a mediator and therapeutic target for tissue fibrosis.
450 Using the liver as a model of chronic inflammation-associated fibrogenesis and carcinogenesis we
451 show that targeting CLDN1 by highly specific mAbs robustly reduced fibrosis and tumor
452 development in mouse models for NASH and biliary fibrosis; strongly reduces liver fibrosis in *ex*
453 *vivo* patient-derived model; and reversed transcriptomic liver disease signatures predictive of liver
454 fibrosis progression and HCC risk. A key strength of our study is its focus on authentic patient-
455 derived model systems, the consistency of results across complementary model systems, different
456 organs and patient cohorts supporting its validity and translatability into the clinic.

457 Our comprehensive analyses provide the following model for the molecular mechanism of
458 CLDN1 mAb mediated anti-fibrotic effects: hepatocytes and their progenitors are the primary
459 target cells of CLDN1 mAb antifibrotic effect. Our detailed gene expression analyses *in vivo* and
460 in patients revealed that mAb treatment restores the hepatocyte fate by suppressing hepatocyte de-
461 differentiation following epithelial liver injury. Target engagement at the hepatocyte cell
462 membrane interferes with the interaction of CLDN1 with EPCAM, EGFR, ITGA5 as well as the
463 ECM, resulting in inhibition of downstream signaling of well-established pro-fibrotic and pro-
464 carcinogenic signaling pathways across organs, such as SRC and MAPK signaling.

465 The second cell type mediating the anti-fibrotic properties of the mAb are likely
466 aHSCs/HLMFs which express CLDN1 in patients. CLDN1 mAb inhibits TNF- α -NF κ B as well
467 as non-canonical TGF- β signaling resulting in inhibition of myofibroblast activation and
468 differentiation across organs. Cross-talk between liver epithelial cells and fibroblasts modulate the
469 ECM with decreased collagen and fibronectin production. The reprogramming of hepatocytes and
470 its microenvironment ultimately results in the attenuation of tissue fibrosis.

471 Our study has some limitations: First, we can not exclude that other signaling pathways or
472 additional mechanistic events are at play mediating the anti-fibrotic effect of the antibodies.
473 Second, while we used a large panel of complementary model systems including patient-derived
474 models, any model system only partially recapitulates pathogenesis of fibrosis in patients.

475 The large majority of liver disease therapeutics target metabolism, inflammation or cell
476 death, which are relevant in the early stage of disease(42, 43). A key differentiator of CLDN1-
477 specific mAb is the combination of robust anti-fibrotic and HCC preventive effect, which
478 addresses the key unmet medical need in advanced liver fibrosis.

479 Our study also provides the opportunity to develop companion biomarkers such as TIMP1 ,
480 a clinically applied read-out for liver fibrosis as part of the ELF score, or CTGF, a biomarker
481 candidate for fibrosis across organs(44, 45). Furthermore, the potent effect on the PLS may enable
482 to stratify patients at high risk for HCC and the highest need for treatment.

483 Our data demonstrate that the administration of the antibody is safe without detectable
484 adverse and off-target effects. Safety studies in non-human primates demonstrate that even
485 repeated high dose administration does not induce any major adverse effects and support further
486 clinical development in humans.

487 Beyond the liver, our *in vivo* data suggest that CLDN1 is also a candidate target for kidney
488 and lung fibrosis(3, 4). Our functional studies suggest common mechanisms across organs as
489 demonstrated by similar inhibition profiles of lung fibroblast differentiation by CLDN1 mAb via
490 interference with TNF- α -NF κ B signaling. However, given the expression of CLDN1 in organ-
491 specific cell types in the kidney(37) or lung(46), it is likely that also additional organ-specific
492 mechanisms are at play.

493 Collectively, the development of CLDN1-specific mAb provides an opportunity for the
494 clinical development of a first-in-class compound for treatment of organ fibrosis, a major and
495 rapidly growing unmet medical need world-wide.

496

497 **MATERIAL AND METHODS**

498 ***Study design.*** Computational transcriptomic analyses were conducted in publicly available
499 and own patient cohorts of chronic liver disease. Overall and cell-type specific target expression
500 was characterized by immunohistochemistry, immunofluorescence and in situ hybridization of
501 multiple non-fibrotic and fibrotic tissues of different patient cohorts. *In vivo* genetic knockdown
502 studies using siRNA were performed to validate CLDN1 as a driver of liver fibrosis. NjCLDN1
503 targeted by highly specific mAbs was evaluated as a target to treat fibrosis in a large set of *in vivo*
504 and *ex vivo* models. The molecular mechanism of action was studied by co-immunoprecipitation
505 studies, transcriptomic and proteomic analyses in cell-based models of chronic liver disease,
506 including patient-derived 3D *ex vivo* models, such as spheroids and liver organoids. Finally, in
507 preparation for clinical translation, the pharmacological and safety properties of a humanized
508 CLDN1 antibody was validated in non-human primates. Experiments were partially blinded and
509 performed in triplicates in at least three independent experiments, unless otherwise stated. Patient
510 tissues for *ex vivo* and *in vitro* studies were randomly assigned. Additional methods are available
511 in the supplementary materials.

512 ***Human subjects and patient cohorts.*** For transcriptomic analyses and *ex vivo* perturbation
513 studies human liver tissue samples were obtained from patients who had undergone liver resections
514 for HCC, colorectal cancer metastasis, or cholangiocellular carcinoma between 2014 and 2022 at
515 Strasbourg University Hospitals, France (DC-2016-2616 and RIPH2 LivMod IDRCB 2019-
516 A00738-49, ClinicalTrial NCT04690972). All patients provided written informed consent and the
517 protocol followed the ethical principles of the declaration of Helsinki and was approved by the
518 local independent ethics committees. For CLDN1 expression analysis, liver tissues were obtained
519 from local biobanks of the Department of Imaging & Pathology, KU Leuven, the University

520 Hospital Geneva, Pathology at Hvidovre University Hospital, Denmark or Indivumed, Hamburg.
521 Demographic data and clinical characteristics of patients enrolled are summarized in table S1-S2
522 and S5-S6, respectively. Datasets of clinical cohorts with chronic liver disease (GSE34798,
523 GSE83148, GSE49541), chronic kidney disease (GSE11585 and GSE60685), IPF (GSE2052,
524 GSE53845, GSE24206) were selected following comprehensive database analysis, where we
525 identified CLDN1 as part of the microarray data. GSE34798, GSE83148, GSE49541, GSE34798,
526 GSE115857, GSE53845 and GSE24206 were analysed using *shinyGEO*(47) with expression
527 values shown as log₂ expression. All other microarray data were analysed using signal intensity
528 values. Liver scRNA-seq data (GSE124395 and GSE136103) and snRNA-seq data (GSE185477)
529 were obtained as Seurat objects and investigated.

530 ***Bioinformatic and statistical analyses.*** Human RNA-seq data was mapped using HISAT2
531 to the human genome hg19. Mouse RNA-seq data was mapped to the mouse genome mm10 and
532 annotated using the Gencode vM15 gene annotation. Data from humanized mice were mapped
533 similarly, but to an artificial genome consisting of all human (hg19) and mouse (mm10)
534 chromosomes, and only reads mapping to human chromosomes were kept for further analysis as
535 described(48). Reads were counted with htseq-count, and a differentially expression analysis was
536 performed with DESeq2 applying GENCODE 19. Gene Set Enrichment Analysis (GSEA) was
537 used for unbiased pathway analysis using Molecular Signature Database (MSigDB)(49). Unbiased
538 assessment of HALLMARK(49), gene ontology and curated gene sets(49) were used for primary
539 screening of clinically relevant signaling pathways and cell circuits in NASH liver tissue
540 microarray data (GSE49541), that were then subsequently analyzed in RNA-seq data of our NASH
541 mouse models. HALLMARK pathways were further analysed in an unbiased way in RNA-seq
542 data from two NASH mouse models. GSEA of hepatocyte and EPCAM⁺ bile duct cell signature

543 gene sets (C8, MSigDB, derived from scRNA-seq studies in human liver(15)) were used for
544 evaluation of hepatocyte cell fate. Results from GSEA were adjusted for the false discovery rate
545 (FDR). FDR<0.25 or FDR<0.05 was considered statistically significant. All other data was
546 compared using Student's t-test when normally distributed (Shapiro-Wilk test) or non-parametric
547 tests (U-test, Kruskal-Wallis test) when non-normally distributed. Functional results in patient-
548 derived cells were compared using Wilcoxon matched paired test. P <0.05 and FDR<0.25 were
549 considered statistically significant.

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593

594

595 **AUTHOR CONTRIBUTIONS**

596 T.F.B. initiated and coordinated the study. H.E.S., N.R., A.S., E.C., L.M., C.T, V.G.M.,
597 M.A.O., S.C., F.H.T.D., Z.N., N.A., C.P., L.H., S.C.D, M.H., A.L., F.D.Z. C.S., J.L., M.B.Z. and
598 T.F.B. designed or performed experiments and analyzed data. A.S., D.H. and S.C.D. analyzed
599 histopathology of mouse liver tissue. G.T. designed CLDN1 expression analyses and functional
600 studies. Z.N., A.L., F.J. and I.D. performed or analyzed epigenetic experiments. S.M., T.O., T.R.,
601 M.V., C.D.V., E.D.G. and B.M.V performed CLDN1 expression analyses by IHC, IF or ISH in
602 human or mouse liver tissue. Y.H. analyzed gene expression of the prognostic liver signature in
603 part of the cell lines. F.J., H.E.S. and N.R. performed computational analyses, E.F. and P.P.
604 prepared liver resections for *ex vivo* models. B.C.F., M.H. and A.T. co-designed animal
605 experiments and edited the manuscript. A.C. and J.S. performed structural modeling of CLDN1
606 mAb binding to CLDN1. G.E., M.M., R.I., T.S. designed and conducted the experiments in non-
607 human primates. G.T. helped generate figures. M.B.Z, C.S., J.L. and O.K. edited the manuscript.
608 N.R., H.E.S., E.C., A.S. and T.F.B. designed the figures and wrote the manuscript.

609

610 **COMPETING INTERESTS**

611 Inserm, the University of Strasbourg, the Strasbourg University Hospitals and the Institut
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625 Transfert for the authors of the University of Strasbourg and Inserm.

626

627 **DATA AND MATERIALS AVAILIBILITY**

628 All data associated with this study are available in the main text or the supplementary
629 materials. Raw data from figures are in data file S1. Transcriptomic data reported in this paper
630 have been deposited at the Gene Expression Omnibus database (GEO) with accession numbers
631 GSE174542, GSE174543, and GSE206581. FRG mice are available under a material transfer
632 agreement with Yecuris. Recombinant HCV strains and Huh7.5 cell lines for infection studies are
633 available under a material transfer agreement with Apath LLC.

634

635 **SUPPLEMENTARY MATERIAL**

636 • **Materials and Methods**

637 • **Figure S1-S19**

638 • **Table S1-S18**

639 • **Data File S1-S2**

640 • **References (51-91)**

641

642

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984 **FIGURE LEGENDS**

985 **Figure 1. CLDN1 expression is upregulated in chronic liver disease. A.** *CLDN1*
986 upregulation in liver tissues of patients with chronic HCV, HBV infection, or NASH. **B.** *CLDN1*
987 expression in livers of patients with NASH with mild (F0-1) or advanced fibrosis (F3-4) and liver
988 tissues of transplanted HCV-infected patients with stable or progressive fibrotic disease. **C.**
989 *CLDN1* expression in healthy liver at the single-nucleus level. ($p < 0.0001$, U-test). **D.** *CLDN1*
990 expression along the cholangiocyte-bipotent progenitor cells-hepatocyte pseudotime trajectory by
991 Slingshot in a combined scRNA-seq and snRNA-seq dataset(50). **E.** *CLDN1* expression at the
992 single-cell level in human cirrhotic and healthy liver cells. Differential expression analysis
993 identified *CLDN1* as a marker of mesothelial cells, hepatocytes and cholangiocytes ($p < 0.0001$, U-
994 test, respectively). **F.** Enhanced *CLDN1* expression in hepatocytes at the stromal-epithelial
995 interface of HCV cirrhotic nodule. Scale bars=1 mm. **G.** RNA ISH showing *CLDN1* expression in
996 a subpopulation of *COL3A1*⁺ stellate cells in NASH-associated liver cirrhosis. Scale bars=50 μ m
997 (low resolution) and 20 μ m (high resolution image). **H.** Staining of *CLDN1* with α SMA by
998 immunofluorescence and CK19 and EPCAM by immunohistochemistry showing *CLDN1*
999 expression in cholangiocytes, EPCAM⁺ bile duct cells and stellate cells. Scale bars=50 μ m. **I.**
1000 Upper panel: Computationally predicted structural model of the non-junctional *CLDN1*/*CLDN1*
1001 mAb complex. Lower panel: Absent accessibility of the epitope targeted by the *CLDN1* mAb in
1002 tight junctions. **J.** Expression of total *CLDN1* and nj*CLDN1* in mild (F0-2, $n=8$) or advanced liver
1003 fibrosis (F3-4, $n=10$) using an antibody targeting the C-terminal domain of *CLDN1* (total *CLDN1*)
1004 or mAb ALE.F02 targeting nj*CLDN1*. **K.** Co-staining of *CLDN1* and EPCAM in ductular
1005 reactions. Scale bars=65 μ m (F2) and 100 μ m (F4). **L.** Regulation of nj*CLDN1* expression by TNF-

1006 α . HLMFs and primary human hepatocytes (PHHs) were treated with TNF- α , IKK-16 or TNF- α
1007 + IKK16 and CLDN1 expression analyzed by flow cytometry using anti-CLDN1 mAb H3L3 ($n=3$
1008 independent experiments with 2-4 replicates per condition). Δ MFI is shown as fold change
1009 compared to untreated cells. **M.** The *CLDN1* locus with the indicated CUT&Tag and ChIP-seq
1010 tracks. Red boxes identify p65-bound sites that coincide with Encode-defined cis-Regulatory
1011 Elements (cCREs) both upstream and downstream of *CLDN1* with the density of previously
1012 identified ChIP-seq RELA binding sites (peak regions) as listed in ReMAP2022. **** $p<0.0001$,
1013 *** $p<0.001$, ** $p<0.01$, * $p<0.05$ U-test (**A-B, J**), U-test with Bonferroni correction (C, E), and
1014 Student's t-test (**L**). MFI=Mean fluorescence intensity; MP=mononuclear phagocyte.
1015

1016 **Figure 2. Targeting CLDN1 by siRNA or mAbs reduces liver fibrosis in a patient-**
1017 **derived mouse model. A-C** *CLDN1* knockdown by GalNAc-siRNA reduces liver fibrosis in
1018 humanized liver fibrosis mouse model. **A.** Illustration of the experimental approach. **B.** CLDN1
1019 expression, fibrosis assessment in humanized areas, and tumor nodule count according to the
1020 treatment group (siCTRL *n*=6, siCLDN1 *n*=5). **C.** Representative images of human CLDN1
1021 staining, Sirius red, fibronectin-1 (FN1) immunostaining and macroscopy of GalNAc siCLDN1
1022 and siCTRL mice. Scale bar=50µm, 250µm, 50µm respectively. **D-H.** CLDN1 mAb treatment
1023 reduced liver fibrosis and HCC. **D.** Study protocol of humanized mouse NASH model treated with
1024 CLDN1 mAb. **E-F.** Fibrosis assessment in the total liver tissue and humanized areas, and tumor
1025 nodule count of experiment 1 (**E**, Control *n*=3, CLDN1 mAb *n*=4), and experiment 2 (**F**, Control
1026 *n*=10, CLDN1 mAb *n*=10) which were performed independently. Sample size in FN1 experiment
1027 2 (Control *n*=8, CLDN1 mAb *n*=7). Control group was treated with i.p. injections of vehicle. **G.**
1028 Representative images of FAH, Sirius red, FN1 staining and macroscopic images according to
1029 treatment groups (experiment 1). Scale bars=250µm, 250µm, 50µm, respectively. **H.** Gene
1030 expression of fibrosis markers *Colla1* and *Timp1* (experiment 1). Bars show mean ±SEM.
1031 **p*<0.05, ***p*<0.01, U-test (**B**, **E**, **F**), t-test (**H**), respectively. FAH=fumarylacetoacetate hydrolase;
1032 hCLDN1= human CLDN1.
1033

1034 **Figure 3. Targeting non-junctional CLDN1 reduces fibrosis and tumor development**
1035 **in mouse models for liver and biliary fibrosis. A.** Study protocol of DEN-CDA-HFD NASH
1036 fibrosis mouse model and mAb treatment. **B.** Representative images of Sirius Red and fibronectin-
1037 1 (FN1) staining in mouse livers. Scale bars=250µm and 50µm, respectively. **C.** Collagen
1038 proportional area (CPA) (Control *n*=18, CLDN1 mAb *n*=20) and FN1 quantification (Control
1039 *n*=15, CLDN1 mAb *n*=16) in representative areas according to the treatment group. **D.** Gene
1040 expression of fibrosis markers *Coll1a1* (Control *n*=5, CLDN1 mAb *n*=5) and *Acta2* (Control *n*=4,
1041 CLDN1 mAb *n*=5). **E.** Liver fat proportional area and NAFLD activity score according to the
1042 assigned treatment (Control *n*=18, CLDN1 mAb *n*=20). **F.** Quantification of liver immunostaining
1043 of CD3⁺ T cells, and CD68⁺ macrophages (Control *n*=15, CLDN1 mAb *n*=16). **G.** Representative
1044 images of macroscopy and HSP70⁺ areas in mouse livers. Scale bars=500µm. **H.** Macroscopic (left
1045 panel) and histological (right panel) assessment of tumor occurrence in mouse livers (Control
1046 *n*=18, CLDN1 mAb *n*=20). **I.** Number and size of tumor nodules and proportion of HSP70⁺ tumors
1047 in mice livers (Control *n*=18, CLDN1 mAb *n*=20). **J.** Study protocol of DDC mouse model for
1048 biliary fibrosis. **K.** Sirius red and FN1 staining in CLDN1 mAb- or Control-treated DDC mice.
1049 Representative images are shown. **L.** Quantification of CPA and FN1-positive area in all mice. **M.**
1050 Ishak score in CLDN1 mAb- or control-treated DDC mice. All control groups were treated with
1051 i.p. injections of vehicle. *****p*<0.0001, t-test. Bars show mean ±SEM. **p*<0.05, ***p*<0.01,
1052 ****p*<0.001, *****p*<0.0001, U-test, respectively. H&E=Haemotoxylin and Eosin; HSP70=Heat-
1053 shock protein 70; SEM=standard error of the mean.

1054

1055 **Figure 4. CLDN1-specific mAb effects in patient-derived *ex vivo* models of chronic**
1056 **liver disease. A.** Illustration of Organovo ExVive fibrosis model. **B.** Images of Trichromic Masson
1057 and H&E staining in Organovo ExVive tissues sections treated with CLDN1 mAb or control mAb
1058 ($n=4$ biological and $N=8$ technical replicates per condition). Macrovascular steatosis is indicated
1059 by green and microvascular steatosis by red arrows. Scale bars= $40\mu\text{m}$. **C.** Quantification of
1060 collagen proportional area in Organovo ExVive tissue. **D.** Illustration of patient-derived liver
1061 spheroids. **E.** Immunostaining of ASPGR1, CD31, CD68 and α -SMA in patient-derived liver
1062 spheroids. Staining with anti-mouse secondary antibodies were used as a control. Spheroids were
1063 visualized by Celigo imaging cytometer. Scale bar= $500\mu\text{m}$. **F.** Gene expression of *COL1A1*,
1064 *CTGF* ($n=3$ independent experiments with $n=4$ biological replicates per condition) in liver
1065 spheroids exposed to a TGF- β , FFA and LPS and treated with either CLDN1 mAb or control mAb.
1066 **G.** Total collagen deposition in patient-derived liver spheroids stimulated with FFA, LPS, and
1067 TGF- β and treated with CLDN1 mAb, control mAb or resmetirom ($n=8$ different donors with 1-3
1068 replicates per condition). **H.** Modulation of PLS to good (green) or poor (orange) prognosis status
1069 (51) in precision cut liver slices. Significance (FDR, Kolmogorov-Smirnov test) of induction (red)
1070 or suppression (blue) of PLS poor- or good-prognosis genes is shown. Bars show mean \pm SEM.
1071 * $p<0.05$, **** $p<0.0001$, t-test (C), U-test (F, G). ECs=Endothelial cells; H&E=Haemotoxylin and
1072 Eosin; HCs=Hepatocytes; HSCs=Hepatic stellate cells; KCs=Kupffer cells; SEM=standard error
1073 of the mean.
1074

1075 **Figure 5. Treatment with CLDN1-specific mAb suppresses liver cell circuits**
1076 **mediating inflammation, fibrosis, and carcinogenesis.** A. Graphical illustration of
1077 methodological approach to assess liver fibrosis associated cell plasticity in bulk RNA-seq data
1078 derived from two fibrosis mouse models. B. Modulation of gene sets characterizing mature
1079 hepatocytes and immature progenitor cells in patients with NASH with mild or advanced fibrosis.
1080 C. Effect of CLDN1 mAb on liver progenitor and mature hepatocyte marker gene sets in
1081 humanized NASH fibrosis mice. D. Differential expression of a gene set characterizing scar-
1082 associated myofibroblasts(*I6*) in NASH patients with mild compared to advanced fibrosis. E.
1083 Effect of CLDN1 mAb on expression of scar-associated myofibroblast marker genes in the regular
1084 NASH fibrosis mouse model. F. Modulation of fibrogenic and carcinogenic signaling pathways
1085 and human cirrhosis gene modules in NASH patients with NASH with mild or advanced fibrosis,
1086 humanized NASH fibrosis mice treated with CLDN1 mAb or control and regular NASH fibrosis
1087 mice treated with CLDN1 mAb or control. Heatmaps illustrate NES of altered gene sets (all
1088 FDR<0.25 except for induction of fibrogenic, KRas signaling and cirrhosis modules #1, #7, #19,
1089 #24 and #23 in humanized mice control tissues and reversal of E2F targets, TGF- β signaling and
1090 cirrhosis modules #1, #7 and #24 in CLDN1 mAb treated NASH fibrosis mice, FDR>0.25). G.
1091 RELA, RELB, and p-P38 positive cells were assessed by immunohistochemistry in DEN-
1092 CDAHFD mice treated with CLDN1 mAb ($n= 16$ animals per staining) or control ($n=15$ animals
1093 per staining). For the RELB analysis, in case of multiple liver specimen from the same mouse, the
1094 maximum value per mouse was considered except in case of >5-fold difference between the
1095 minimum and maximum value where the mean value was used (U-Test). Vertical bars show mean
1096 \pm SEM and single data points. Horizontal bars indicate NES of significantly (FDR<0.25) altered
1097 gene sets. * $p<0.05$, U-test.

1099 **Figure 6. Targeting CLDN1 inhibits pro-fibrotic signaling mediating cell-matrix**
1100 **interaction and plasticity in hepatocytes. A.** Co-immunoprecipitation followed by mass-
1101 spectrometry identified CLDN1 interactants. String analysis with MCL clustering is shown. **B.**
1102 Validation of the main CLDN1 interactants in Huh7 cells by Western Blot analysis. Negative
1103 control is the hepatocyte marker ASGR1. **C.** CLDN1 mAb treatment decreases EGFR
1104 phosphorylation in Huh7 cells (representative results of $n=3$ independent experiments are shown).
1105 **D.** CLDN1 mAb inhibits ERK phosphorylation in Huh7 cells (representative results of $n=4$
1106 independent experiments are shown). **E.** CLDN1 decreases SRC recruitment at the membrane in
1107 Huh7 cells (representative results of $n=2$ independent experiments with $n=2$ biological replicates
1108 are shown). **F.** CLDN1 mAb inhibits SRC phosphorylation in Huh7 spheroids (representative
1109 results of $n=4$ independent experiments are shown). **G.** Effect of CLDN1 mAb on phosphokinase
1110 signaling in the patient-derived NASH liver spheroid model shown in Fig. 4. **H.** Kinome assay of
1111 CLDN1 mAb-treated vs. Control mAb-treated patient-derived spheroids. B-F shows
1112 representative results out of 3 independent experiments. Full-length Western blots are shown in
1113 fig. S16. ASGR1: Asialoglycoprotein Receptor 1; CTRL=Control; FT=Flowthrough;
1114 MCL=Markov clustering.
1115

1116 **Figure 7. CLDN1 mAb modulates liver progenitor cell phenotype and inhibits**
1117 **profibrogenic differentiation of activated stellate cells. A.** Left panel: Representative images of
1118 human cirrhosis-derived primary liver organoids after 4 days of treatment with CLDN1 or Control
1119 mAb. Scale bars=10 μ m. Right panel: Size (measured as organoid area) of CLDN1 mAb-treated
1120 organoids is shown as fold change compared to mean organoid size in Control mAb-treated
1121 organoids (2 independent experiments with $n=3$ and $n=4$ replicates per condition respectively,
1122 $p=0.03$, U-test). **B.** Differentially expressed genes in CLDN1 mAb vs. Control mAb-treated liver
1123 organoids. **C.** Enrichment of gene sets related to epithelial development in CLDN1 mAb and
1124 enrichment of proliferation associated gene sets versus isotype control mAb treated organoids (all:
1125 $FDR<0.0001$, Kolmogorov-Smirnov test). Shown are the most relevant significantly altered
1126 pathways. **D.** Expression of progenitor marker *TACSTD2* in human cirrhosis-derived primary
1127 liver organoids treated with CLDN1 mAb or Control mAb ($n=1$ donor and $n=3$ independent
1128 experiments with $n=3-4$ replicates per condition) is shown as fold change compared to Control
1129 mAb-treated cells ($p=0.04$, U-test). **E.** Effect of CLDN1 mAb on scar-associated myofibroblast
1130 type A and B marker genes (table S11-S12) in patient-derived HLMFs. **F.** Enrichment plot for
1131 TNF- α -NF κ B signaling (HALLMARK_TNFA_SIGNALING_VIA_NFKB) in HLMFs treated by
1132 CLDN1 mAb compared to control mAb. **G.** Representative immunoblots of pS32 I κ B α , I κ B α ,
1133 pS536 p65, p65, H3 histone, and beta tubulin in the cytosolic fraction of HLMFs treated with TNF-
1134 α for 2h ($n=3-4$ independent experiments with 1 replicate per condition, respectively). **H.**
1135 Representative immunoblots of pS465/467 SMAD2/ pS423/425 SMAD3, SMAD2/3,
1136 pThr180/Tyr182 p38, p38, pS473 Akt, Akt, pT202/Y204 ERK1/ pT185/Y187 ERK2, ERK, and
1137 Actin in HLMFs treated with TGF- β . **I.** Quantification of immunoblots of pT202/Y204 ERK1/
1138 pT185/Y187 ERK2, ERK ($n= 6$), pS473 Akt, Akt ($n= 4$) and pThr180/Tyr182 p38, p38 ($n= 6$) in

1139 HLMFs treated with TGF- β (p=0.02, p=0.03 and p=0.03, U-test, respectively). **J.** Expression of
1140 *ACTA2*, *COL1A1* (n=7 different donors and independent experiments with N=2-4 technical
1141 replicates per condition), and *FNI* in HLMFs (n=3 different donors and independent experiments
1142 with N=2-4 technical replicates per condition) treated with CLDN1 mAb or control is shown as
1143 fold change compared to untreated cells (p=0.003, p=0.01 and p=0.02, Wilcoxon-matched paired
1144 test, respectively). Original Western blots are shown in figs. S18-S19. *p<0.05, **p<0.01.
1145

1146 **Figure 8. CLDN1 as therapeutic target in fibrotic kidney and lung diseases. A.** *CLDN1*
1147 gene expression in membranous glomerulonephritis renal tissues(14) and fibrotic kidney tissue
1148 (38) compared to respective healthy kidneys. **B.** *CLDN1* gene expression in pulmonary tissues of
1149 patients with IPF(39). **C.** Illustration of the UUO and bleomycin mouse models of kidney and lung
1150 fibrosis. **D.** Representative images of Sirius-red in kidneys from vehicle and CLDN1 mAb-treated
1151 animals. **E.** Quantification of collagen proportional area in UUO mice treated with vehicle control,
1152 telmisartan or CLDN1 mAb (*n*=8 mice per group). **F.** Representative images of F4/80
1153 immunostaining in kidney tissues of CLDN1 mAb- or control-treated animals. Arrows show
1154 macrophage infiltration. **G.** Representative images of Trichrome masson staining of lung tissue
1155 from vehicle and CLDN1 mAb-treated animals. **H.** Evaluation of pulmonary fibrosis by Ashcroft
1156 score (52) in vehicle- (*n*=14), CLDN1 mAb- (*n*=13) and dexamethasone- (*n*=8) treated animals. **I.**
1157 Representative images of CLDN1 mAb binding to CLDN1 on lung fibroblasts. Scale bar=100µm.
1158 **J.** Kidney fibroblasts and lung fibroblasts were treated with TNF- α (10 ng/mL), IKK-16 (1 µM),
1159 TNF- α + IKK16, or vehicle control and subjected to fluorocytometric analysis of CLDN1 mAb
1160 H3L3 binding, respectively (3 independent experiments with *n*=3 biological replicates per
1161 condition). Δ MFI is shown as fold change compared to untreated cells. **K.** Modulation of gene sets
1162 characterizing lung fibrosis-associated fibroblast differentiation states in CLDN1 mAb- or control
1163 mAb-treated IPF patient-derived fibroblasts. **L.** Enrichment plot for TNF- α -NF κ B signaling
1164 (HALLMARK_TNFA_SIGNALING_VIA_NFKB) in IPF fibroblasts treated by CLDN1 mAb
1165 compared to control mAb. Vertical bars show mean \pm SEM and single data points. Horizontal bars
1166 indicate NES of significantly (FDR<0.25) altered gene sets. **p*<0.05, ****p*<0.001, *****p*<0.0001,
1167 t-test (**A, E, G, J**) or U-test (**B**), respectively. IPF=idiopathic pulmonary fibrosis; MFI=Mean
1168 fluorescence intensity; UUO=unilateral ureteral obstruction.

MATERIALS AND METHODS

Reagents and antibodies. The following reagents were used for *in vitro* experiments in this study: DMSO, oleic acid and palmitic acid (#D8418, #O1383 and P0500, Sigma-Aldrich), IL6 (Sigma-Aldrich), TGF- β (R&D Systems), IFN γ (Thermo Fisher Scientific), PMA (Sigma-Aldrich). Humanized CLDN1 specific mAb H3L3 has been described(10) and was produced by Evitria, Schlieren. Murinized CLDN1 specific mAb (TAR-Rm) was generated by co-transfecting chinese hamster ovary (CHO) cells with plasmids containing appropriate heavy and light chain variants as described(10) by Evitria. For proteomic studies, a fully humanized variant derived from the same original OM-7D3-B3 rat anti-human CLDN1 antibody clone (ALE.F02) was used. Differently from H3L3, the Fc region of the ALE.F02 molecule contains three mutations (L234F, L235E and P331S) that have been introduced to reduce binding to Fc gamma receptors whilst maintaining binding to the neonatal Fc receptor. The isotype control antibodies used were palivizumab IgG4(53) (RRID: AB 2910861, Evitria) and motavizumab (RRID: AB 2910856, Evitria).

CLDN1 expression analysis in liver tissue by immunohistochemistry, immunofluorescence, and in-situ-hybridization. Immunohistochemistry (total CLDN1): For colocalization experiments, double immunostainings were performed on serial sections (3 μ m thick) using Claudin1 antibody (#E-AB-30939, Elabscience) and each of the following 5 different antibodies: rabbit polyclonal anti-human CK19 (RRID: AB 2281020, #ab52625, Abcam) at a 1:400 dilution, rabbit polyclonal anti-human EPCAM (RRID: AB 10984102, #PA5-19832, Invitrogen) at a 1:250 dilution, mouse monoclonal anti-human CD34 (RRID: AB 2074356,

1191 [#343607, DakoCytomation](#)) at a 1:600 dilution and mouse monoclonal anti-human CD68 (RRID:
1192 [AB_2314148, #M0814, DakoCytomation](#)) at a 1:800 dilution. Briefly, 3 µm serial sections of
1193 [paraffin-embedded livers](#) were submitted to the appropriate antigen retrieval and incubated with
1194 [rabbit polyclonal anti-human Claudin1 antibody \(#E-AB-30939, Elabscience,\)](#) at a 1:250 dilution
1195 [1 h at room temperature followed by an anti-rabbit antibody \(RRID: AB_10015288, #111-006-](#)
1196 [045, Jackson Immuno Research\)](#) for 30 min (room temperature) and then liquid diaminobenzidine
1197 [substrate–chromogen system \(DakoCytomation,\).](#) Sections were then incubated with the
1198 [corresponding second primary antibody for 1 h at room temperature followed by the appropriate](#)
1199 [second antibody for 30 min and then by phosphatase alkaline-fast red enzyme system](#)
1200 [\(DakoCytomation\).](#) Counterstaining was performed using Mayer hematoxylin.
1201 [Immunohistochemistry \(non-junctional CLDN1\):](#) Frozen liver sections were air-dried for at least
1202 [45 minutes at room temperature \(RT\) and fixed in zinc formalin for 2 minutes. After rinsing the](#)
1203 [sections in Millipore water for at least 3 minutes, endogenous peroxidase activity was blocked in](#)
1204 [a solution of PBS supplemented with 0.3% H₂O₂ for 20 minutes. The sections were washed in PBS](#)
1205 [tween for 3 min and then blocked with avidin and then biotin for each 30 minutes. After 3 washes](#)
1206 [in PBS tween, CLDN1 mAb Ale-F02 targeting njCLDN1 was diluted in antibody diluent \(Ventana](#)
1207 [antibody diluent, #06440002001, Roche\) supplemented with 10% human serum, and incubated for](#)
1208 [1 hour at room temperature. For co-staining with EPCAM, anti-EPCAM antibody \(RRID:](#)
1209 [AB_10984102, #PA5-19832, Thermo Fisher\)](#) was added at 5 µg/mL. After 3 washes the
1210 [streptavidin complex was applied for 20 minutes at RT. After 3 washes, slices were incubated with](#)
1211 [DAB for 5 minutes at room temperature and then washed in Millipore water for 2 minutes.](#)
1212 [Counterstaining was performed with hematoxylin \(dilution 1/16\) for 15 seconds. Slides were](#)
1213 [thoroughly washed in running tap water for at least 2 minutes. The sections were dehydrated and](#)

1214 all slides were mounted. Immunofluorescence: Multicolor immunofluorescent staining was
1215 performed on formalin-fixed paraffin-embedded sections cut at a thickness of 3 μm. First, slides
1216 were dewaxed and antigen retrieval was performed in Tris-EDTA buffer (pH8) for 30 minutes at
1217 99°C followed by bleaching to remove autofluorescence. Slides were incubated with primary
1218 antibody mixture of anti-alpha-SMA antibody (RRID: AB_2223500, #M0851, Dako, 1/200), anti-
1219 CLDN1 antibody targeting total CLDN1 (E-AB-30939, 1/600, Elabscience) and anti-CD68
1220 (RRID: AB_2616797, # 916104, Biolegend, 1/150) for 4 hours, followed by incubation with
1221 fluorescence-labelled secondary antibody mixture for 30 minutes. Slides were mounted with
1222 mounting medium containing DAPI and images were taken using the Zeiss Axioscan.Z1 slide
1223 scanner. In-situ hybridization: RNAscope duplex ISH was performed on the Leica Biosystems
1224 BOND RX platform, 4.5 μm sections were baked and deparaffinized on the instrument, followed
1225 by target retrieval (30 min at 95°C using Leica Epitope Retrieval Buffer 2) and 5 min protease
1226 treatment (Advanced Cell Diagnostics (ACD)). RNAscope probes (ACD) directed against human
1227 CLDN1 and human COL3A1 were hybridized for 2 h at 42°C using RNAscope 2.5 LS Duplex
1228 Reagent Kit (ACD) followed by RNAscope amplification. Fast red chromogenic detection for
1229 detection of COL3A1 was performed first, followed by green chromogenic detection (ACD) for
1230 detection of CLDN1. Dihydrodipicolinate reductase (dapB), a bacterial gene, was used as a
1231 negative control probe. Sections were counterstained with haematoxylin. Images were acquired
1232 using Olympus UC90 camera and Olympus cellSense Entry 2.3 imaging software. Staining
1233 quantification was performed using QuPath version 0.3.2(54).

1234

1235 *Modeling of the claudin-1/antibody complex.* Generation of a CLDN1 structural model:

1236 To date the structure of CLDN1 has not been solved and no structure is available in the protein
1237 data bank (PDB)(55). We therefore generated an atomistic model by homology modeling.
1238 Sequence analysis revealed that claudin-19 (CLDN19) has a sequence similarity of 57% with
1239 CLDN1 and was therefore selected as a template. A structural model of CLDN1 was generated
1240 and optimized using PRIME, a dedicated pipeline implemented in the Schrodinger suite for
1241 molecular modeling(56, 57). Generation of the antibody model: The structure of the antibody was
1242 generated using the antibody modelling pipeline implemented in the Schrodinger suite for
1243 molecular modeling(58-60). Molecular dynamics simulations: To explore conformational
1244 variability and dynamics of CLDN1, we performed extensive molecular dynamics simulations. An
1245 atomistic model of CLDN1 in a membrane was build using the OPM webserver(61). In particular
1246 CLDN1 was immersed in a POPC lipid bilayer with a concentration of 0.15M NaCl. Furthermore,
1247 the TIP3P model(62) was used to describe the water molecules whereas all other parts of the
1248 system were described by the OPLS3e force field(63). The full system was then equilibrated using
1249 the following protocol: 1. Brownian Dynamics was run for 100 ps in an NVT ensemble (T=10 K)
1250 applying harmonic restraints on solute heavy atoms (force constant 50 kcal/mol/Å²); 2. NVT
1251 (T=10K) MD simulation of 12 ps in NVT ensemble conserving the same restraints applied in 1.;
1252 3. NPT (T=300K and P=1atm) MD simulation (12 ps) conserving the same restraints applied in
1253 1.; 4. NPT (T=300K and P=1atm) MD simulation (24 ps) without restraints. Pressure and the
1254 temperature were fixed at 300 K and 1 atm by the Martyna-Tobias-Klein barostat(64) and the
1255 Nose-Hoover chain thermostat(65), respectively. Three independent production runs of 1 μs were
1256 performed. The DESMOND software in its GPU implementation was used as simulations
1257 engine(66). Last, a cluster analysis was run to extract the most relevant conformations from the

1258 MD trajectories. This analysis was carried out with the tclust program(67). The CLDN1 backbone
1259 atoms were considered for both alignment and clustering, the optimal number of clusters was
1260 automatically determined using the “elbow” method with kmeans(68). *Modeling of*
1261 *CLDN1/antibody complex*: Cluster analysis identified six different clusters. However, only two
1262 included more than 20% of the conformations sampled during molecular dynamics. The centers of
1263 these two clusters were, therefore, used for the modelling of the structure of the Claudin-1/antibody
1264 complex. CLDN1/antibody docking was simulated using the Haddock v2.4 webserver(69, 70) as
1265 described by and the definition of the epitope given in(9). Two complex structures, one for each
1266 representative CLDN1 structure, were selected for further investigation. Next, to optimize the
1267 CLDN1/antibody interface and account for induced-fit effects on the proteins, two complexes were
1268 simulated by MD for 500ns using the same set-up described before, and the trajectories analyzed
1269 by cluster analysis. Last, the interaction free energy (ΔG) for the most representative structure
1270 from the two largest clusters were computed using the PRODIGY software(72) and the model with
1271 the best (more negative) ΔG was selected as the final model of the Claudin-1/Antibody complex.

1272
1273 *Retrogenix study.* Retrogenix’s cell microarray technology was performed as
1274 described(73). Briefly, 5484 expression vectors, encoding both ZsGreen1 and a full-length human
1275 plasma membrane protein or a cell-surface tethered human secreted protein were arrayed in
1276 duplicate across 16 microarray slides (‘slide-sets’) for a primary screen. An expression vector
1277 (pIRES-hEGFR-IRES-ZsGreen1) was spotted in quadruplicate on every slide and was used to
1278 ensure that a minimal threshold of transfection efficiency had been achieved or exceeded on every
1279 slide. Human HEK293 cells (RRID: CVCL_0045) were used for reverse transfection/expression.

1280 The test antibody was added to each slide after cell fixation, giving a final concentration of 2
1281 µg/mL. Detection of binding was performed by using AlexaFluor 647 labelled anti-human IgG Fc
1282 detection antibody (RRID: AB 2563330, #409320, BioLegend). Fluorescent images were
1283 analyzed and quantitated (for transfection) using ImageQuant software. A protein ‘hit’ was defined
1284 as a duplicate spot showing a raised signal compared to background. Hits were classified as
1285 ‘strong, medium, weak, or very weak’, depending on the intensity of the duplicate spots. To
1286 confirm the hits and assess specificity, vectors encoding all hits identified in the primary screens,
1287 plus vectors encoding CD20 and EGFR, were arrayed and expressed in HEK293 cells (RRID:
1288 CVCL_0045) on new slides. Confirmation/specificity screens and analyses were carried out as for
1289 primary screening except that identical slides were treated, after cell fixation, with the test antibody
1290 individually at the same concentration as before (2 µg/mL), 1 µg/mL Rituximab biosimilar, or no
1291 test antibody/secondary only (n=2 slides per treatment).

1292

1293 *Isolation of primary liver cells.* Mouse: Primary Mouse Hepatocytes (PMH) were isolated
1294 from fresh non-diseased mouse liver tissue, as described(74). Human: Isolation of PHH and non-
1295 parenchymal cells from patients’ liver tissue (table S12) was performed as previously
1296 described(75). Briefly, human liver tissue samples from surgical interventions were digested using
1297 a two-step EGTA/collagenase perfusion technique. PHH were depleted by initial centrifugation at
1298 50xg and nonparenchymal cells were further purified by serial centrifugation at different speed
1299 and density gradient centrifugation. Fast attachment of Kupffer cells to culture plates as well as
1300 magnetic separation of endothelial cells using CD31 microbeads (CD31 MicroBead Kit, human,
1301 Miltenyi) further allowed separation and cultivation of HSCs(75).

1302

Binding studies of murinized and humanized CLDN1 specific mAbs by flow cytometry.

Binding of murinized and humanized CLDN1 mAb to cells was analyzed by flow cytometry with $\sim 1 \times 10^5$ cells in triplicate per condition. PHH and PMH (primary antibody staining): Isolated PHH and PMH were incubated with increasing concentrations of humanized CLDN1 mAb H3L3 or murinized mAb CLDN1 TAR-Rm (0.01-100 $\mu\text{g}/\text{mL}$), respectively. 293-T cells (primary antibody staining): 293-T cells (RRID: CVCL_4U22) were transfected with plasmids encoding for human or mouse CLDN1 fused with cerulean fluorescent protein or empty plasmid fused with cerulean fluorescent protein (kindly provided by M. Evans, Mount Sinai Hospital, New York). Transfected cells were incubated with increasing concentrations of humanized CLDN1 mAb H3L3, murinized CLDN1 mAb TAR-Rm or the respective isotype control antibodies. Activated hepatic stellate cells (primary antibody staining): Isolated HSCs were differentiated into activated HSCs (aHSCs) within 10 days of culture on plastic (76). Phenotypic identity was subsequently confirmed by α -SMA-positive staining using immunofluorescence (see below). For flowcytometric analysis of CLDN1 mAb binding under conditions of inflammation, transdifferentiated aHSCs were treated with TNF- α (10 ng/mL), IKK-16 (1 μM) or TNF- α (10 ng/mL) + IKK-16 (1 μM) for 24 h before incubation with humanized CLDN1 mAb H3L3 or isotype control mAb at 10 $\mu\text{g}/\text{mL}$. Secondary antibody staining (all cell types): After incubation with the respective mAbs concentrations for 1h, all cells were washed and incubated with phycoerythrin (PE)-conjugated species-specific (human or mouse) secondary antibodies (RRID: AB_2337676, #109-116-088 or RRID: AB_2338629, #115-116-146, Jackson Immuno Research) at 4 $^{\circ}\text{C}$ for 45 min to allow detection of binding. Cells were subsequently washed and fixed with 2% paraformaldehyde (PFA). Data were acquired using Cytotflex B2R2V0 (Beckman Coulter) and analyzed using CytExpert 2.1 and FlowJo v10 (Beckman Coulter). All experiments were repeated in at least 3 independent experiments in

1326 triplicate. In case of studies with patients' material, independent experiments with cells derived
1327 from at least 3 different donors were performed in triplicate. CLDN1 expression was calculated
1328 as the difference of the mean fluorescence intensities of cells stained with CLDN1 mAb and cells
1329 stained with the isotype control mAbs. The kinetics of the interaction between humanized or
1330 murinized mAb against human or mouse CLDN1, respectively, were determined by gating in
1331 cerulean-positive cells using FlowJo and the Michaelis-Menten mathematical model using R 3.5.1
1332 (<http://www.R-project.org/>).

1333
1334 *Liver fibrosis mouse models.* All experiments were performed at the animal facility of
1335 Inserm U1110 according to local laws and ethics committee approval (institutional protocol
1336 approval number APAFiS #3559, #7216 and #32429). The mice were housed in individually
1337 ventilated cages with 12h/12h light/dark cycles and *ad libitum* access to food and water.
1338 *Pharmacokinetics studies.* Three C3H male mice (RRID:MGI:6197584, 6-8 weeks old) were i.p.
1339 injected with 500 µg of murinized CLDN1 specific mAb TAR-R-mIgG. At day 1, 3, 8, and 15
1340 after injection, 100 µL blood was harvested under general anesthesia (isoflurane 3%) by retro-
1341 orbital puncture with dry capillaries. Serum concentrations of the murinized CLDN1 specific mAb
1342 were quantified by flow cytometry as described(77). Briefly, 3x10⁴ CLDN1-overexpressing
1343 Huh7.5.1 cells were incubated for 30 min at 4 °C with 20 µL of 1/50-diluted serum or serial
1344 concentrations (0, 0.1, 0.3, 1, 3, 10, and 30 µg/mL) of CLDN1-specific mAb TAR-R-mIgG in
1345 1:50-diluted serum from an untreated C3H mouse. After extensive washing, cells were labelled
1346 with PE-conjugated goat-anti-mouse Abs (RRID: AB 2338629, #115-116-146, Jackson
1347 ImmunoResearch Laboratories) and fixed with 2% paraformaldehyde. Cells were analyzed on a
1348 BD LSRII FACS. To determine the mAb concentration at each time point, the PE mean

1349 fluorescence intensity (MFI) of all viable cells in experimental samples were compared with that
1350 of the titration curve. The mAb serum concentrations were then plotted against time and the half-
1351 life was calculated for each mouse using its regression curve. *DEN-CDA-HFD model*: Forty 7-
1352 week old male C57BL/6J mice (RRID: IMSR_JAX:000664, Charles River Laboratories) received
1353 a single i.p. injection of DEN (100 mg/kg, Sigma-Aldrich) and were subsequently fed with the
1354 CDA-HFD (A06071302, Research Diet) after 3 weeks. After 6 weeks of diet, the mice were
1355 randomized in 2 groups, receiving weekly i.p. injections of 500 µg of either CLDN1 specific mAb
1356 or vehicle control for 16 weeks. After 16 weeks of treatment, all mice were sacrificed, the blood
1357 was sampled and the liver as well as other major organs (brain, heart, lung, kidney, stomach,
1358 intestine, spleen, bladder and skin) were harvested and underwent macroscopic and microscopic
1359 examination (fig. S8). *Humanized liver NASH mouse model*: *Fah^{-/-}/Rag2^{-/-}/Il2rg^{-/-} (FRG) –*
1360 *NOD* (RRID: IMSR_JAX:018454) breeding mice were kept at the Inserm Unit 1110 SPF animal
1361 facility and maintained with 16 mg/L of 2-(2-nitro-4-trifluoro-methyl-benzoyl)-1,3
1362 cyclohexanedione (NTBC; Swedish Orphan Biovitrum) in drinking water. Six-week-old mice
1363 were intravenously injected with 1.5×10^9 plaque forming units (pfu) of an adenoviral vector
1364 encoding the secreted form of the human urokinase-like plasminogen activator (Ad-uPA)(21).
1365 Forty-eight hours later, 10^6 PHH were injected intrasplenically via a 27-gauge needle. For the
1366 procedure, the mice were kept under gaseous isoflurane anesthesia and received a subcutaneous
1367 injection of buprenorphine at the dose of 0.1 mg/kg. After transplantation, NTBC administration
1368 was gradually decreased and completely withdrawn in 7 d. Transplant success was evaluated 2
1369 months after the procedure by dosing human albumin in mouse serum as previously described(77).
1370 The mice successfully transplanted were fed with CDA-HFD for 16 weeks and then treated with
1371 humanized CLDN1-specific mAb 500 µg or vehicle for additional 8 weeks. For the *GalNAc*

1372 siRNA *in vivo* knockdown study, mice successfully transplanted with PHH were fed with CDA-
1373 HFD for 12 weeks followed by subsequent subcutaneous injections of *in vitro* validated siRNA
1374 targeting the human *CLDN1* or siCTRL (3 mg/kg/week) for 8 weeks. DDC (3,5-
1375 Diethoxycarbonyl-1,4-Dihydrocollidine) mouse model: Seven-week-old C57BL/6J male mice
1376 (RRID: IMSR JAX:000664, Charles River Laboratories) were fed a 0.1% DDC-supplemented
1377 diet (R03-25, Safe). One week after beginning the 0.1% DDC diet, the mice were randomly
1378 assigned to either the treatment group (IP injection of 25 mg/kg/week of H3L3 anti-CLDN1 mAb)
1379 or control group (PBS) for three weeks. Mice were sacrificed after 4 weeks of 0.1% DDC diet and
1380 plasma and livers were harvested for subsequent analyses. *ELISAs on mouse serum*. CRP was
1381 measured in collected plasma of the humanized mice using Human C-Reactive Protein/CRP
1382 Quantikine ELISA Kit (#DCRP00, R&D Systems) according to the manufacturer's instructions.

1383

1384 **Mouse liver tissue staining analysis**

1385 *Histological analysis*. All organs were immediately fixed in a 10% formalin solution after
1386 harvesting and subsequently included in paraffin. Liver slices stained with hematoxylin & eosin
1387 (H&E) and Sirius Red were obtained for all mice. For immunohistochemistry staining, the
1388 following antibodies were used: CD11c (RRID: AB_2800282, #97585, Cell Signaling), CD3
1389 (RRID: AB_1956722, #MA1-90582, Invitrogen), CD4 (RRID: AB_2573008, #14-9766-82,
1390 eBioscience), CD8a (RRID: AB_2572861, #14-0808-82, Invitrogen), CLEC4F (RRID:
1391 AB_2081339, #AF2784, R&D Systems), Fibronectin-1 (RRID: AB_732380, #ab45688, Abcam),
1392 LY6C (RRID: AB_302004, #ab15627, Abcam), MHC II (RRID: AB_10006678, #NBP1-43312,
1393 Novus Biologicals), MPO (RRID: AB_307322, #ab9535, Abcam), REL A (RRID: AB_535932,
1394 #NB_100-2176, Novus Biologicals), REL B (RRID: AB_632341, #sc-226, Santa Cruz), pP38

1395 (RRID: AB 2139682, #4511, Cell Signaling), HSP70 (RRID: AB 1150514, #IMG-80181,
1396 IMGENEX), FAH (RRID: AB_2678806, #HPA044093, Sigma), α -SMA (RRID: AB_476701,
1397 #A2547, Sigma). Staining quantification was performed on entire histological slide or on 5 to 10
1398 consecutive images at 10x or 20x magnification per staining Images were analyzed using ImageJ
1399 software v1.51j8 (Rasband W, National Institutes of Health, USA) or QuPath version 0.3.2(54).

1400 For the collagen proportional area quantification in humanized areas, two consecutive liver
1401 cuts were stained with FAH and Sirius Red. The corresponding FAH-positive area in the Sirius
1402 Red histological slide was selected as region of interest and then the collagen proportional area
1403 quantified using ImageJ software(78). For quantification of other immunostaining in
1404 representative humanized area, the humanized areas were selected from the hematoxylin staining
1405 based on the phenotype of human hepatocytes showing a brighter cytoplasm and different nucleus
1406 and cytoplasm size compared to mouse ones(21). All the available tissue slides were analyzed with
1407 two exceptions: for α -SMA staining in the DEN-CDAHFD model samples were selected on
1408 fibrosis phenotype, for fibronectin-1 analyses of CLDN1 mAb-treated humanized NASH mice of
1409 experiment 2, outliers were excluded according to the Rosner test.

1410 In situ hybridization of mouse liver tissues: RNAscope ISH was performed on the Leica
1411 BOND III platform, 5 μ m sections were baked and deparaffinized on the instrument, followed by
1412 target retrieval (30 min at 95°C using EDTA Buffer) and 15 min protease treatment. RNAscope
1413 probes (ACD) directed against mouse and *Ccl2*, *Ccl20*, *Cxcl10* were hybridized for 2 h at 42°C.
1414 Staining was performed using BOND RNAscope Brown Detection (DS981) and slides were
1415 counterstained with haematoxylin. Staining quantification was performed using QuPath version
1416 0.3.2(54).

1417

1418 *Kidney fibrosis (unilateral ureteral obstruction model, UUO) mouse model:* The
1419 experiment was conducted by SMC laboratories (Japan) according to local laws and following
1420 ethics committee approval (SLMC053-1906-8 (UUO): U015). Seven-week-old female C57BL/6J
1421 mice (RRID: IMSR_JAX:000664) were obtained from Japan SLC, Inc. and housed and cared for
1422 in accordance with the Japanese Pharmacological Society Guidelines for Animal Use at SMC
1423 laboratories, Japan. Animals were housed and fed with a normal diet (CE-2; CLEA Japan) under
1424 controlled conditions. On day 0, UUO surgery was performed under mixed anesthetic agents
1425 (medetomidine, midazolam, butorphanol). Mice were randomized to receive CLDN1 mAb
1426 (500 µg in 100 µL/mouse, n=8) or vehicle (100 µL, n=8) which were administered
1427 intraperitoneally of twice weekly for 14 days. Telmisartan (30 mg/kg, n=8) was administered
1428 orally once daily for 14 days. The animals were sacrificed by exsanguination through direct cardiac
1429 puncture under isoflurane anesthesia (Pfizer Inc.) at day 14. For plasma biochemistry, non-fasting
1430 blood was collected in polypropylene tubes with anticoagulant (Novo-Heparin, Mochida
1431 Pharmaceutical Co. Ltd.) and centrifuged at 1,000xg for 15 min. at 4 °C. The supernatant was
1432 collected and stored at -80 °C until use. Plasma urea nitrogen was measured by FUJI DRI-CHEM
1433 7000 (Fujifilm). *Histological and image analysis.* To visualize collagen deposition, kidney
1434 sections were stained using picro-Sirius red solution (Waldeck). For quantification of interstitial
1435 fibrosis area, bright field images in the corticomedullary region were captured using a digital
1436 camera (DFC295) at 200-fold magnification, and the positive areas in 5 fields/section were
1437 measured using ImageJ software. For immunohistochemistry, sections were cut from paraffin
1438 blocks and deparaffinized and rehydrated. Endogenous peroxidase activity was blocked using
1439 0.3% H₂O₂ for 5 min., followed by incubation with Block Ace (Dainippon Sumitomo Pharma Co.
1440 Ltd.) for 10 min. The sections were incubated with a 100-fold dilution of anti-F4/80 antibody

1441 (RRID: AB 1227368, #T-2006, BMA Biomedicals) at room temperature for 1 hour. After
1442 incubation with secondary antibody (#31470, HRP-Goat anti-rat antibody, Invitrogen), enzyme-
1443 substrate reactions were performed using 3, 3'-diaminobenzidine/H₂O₂ solution (Nichirei
1444 Bioscience Inc., Japan). For quantitative analysis of inflammation areas, bright field images of
1445 F4/80-immunostained sections were captured using a digital camera (DFC295) at 200- and 400-
1446 fold magnifications.

1447

1448 *Lung fibrosis (Bleomycin-induced) mouse model.* The experiment was conducted by
1449 SMC laboratories (Japan) according to local laws and following ethics committee approval
1450 (SLMP052-1906-7 (IPF): B048). Six-week-old female C57BL/6J mice (RRID:
1451 IMSR JAX:000664) were obtained from Japan SLC, Inc. and housed and cared in accordance
1452 with the Japanese Pharmacological Society Guidelines for Animal Use at SMC laboratories.
1453 Animals were housed and fed with normal diet (CE-2; CLEA Japan) under controlled conditions.
1454 On day 0, mice were anesthetized with a mixture of medetomidine (Nippon Zenyaku Kogyo),
1455 midazolam (Sandoz K.K.) and butorphanol (Meiji Seika Pharma) anesthesia and intratracheally
1456 administered BLM (Nippon Kayaku) in saline at a dose of 3 mg/kg, in a volume of 50 μ L per
1457 animal using a Microsprayer (Penn-Century). Mice were randomized to receive CLDN1 mAb
1458 (500 μ g/mouse and 5 mL/kg, $n=9$) or vehicle (5 mL/kg, $n=9$) which were administered
1459 intraperitoneally twice weekly from day 0 to 20. Dexamethasone (0.25 mg/kg, $n=9$) was
1460 administered orally once daily from day 0 to 20. The animals were sacrificed at day 21 by
1461 exsanguination through the abdominal aorta under a mixture of medetomidine, midazolam and
1462 butorphanol anesthesia. *Histological and image analysis.* Right lung tissues prefixed in 10%
1463 neutral buffered formalin were embedded in paraffin and sectioned at 4 μ m. For Masson's

1464 Trichrome staining, the sections were stained with Masson's Trichrome staining Kit (Sigma)
1465 according to the manufacturer's instructions. The degree of pulmonary fibrosis was evaluated
1466 using the Ashcroft score(52).

1467
1468 *Non-human primate study.* The experiment was performed by Charles River Laboratories
1469 from February to April 2020 according to local laws and following ethics committee approval
1470 under study number CRL 20229915. The animals were housed in the Charles River Laboratories
1471 France Safety Assessment SAS test facility (France). This dose-ascending study was performed
1472 with the fully humanized variant Ale-F02. Five groups of cynomolgus monkey aged from 29 to 33
1473 month were included in the study. Groups 1 to 3 included 1 animal while groups 4 and 5 two
1474 animals per group. Animals in group 1 were treated with an intravenous CLDN1-specific mAb
1475 injection at 0.3 mg/kg, in group 2 at 3 mg/kg, in group 3 at 15 mg/kg, in group 4 at 60 mg/kg, in
1476 group 5 at 150 mg/kg. The groups 1 to 3 received a single administration, while groups 4 and 5, a
1477 weekly injection for a total of 4 doses (Day 1, 8, 15 and 22). The animals were observed for 6
1478 weeks. Parameters monitored included morbidity/mortality, clinical signs, injection site
1479 observations, body weights, food consumption and clinical pathology parameters (hematology,
1480 coagulation, clinical chemistry and urinalysis). All animals were sampled for toxicokinetic on Day
1481 1, before and at various time points after dosing and on Days 7, 14, 21 and 29 as well as Day 42
1482 for animal 1 to 3 only.

1483 Pharmacokinetic modeling was performed by LYO-X (Allschwil); in brief, for parameter
1484 estimation and diagnostic plots, Monolix Suite 2019R2, and for the human PK-binding
1485 simulations, Simulx (Monolix Suite 2019R2), mlxR 4.1.0 (Lavielle 2019) and R 3.6.0 (R
1486 Development Core Team 2008) were used.

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Functional assessment of the murinized CLDN1-specific mAb. Mouse CLDN1-transfected 293-T cells were pre-incubated with murinized CLDN1 mAb or its corresponding murinized isotype control mAb (100 µg/mL) for 1 h at 37 °C and subsequently exposed to HCV pseudoparticles (HCVpp) for 4 h at 37 °C, as described(79). HCVpp entry was analyzed by measuring intracellular luciferase activity after 72 h (relative light units, RLU). Inhibition was expressed as a percentage relative to cells treated with the corresponding murinized isotype control mAb as described(77).

RNA extraction from human and murine liver tissue. Liver cells were lysed in TRI-reagent (Molecular Research Center) using GentleMACS Octo Dissociator, and RNA was purified using Direct-zol RNA MiniPrep (Zymo Research) according to the manufacturer’s instructions. RNA quantity and quality were assessed using NanoDrop (ThermoScientific). Gene expression profiling was performed using 250-500 ng total RNA.

Prognostic liver signature expression analyses. Profiling of the prognostic liver signature (PLS) was performed using Nanostring nCounter assay as described(80). Induction or suppression of the PLS in gene expression data was determined as previously reported using the Gene Set Enrichment Analysis (GSEA)(81), implemented in GenePattern genomic analysis toolkits. False discovery rate (FDR) <0.25 was regarded as statistically significant(81). PLS was always determined by using control cells, control animals, or control patient-derived tissues as references. Results are presented as simplified heatmaps showing the classification of PLS global status as poor or good prognosis and the significance of induction/suppression of PLS genes (log10 of FDR

1510 values). Global status corresponds to the difference between low-risk and high-risk gene
1511 enrichments.

1512

1513 *ChIPseq and CUT&TAG assay.* LX2 cells (RRID: CVCL_5792) were incubated 24h with
1514 or without 100 ng/mL TNF- α (Peprotech, #300-01A) and scraped. The CUT&Tag assay against
1515 H3K27ac (RRID: AB_2561016, #39133, Active motif) or NF- κ B p65 subunit (RRID:
1516 AB_10828935, #6956, Cell Signaling Technology) were performed following the manufacturer's
1517 instructions (Active motif CUT&Tag-IT Assay Kit, #53165, #53160). Briefly, 1×10^6 cells per
1518 conditions were used. The cells were washed 2 times before binding on Concavalin A beads and
1519 then incubated overnight with primary antibody at the recommended dilution (1:50) or no primary
1520 antibody (negative control). The next day the corresponding secondary antibody, guinea pig anti-
1521 rabbit antibody or rabbit anti-mouse antibody (RRID: AB_11024108, NBP1-72763, Novus
1522 Biologicals or AB_228419, #31188, Thermo Fisher) were used at 1:100 dilution in digitonin buffer
1523 and incubated at room temperature for 1 hour. The CUT&Tag-IT Assembled pA-Tn5
1524 Transposomes were incubated for 1 hour at room temperature before tagmentation. Cells were
1525 resuspended in tagmentation buffer and incubated at 37°C for 1 hour, then the tagmentation
1526 process was stopped by addition of EDTA and SDS. Protein digestion was performed by the
1527 addition of 80 μ g/mL of proteinase K and incubated at 55°C for 60 minutes. DNA was retrieved
1528 using DNA purification columns following the manufacturer's instruction. Library preparation and
1529 PCR amplification were done using the Kit primers and purified by 2 successive washes with SPRI
1530 beads (1.1 μ L/sample volume). Samples were subjected to paired-end sequencing by the
1531 GenomEast platform on Illumina HiSeq 4000 instrument. The reads were mapped to the HG38
1532 genome by BWA and peak-calling was performed by MACS2. For H3K27ac 48741 and 61571

1533 peaks were detected in absence and presence of TNF- α , respectively, whereas for p65, 16391 and
1534 50160 peaks were detected in absence and presence of TNF- α , respectively. RSAT analyses
1535 (http://rsat.sb-roscoff.fr/peak-motifs_form.cgi) of the 100 bp surrounding the top p65 1000 peaks
1536 in absence and presence of TNF- α confirmed a strong enrichment of the RelA/NF- κ B recognition
1537 motifs in the TNF- α -treated sample.

1538

1539 *Organovo ExVivo Human Liver Tissue NASH fibrosis model.* The study was conducted
1540 by Organovo. PHHs and nonparenchymal cell populations (LECs, HSCs and Kupffer cells)
1541 cultured in conditioned medium (sugars, free fatty acids and inflammatory inducers) were
1542 bioprinted in 3D using the NovoGen Bioprinter platform as described(28). Four NASH induced
1543 ExVivo Human Liver Tissues with Kupffer cells per condition were exposed to Vehicle, CLDN1
1544 mAb H3L3 or isotype control mAb at 10 or 100 μ g/mL daily for 21 days. After 21 days, tissues
1545 were stained with hematoxylin and eosin and Trichromic Masson. Eight sections of each tissue
1546 replicate underwent histological quantification. One image per each of the eight sections for the
1547 four tissue replicates stained with Trichromic Masson underwent fibrosis quantification (total 32
1548 images). Samples treated with isotype and CLDN1 mAbs (10 μ g/mL) were selected for
1549 comparative quantitative analyses. Image analysis was performed using ImageJ software.

1550

1551 *Patient-derived liver spheroids and tumorspheres.* Liver tissues from patients with or without
1552 chronic liver disease (table S7) were gently digested using a two-step digestion method as
1553 described(25, 75). The sample was then washed with PBS 1x and loaded on a 70 μ m cell strainer.
1554 Digested tissue was gently smashed, and the cell strainer washed with up to 10 mL PBS 1x.

1555 Collected cell clusters were further filtered through a 0.45 μ m filter and centrifuged for 5 min at
1556 800xg. The cell pellet containing all liver cell types was then re-suspended in Mammocult basal
1557 medium (StemCell), supplemented with human proliferation supplement (3.4%), hydrocortisone
1558 (0.056%) and heparin (0.011%) and cultured in 96-well ultra-low attachment plates (Corning,
1559 Sigma Aldrich). Cell characterization in spheroids by immunofluorescence: Spheroids were fixed
1560 with formaldehyde (4% for 2 hours), permeabilized with Triton 0,5%, blocked with 5% FBS, and
1561 incubated with ASGPR1- PE (REA608, Miltenyi, 1:50), α SMA (RRID: AB_2223021, #ab5694,
1562 Abcam, 1:50), CD68 (RRID: AB_1089059, #333801, Biolegend, 1:50) or CD31-FITC (RRID:
1563 AB_2160882, #3528, Cell signaling, 1:50) overnight. Respective species-specific secondary
1564 antibodies (RRID: AB_2338078, #111-605-144 or AB_2338840, #115-545-003, Jackson Immuno
1565 Research) were added for 1h, followed by washing steps. Spheroids were visualized by Celigo
1566 imaging cytometer. Spheroid fibrosis model: 150.000-200.000 cells per well were suspended in
1567 Mammocult complete medium (STEMCELL Technologies) supplemented with 20% donor-
1568 derived serum (or 20% FBS if donor-derived serum was not available) and seeded on a 96-well
1569 low-attachment plate (Corning), then spun at 300g for 5 minutes and incubated overnight. The day
1570 after, spheroids were treated with Mammocult complete medium plus 20% serum supplemented
1571 with a 2-fold concentrated mixture of oleic acid, palmitic acid, TGF- β , LPS, and either CLDN1
1572 monoclonal antibody or isotype control antibody to reach the final concentrations of 800 μ M, 400
1573 μ M, 10 ng/mL, 100 ng/mL, and 10 μ g/mL, respectively. Vehicle-treated spheroids served as
1574 control. Seventy-two hours later, spheroids were lysed, and RNA was extracted using Arcturus
1575 PicoPure RNA Isolation Kit (Applied Biosystems). Subsequently, total RNA was reverse
1576 transcribed (H Minus First Strand cDNA synthesis Mix, ThermoScientific) on a Thermocycler

1577 (Bio-Rad T100, Bio-Rad, Hercules). Quantitative PCR was performed on the CFX96 Touch Real-
1578 Time PCR Detection system with 10 μ L reaction volumes containing 5 μ L SYBR Green 2x mix
1579 (Bio-Rad), 2 μ L of RNase-free water and 250 nM gene specific sense and antisense primers. For
1580 qPCR analyses Prime PCR SYBR Green Assays for *COL1A1*, *COL3A1* and *COL4A1* (Biorad)
1581 were applied according to the manufacturer's instructions. qPCR for *CTGF* and *TACSTD2* was
1582 analysed with the following primer sequences: *CTGF*: Fw: 5'-ACC GAC TGG AAG ACA CGT
1583 TTG, Rv: 5'-CCA GGT CAG CTT CGC AAG G; *TACSTD2*: Fw: 5'-CCT GAA CGC AGT TTG
1584 GAT GTC-3', Rv: 5'-GTA AGG GCA AGC TGA AGA ATA AAT AGA (82). Gene expression
1585 were normalized to the housekeeping gene *HPRT1* (Fw: 5'-CTG GAA AGA ATG TCT TGA TTG
1586 TGG, Rv: 5'-TTT GGA TTA TAC TGC CTG ACC AAG) using the $\Delta\Delta$ Ct method (83).
1587 Assessment of collagen deposition in spheroids: Healthy liver tissue (**table S7**) was processed into
1588 multicellular spheroids, stimulated with FFA (100 ng/ml), LPS (100 ng/mL), and TGF- β (10
1589 ng/mL) and then treated with resmetirom (10 μ M), isotype control antibody (10 μ g/mL), or
1590 CLDN1 mAb (10 μ g/mL) for 4 days. Total collagen deposition was quantified using Total
1591 Collagen Assay Kit perchlorate-free (Abcam), according to the manufacturer's instructions.

1592
1593 ***Patient-derived liver organoids:*** Organoid establishment and expansion: Organoids were
1594 established from non-tumorous cirrhotic liver tissue from a patient undergoing liver resection for
1595 HCC as described(36). Briefly, liver tissue was dissociated using Tumor dissociation kit, human
1596 (#130-095-929, Miltenyi Biotec) and the gentleMACS Octo Dissociator (Miltenyi) according to
1597 the manufacturer's instructions. Total cell population was passed through a 75 μ m cell filter and
1598 washed 2 times with PBS and centrifuged at 500g, 5 min, 4°C. 10 x 10³ cells were then re-

1599 suspended in 50 μ L Corning Matrigel Growth Factor Reduced Basement Membrane Matrix
1600 (#354230, Corning) and seeded as domes onto 24 well plates. Following incubation for 15 min at
1601 37°C, 500 μ L prewarmed initiation medium was added to each well. Once formation of organoids
1602 was microscopically detected, initiation medium was refreshed by pre-warmed expansion medium
1603 and medium was replaced every 2-3 days. Organoids were passaged every 1-2 weeks and cells of
1604 passage 4 were used for perturbation studies. Organoid perturbation study (3 independent
1605 experiments): Organoids from passage 3 were harvested and washed. Pre-washed organoid cell
1606 pellet was pre-incubated with 10 μ g/ml CLDN1 mAb or isotype control mAb in PBS for 1h at
1607 room temperature. The cell pellet was then washed once with PBS and resuspended in Matrigel
1608 and seeded as 25 μ L domes onto 48-well plates. After solidification of Matrigel for 15 minutes at
1609 37°C, 500 μ L prewarmed initiation medium supplemented by 10 μ g/ml CLDN1 mAb or isotype
1610 control was added to each well. The experiment was performed with 3-4 replicates per condition.
1611 Organoids were incubated for 4 days at 37°C, representative microscopic images were collected
1612 and organoids were harvested for RNA extraction using Qiagen RNeasy Mini extraction kit
1613 (#4106, Qiagen) according to the manufacturer's instructions. Organoid size diameter was
1614 measured in representative microscopic images at 50-100x magnification using ImageJ.

1615
1616 *Precision cut ex vivo liver slice culture.* Liver tissue slices (200-500 μ m-thick) were
1617 prepared from surgically resected non-tumorous liver tissues from patients with NASH who
1618 underwent liver resection for HCC (**Fig. 4H-I**) (table S7) and from patients without liver disease
1619 undergoing liver resection for metastasis of colorectal cancer (fig. S12D) (table S8). The slices
1620 derived from adjacent non-tumorous tissue were cultured with CLDN1 specific mAb or isotype

1621 control mAb (10 µg/mL) for 24 h and harvested for gene expression analysis, as described above.
1622 Gene expression data from non-diseased liver tissues (University Strasbourg NASH cohort,
1623 table S1) were used as reference controls to verify the induction of the PLS in the studied NASH
1624 patients. For non-diseased tissues, the PCLS were cultured in William's E complete medium
1625 supplemented with 20% patient serum and were subjected to LPS treatment (100 ng/mL) to induce
1626 inflammation and to treatment with control (palivizumab IgG4(53)) or CLDN1 mAb (50 µg/mL).
1627 After 3 days, the tissues were harvested and proteins extracted to assess effect of the treatment on
1628 EGFR and SRC phosphorylation.

1629
1630 *Genome wide RNA-seq analyses.* RNA-Seq libraries were generated from 300 ng of total
1631 RNA using TruSeq Stranded mRNA Sample Preparation Kit (Illumina, Part Number RS-122-
1632 2101). Briefly, following purification with poly-T oligo attached magnetic beads, the mRNA was
1633 fragmented using divalent cations at 94 °C for 2 min. The cleaved RNA fragments were copied
1634 into first strand cDNA using reverse transcriptase and random primers. Strand specificity was
1635 achieved by replacing dTTP with dUTP during second strand cDNA synthesis using DNA
1636 Polymerase I and RNase H. Following addition of a single 'A' base and subsequent ligation of the
1637 adapter on double stranded cDNA fragments, the products were purified and enriched with PCR
1638 (30 sec at 98 °C; [10 sec at 98 °C, 30 sec at 60 °C, 30 sec at 72°C] x 12 cycles; 5 min at 72°C) to
1639 create the cDNA library. Surplus PCR primers were further removed by purification using
1640 AMPure XP beads (Beckman Coulter) and the final cDNA libraries were checked for quality and
1641 quantified using 2100 Bioanalyzer (Agilent). Libraries were sequenced on the Illumina HiSeq
1642 4000 as Single-Read 50 base reads following Illumina's instructions. Image analysis and base
1643 calling were performed using RTA v2.7.3 and bcl2fastq v2.17.1.14.

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In vitro perturbation studies on human activated stellate cells (aHSCs). Isolated human hepatic stellate cells (HSCs) (75) were seeded at a density of 5×10^4 cells/cm² in DMEM with 10% FBS on collagen-coated 12-well plates. After 10 days of cultivation on plastic, all cells showed a myofibroblast-like phenotype consistent with an activated stage (76). At this stage (10d of culture), identity and purity of aHSCs were validated by expression of α -SMA (RRID: AB_2223021, Abcam), as assessed by immunofluorescence (see below). For analysis of CLDN1 mAb effects on aHSCs activation markers, primary aHSCs were seeded at 5×10^4 cells/cm² in 12-well plates and treated with CLDN1 mAb (50 μ g/mL) or vehicle control for 3 days. aHSCs were derived from n=7 different donors (table S12) and experiments were performed in triplicate per condition and donor.

Immunofluorescence on isolated cells. Cells were seeded onto 8-chamber cover glasses (Lab-Tek II #1.5, Sigma-Aldrich). The next day, cells were washed twice with PBS and fixed with 4% PFA for 15 min at room temperature, followed by permeabilization with 0.1% Triton-X for 10 min. After two washing steps, cells were blocked for 30 min with 10% FBS. Primary antibody staining with anti- α -SMA Ab (RRID: AB_2223021, #ab5694, Abcam, 1:100) or anti-CD68 (#CSB-PA282654, CUSABIO, 1:100) and CLDN1 mAb H3L3 or control mAb (10 μ g/mL, respectively) was performed overnight at 4 °C. Cells were washed with PBS and incubated with goat anti-human Alexa Fluor 488 and/or goat anti-rabbit Alexa Fluor 647 secondary antibodies (RRID: AB_2337831, #109-515-003 or AB_2338078, #111-605-144, Jackson Immuno Research) at a dilution of 1:200. Nuclear staining was done using DAPI (1 μ g/mL) and cells were visualized

1666 using epi-fluorescence and confocal microscopy. Results were confirmed in at least 3 independent
1667 experiments.

1668
1669 *Gene expression analyses in 2D cell culture experiments.* Total RNA extraction from 2D
1670 cell cultures was performed using RNAeasy Mini Kit (Quiagen) according to the manufacturer's
1671 instructions. Subsequently, 100-500 ng RNA was reverse-transcribed (H Minus First Strand cDNA
1672 synthesis Mix, ThermoScientific) on a Thermocycler (Bio-Rad T100, Bio-Rad, Hercules).
1673 Quantitative PCR was performed on the CFX96 Touch Real-Time PCR Detection system with 20
1674 μL reaction volumes containing 10 μL SYBR Green 2x mix (Bio-Rad), 4 μL of RNase-free water
1675 and 250 nM gene specific sense and antisense primers. The primer sequences were as follows:
1676 *ACTA2* Fw: 5'-TGA AGA GCA TCC CAC CCT, Rv: 5'-ACG AAG GAA TAG CCA CGC;
1677 *COL1A1*: Fw: 5'-CCT CAA GGG CTC CAA CGA G, Rv: 5'-TCA ATC ACT GTC TTG CCC
1678 *CA*; *TNFA*: Fw: 5'-GAG GCC AAG CCC TGG TAT G, Rv: 5'-CGG GCC GAT TGA TCT
1679 *CAG C*; *IL6*: Fw: 5'-ACT CAC CTC TTC AGA ACG AAT TG, Rv: 5'-CCA TCT TTG GAA
1680 *GGT TCA GGT TG*; *TIMP1*: Fw: 5'-GCC CAG AGA GAC ACC AGA GAA C, Rv: 5'-CTA
1681 *TCA GCC ACA GCA ACA AC AGG*. All gene abundances were normalized to housekeeping
1682 genes *HPRT1* (Fw: 5'-CTG GAA AGA ATG TCT TGA TTG TGG, Rv: 5'-TTT GGA TTA TAC
1683 *TGC CTG ACC AAG* in HLMFs) and *GAPDH* (Fw: 5'-GTC TCC TCT GAC TTC AAC AGC G,
1684 Rv: 5'-ACC ACC CTG TTG CTG TAG CCA A) using the $\Delta\Delta C_t$ method(83).

1685
1686 *Western blot analyses of signaling in 2D culture.* TNF- α signaling: 1×10^6 aHSCs were
1687 treated with CLDN1 mAb (10 μg/mL) or control mAb (10 μg/mL) for 3 days in serum free media.
1688 At day 3 post-treatment, aHSCs were treated again with CLDN1 mAb (10 μg/mL) or control mAb

1689 (10 µg/mL) for an additional 6h, followed by TNF-α stimulation (10 ng/mL) for 2 hours.
1690 Cytoplasmic and nuclear cell fractions were then isolated using the Cell Fractionation Kit (Abcam,
1691 ab109719) according to the manufacturer's instructions. Briefly, cells were collected, resuspended
1692 in buffer A and permeabilized with detergent I. Following incubation for 7 minutes on a rotator at
1693 RT and centrifugation at 10,000 x g for 1 min, the resultant supernatant fraction was collected and
1694 considered the cytosol fraction. Following resuspension in buffer A and solubilization with
1695 detergent II, the cytosol-depleted pellet was centrifuged at 10,000 x g for 1 minutes. Supernatant
1696 was discarded and cell pellet was resuspended in buffer A and considered the nuclear fraction.
1697 Protein expression in each fraction was assessed by immunoblotting, with histone H3 (RRID:
1698 AB 302613, #ab1791, Abcam) and tubulin (RRID: AB 1952434, #GTX101279, GeneTex)
1699 antibodies being used as markers for nuclear and cytoplasmic fraction, respectively. For
1700 immunoblotting, the following primary antibodies were used: p65 (RRID: AB_628017, #sc-8008,
1701 Santa Cruz Biotechnology), P-p65 (RRID: AB 331284, #3033, Cell Signaling technology), IκB
1702 (RRID: AB 390781, #4814, Cell Signaling technology), and P-IκB alpha (RRID: AB 561111,
1703 #2859, Cell Signaling technology). TGF-β signaling: 1x10⁵ aHSCs were treated with CLDN1
1704 mAb (10 µg/mL) or control mAb (10 µg/mL) for 3 days in serum free media. At day 3 post-
1705 treatment, aHSCs were stimulated with TGF-β (10 ng/mL) for 1 hour to assess SMAD2/3, p38 and
1706 ERK pathways, or 2 hours to assess Akt pathway. RepSox (2 µM, ab142139, Abcam), SML0543
1707 (10 µM, Sigma Aldrich), U0126 (10 µM, Sigma Aldrich) or Wortmannin (10 µM, W3144, Sigma
1708 Aldrich) were used as inhibitors of SMAD2/3, p38, ERK and Akt signaling, respectively. The
1709 following primary antibodies were used: SMAD 2/3 (RRID: AB 10889933, #8685, Cell Signaling
1710 technology), P-SMAD 2/3 (RRID: AB_2631089, #8828, Cell Signaling technology), p38 (RRID:
1711 AB 330713, #9212, Cell Signaling technology), P-p38 (RRID: AB 2139682, #4511, Cell

1712 Signaling technology), ERK1/ERK2 (RRID: AB_2140121, #MAB1576, R&D Systems), P-
1713 ERK1/ERK2 (RRID: AB_354539, #AF1018, R&D Systems), Akt (RRID: AB_915783, #4691,
1714 Cell Signaling technology) and P-Akt (RRID: AB_2315049, #4060, Cell Signaling technology).
1715 EGFR signaling: 1x10⁵ Huh7 were treated with CLDN1 mAb (10 µg/mL) or control mAb (10
1716 µg/mL) for 2 days. Medium was then changed to a serum free one, and cultures were treated for 1
1717 additional day with CLDN1 mAb (10 µg/mL) or control mAb (10 µg/mL). Huh7 were stimulated
1718 with EGF (10 ng/mL) for 6 hours. Erlotinib (50 nM) was used as inhibitor of EGFR signaling. The
1719 following primary antibodies were used: EGFR (RRID: AB_331707, #2232, Cell Signaling
1720 technology) and p-EGFR (RRID: AB_2096270, #3777, Cell Signaling technology). SRC (RRID:
1721 AB_2106059, #2109, Cell signaling technology), P-SRC (Tyr416) Antibody (RRID: AB_331697,
1722 #2101, Cell Signaling technology). Secondary antibodies used were peroxidase AffiniPure goat
1723 anti-rabbit IgG (H+L) mAb (RRID: AB_2307391, #111-035-144, Jackson ImmunoResearch) or
1724 ECL mouse IgG, HRP-linked whole Ab (RRID: AB_772210, #NA931, Amersham). Protein
1725 immunodetection of the membranes was performed with Clarity ECL Western Blot Substrate
1726 (Biorad) in a ChemiDoc MP Imaging System (Biorad).

1727
1728 *Cell-based models for chronic liver disease.* Huh7.5.1 (RRID: CVCL_E049) and LX2
1729 stellate cells (RRID: CVCL_5792) were cultured in Dulbecco's Modified Eagle Medium (DMEM)
1730 containing 10% fetal bovine serum (FBS) and 1% DMSO for differentiation (Huh7.5.1^{dif} cells) as
1731 described(84-86). NTCP-overexpressing HepG2 (HepG2-NTCP, RRID: CVCL_JY40) cells were
1732 selected using puromycin and cultured in DMEM with 10% FBS as previously described(87).
1733 HCV: DMSO-differentiated Huh7.5.1^{dif} cells were plated in 6-well plates and infected with
1734 HCVcc Jc1 (genotype 2a/2a) as described (86). HCV infection was assessed at day 10 by qRT-

1735 PCR of intracellular RNA as described (86). CLDN1 mAb or control mAb (palivizumab
1736 IgG4(53)) (10 µg/mL, respectively) were added for 3 days after HCV infection. HBV: HepG2-
1737 NTCP cells (RRID: CVCL JY40) were plated in 12-well plates and infected with HBV purified
1738 from patient serum(87) in presence of CLDN1 mAb or control mAb (10 µg/mL, respectively).
1739 HBV infection was assessed at day 7 post-infection by qRT-PCR quantification of HBV pre-
1740 genomic RNA (pgRNA)(87). FFA-NASH model: DMSO-differentiated Huh7.5.1^{dif} cells co-
1741 cultured with LX2 cells (20%) were plated in 12-well plates and exposed to FFA (800 µM oleic
1742 acid and 400 µM palmitic acid) for 48 hours as described(88). CLDN1 mAb or control mAb
1743 (10 µg/mL, respectively) were added for 3 days after FFA treatment. Ethanol-ALD model:
1744 DMSO-differentiated Huh7.5.1^{dif} cells were plated in 6-well plates and exposed to ethanol (40
1745 mM) in presence of CLDN1 mAb or control mAb (10 µg/mL, respectively) for 10 days. Fresh
1746 medium containing ethanol and mAbs was replenished daily. Each cell culture model was assessed
1747 in at least three independent experiments, performed in triplicate.

1748
1749 *Analysis of phosphokinase phosphorylation.* Phosphokinase phosphorylation was
1750 assessed in cell lysates derived from the NASH *in vitro* model using the Proteome Profiler Human
1751 Phosphokinase Array Kit (R&D Systems Inc.), according to the manufacturer's instructions.
1752 Phosphokinases were assessed using biotinylated detection antibodies followed by
1753 chemiluminescence detection.

1754
1755 *Pamgene kinase activity profiling in patient-derived spheroids.* Protein tyrosine kinase
1756 (PTK) and serine-threonine kinase (STK) activities in the patient-derived 3D model of fibrosis
1757 were assessed by PamGene Assay (PamGene International BV). Patient-derived spheroids were

1758 established and treated with a profibrogenic cocktail as described above. After the 72h treatment,
1759 4 spheroids were pooled in each biological quadruplicate and proteins were extracted using M-
1760 PER Mammalian Protein Extraction Reagent (ThermoFisher Scientific). Samples were then spun
1761 at 16000g for 15 minutes to remove debris. One μ g protein were applied on PamChip4 arrays
1762 containing 196 (PTK) or 144 (PTK) peptides harboring kinase targets/substrates. Fluorescence-
1763 labelled antibodies against phosphoresidues were used to measure kinase activity in the samples.
1764 Phosphosites were selected for subsequent analyses if their signals were statistically significantly
1765 different between conditions (by t-test or ANOVA). The signal per phosphosite is the result of the
1766 net phosphorylation attributed to the activity of one or more kinases (89, 90). Instrument operation
1767 and imaging were controlled by the EVOLVE 2.0 software and quantified using BioNavigator 6.3
1768 (BN6; PamGene International BV). Signal intensities at multiple exposure times were integrated
1769 by linear regression (S100), Log2-transformed, and normalized using a Combat correction model
1770 for batch correction where the scaling parameters (mean and sd) were estimated using an empirical
1771 Bayesian approach. By interpreting kinase activity at multiple phosphosites, an Upstream Kinase
1772 Analysis (UKA) was performed. Exploiting knowledge on specific kinase-to-substrate
1773 relationships from publicly available databases, UKA algorithm predicts differential kinase
1774 activity in the test condition compared to the control. Kinase statistic represents the change in
1775 kinase activity, indicating inhibition if <0 and activation if >0 . Mean specificity score represents
1776 the specificity of the change in kinase activity, higher scores highlighting a lower chance of
1777 observations descending from random set of peptides(91). The hits of our PamGene assay were
1778 then ordered by Median Final Score, a synthetic measure of both target robustness and
1779 significance. The 20 top hits were plotted in a heatmap comparing Mean Kinase Statistics of our

1780 model vs those of a recently published liver kinome atlas, established with the same technique
1781 (34).

1782
1783 *CLDN1 knockout using CRISPR-Cas9 technology.* Huh7.5.1 stably expressing Cas-9
1784 endonuclease (Huh7.5.1-Cas9) were DMSO-differentiated for 7 days (Huh7.5.1-Cas9^{diff}), and then
1785 either co-cultured with LX-2 stellate cells (RRID: CVCL 5792, 20%) and treated with free fatty
1786 acids (FFA; 800 μ M oleic acid and 400 μ M palmitic acid) or infected using HCV Jc1. After 3
1787 (FFA treatment) or 7 days (HCV Jc1 infection), cells were transduced with lentiviruses expressing
1788 control single guide RNA (sgRNA) or sgRNA targeting *CLDN1* gene expression (sgCLDN1).
1789 Expression plasmids were provided by Dr. David Root (Broad Institute of Harvard and MIT,
1790 Cambridge, USA). Transduced cells were selected under hygromycin treatment (500 μ g/mL) for
1791 3 days and lysed using iScript RT-qPCR sample preparation reagent. The HCV- or FFA-induced
1792 PLS was analyzed using nCounter Nanostring technology in cell lysates. In parallel, cells were
1793 used to analyze *CLDN1* abundance by flow cytometry using a *CLDN1*-specific mAb (H3L3,
1794 10 μ g/mL).

1795
1796 *CLDN1 knockdown using GalNac technology.* The following siRNA sequences were
1797 designed to target human *CLDN1* or used as non-targeting siRNA (CTRL): *siRNA CLDN1*: 5'-
1798 UAACAUUAGGACCUUAGAAUU-3' *siRNA CTRL*: 5'-UAAGGCUAUGAAGAGAUAC-3'.
1799 siRNA were then coupled to GalNac group (Creative Biogene Inc.) for *in vivo* delivery. To validate
1800 the efficacy of GalNac siRNA, Huh7 cells were transfected with 12 pmol of regular siRNA or
1801 GalNac siRNA and the corresponding CTRLs by using lipofectamine RNAi Max (Invitrogen,

1802 Cat#13778-150) following the manufacturer's instructions. Knock-down was validated by
1803 detecting CLDN1 at the cell surface by flow cytometry (see above).

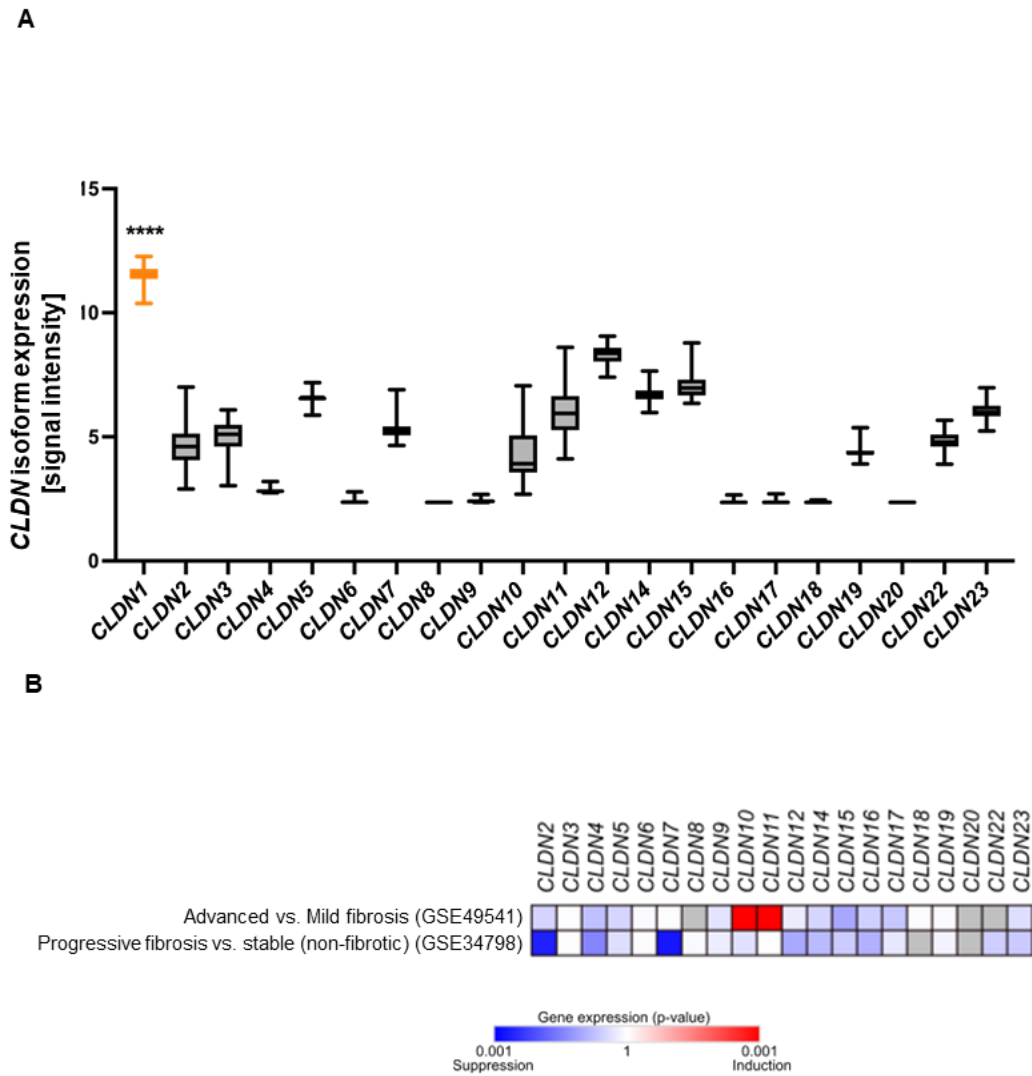
1804
1805 **CLDN1 co-immunoprecipitation.** For co-immunoprecipitation (co-IP), 5.10⁸ Huh7 cells
1806 (RRID: CVCL-0336) were harvested by scraping in cold PBS^{-/-} and plasma membranes were
1807 extracted by using "Plasma Membrane Protein Extraction Kit (Abcam ab65400) according to
1808 manufacturers' instructions. Approximately 100 µg of plasma membranes were resuspended in
1809 250 µL of co-IP buffer (10 mM Tris HCl pH 7.4, .15 M NaCl, 1 mM EDTA, 1 mM EGTA pH 8,
1810 0.1 % NP40, glycerol 10 % in H2O milliQ water supplemented with protease inhibitors). For WB
1811 analysis, 50 µL of the total plasma membrane proteins were conserved and used as "Input" control.
1812 In parallel, 50 µL of magnetic Dynabeads (ThermoFisher) were coupled to 2 µg of CLDN1
1813 antibody targeting the C-terminus part of the protein to capture membrane interactants (rabbit anti-
1814 human CLDN1 antibody, ab211737, Abcam) or CTRL antibody (Recombinant Rabbit IgG,
1815 monoclonal Isotype Control, RRID: AB_2687931, ab172730, Abcam) 10 min at RT. Dynabeads
1816 were then mixed with 150 µL of co-IP buffer and 100 µL of plasma membranes and incubated at
1817 4°C on an orbital shaker. Lasst, Dynabeads were harvested using a magnet (the "Flow-through"
1818 was conserved for WB analysis) and washed 3 times with cold PBS^{-/-}. CLDN1 interactants were
1819 eluted in 1X Laemmli buffer by heating the beads 5 min at 95°C. CLDN1 IP was validated by WB
1820 analysis before analyzing the interactants by mass-spectrometry (IGBMC proteomic platform,
1821 Illkirch-Graffenstaden).

1822
1823 **Single-cell and single nucleus RNAseq analyses.** Single-cell (sc) RNAseq data: Based on
1824 the clustering and data normalization of the whole human liver cell atlas (GSE124395)(15), we

1825 analyzed *CLDN1* expression in different cell types. Thereby, we used RaceID methods to draw
1826 expression t-SNE maps and ggplot2(92) to draw corresponding boxplots of normalized expression
1827 values. Based on the clustering and provided data of the fibrotic liver cell atlas (GSE136103), we
1828 analyzed *CLDN1* expression in different cell types. We used Seurat(93) to generate expression
1829 UMAPs and violin plots, and ggplot2(92) to generate correlation figures. Single nucleus (sn)
1830 RNAseq data: Based on the clustering of the whole snRNA-seq data set as published in(21), we
1831 analyzed *CLDN1* expression using Seurat(93). We extracted all epithelial cell clusters
1832 (hepatocytes, cholangiocytes and bipotent progenitor cells) and performed a pseudotime analysis
1833 applying slingshot(50). Cell types were annotated based on specific marker gene expression
1834 including *ALB* (hepatocytes), *EPCAM* (bipotent progenitor cells), and *CK19* (cholangiocytes). An
1835 expression plot overlayed with the resulting pseudotime trajectories was generated using
1836 ggplot2(92).
1837

1838 **SUPPLEMENTARY FIGURES**

1839



1840

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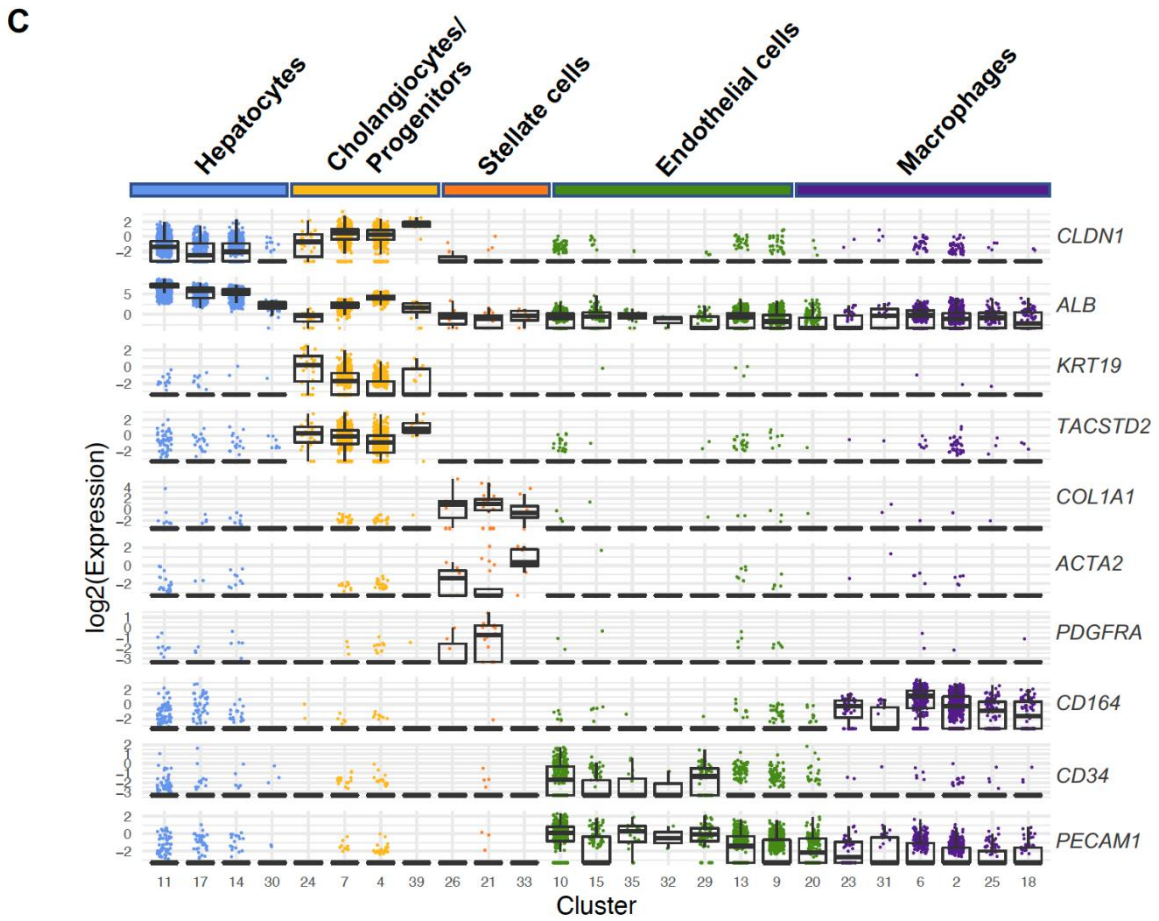
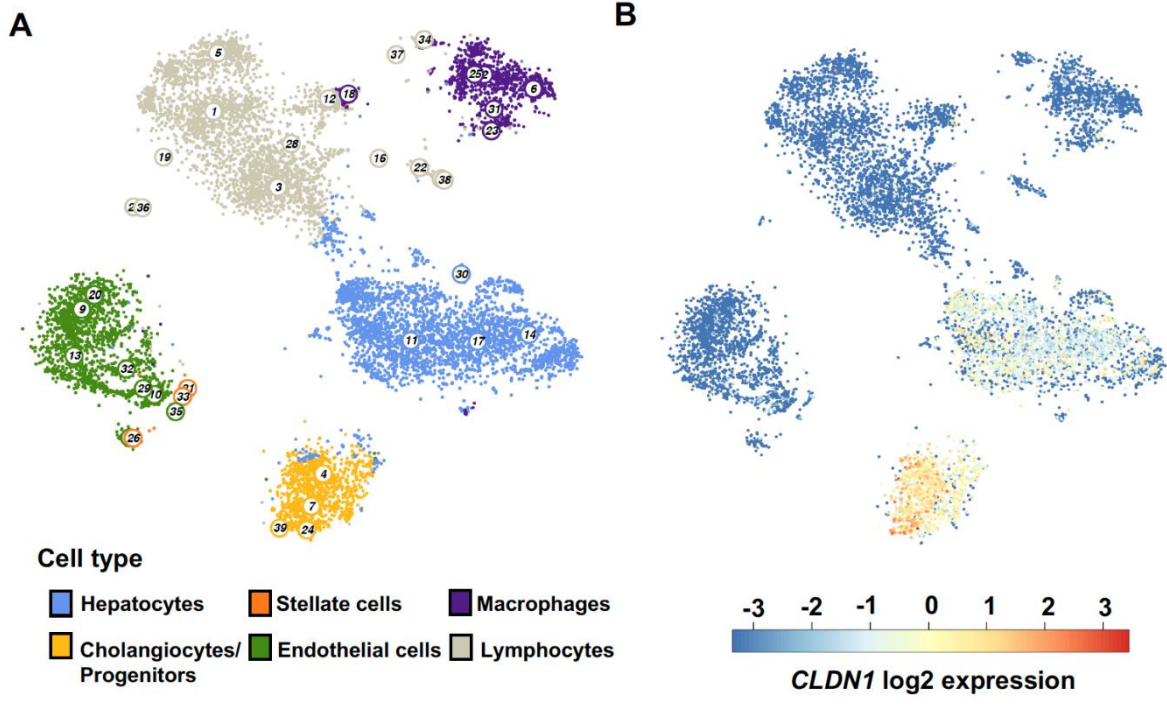
1842 **Figure S1, related to Fig. 1. Expression of different CLDN isoforms in the diseased liver. A.**

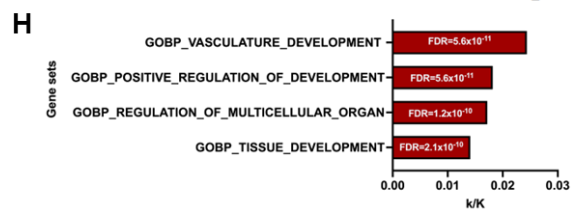
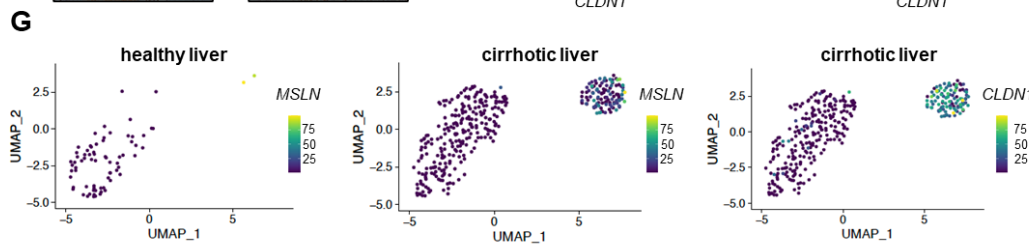
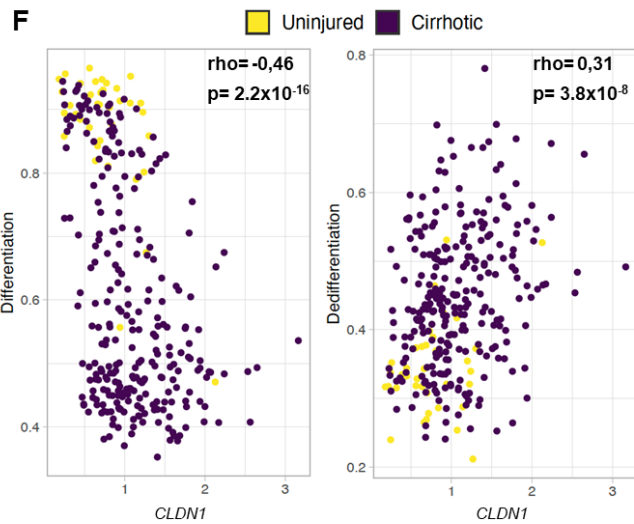
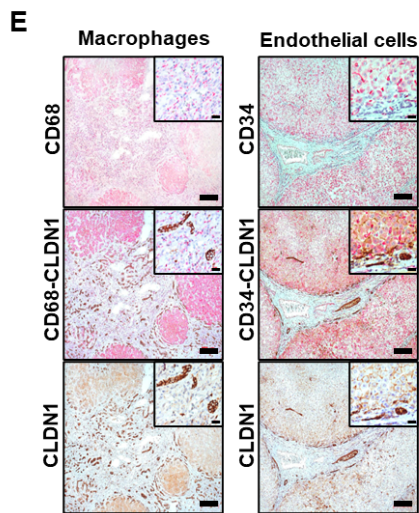
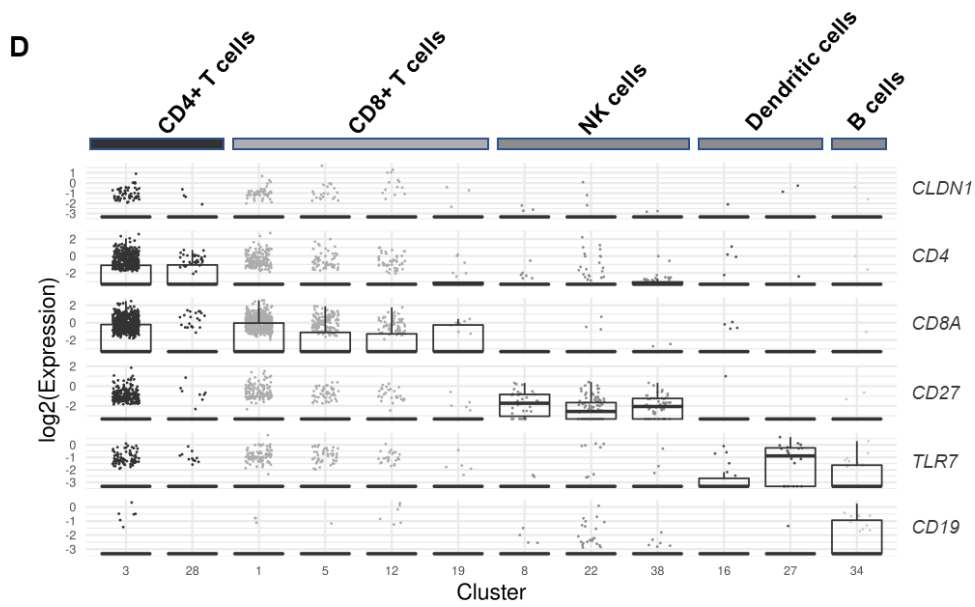
1843 **Expression in NASH fibrotic liver (GSE49541) is shown for all CLDN isoforms. B. Expression of**

1844 **Claudins in livers of patients with NASH with mild (F0-1) or advanced fibrosis (F3-4) (GSE49541,**

1845 **left panel) and liver tissues of HCV-infected patients after liver transplantation with stable or**

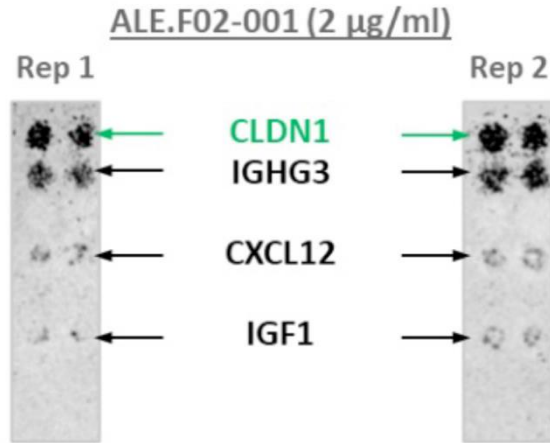
1846 progressive fibrotic disease (GSE34798, right panel). Heatmaps indicate significance of induction
1847 (red) or suppression (blue) compared to control tissue samples of the indicated cohorts. Grey boxes
1848 indicates that the gene is not covered in the data provided. ****p<0.0001, Kruskal-wallis test.
1849 Abbreviations: *CLDN*= Claudin.
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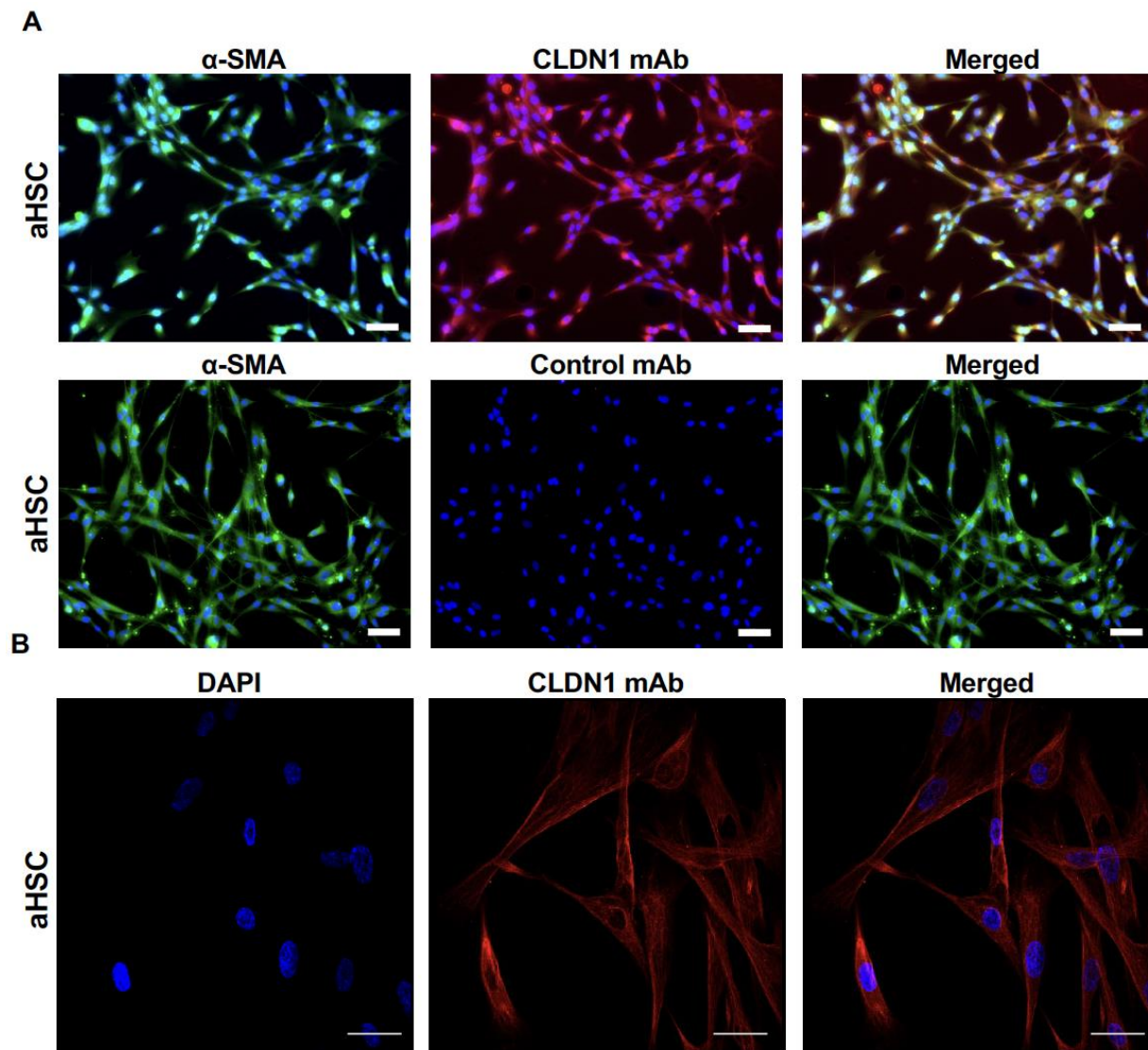
1853 **Figure S2, related to Fig. 1. *CLDN1* expression in different liver cell types analyzed**
1854 **by scRNAseq. A.** t-SNE map of the human liver cell atlas (GSE124395). **B.** *CLDN1* expression
1855 **in the human liver cell atlas (GSE124395).** **C.** *CLDN1* and marker gene expression in specific
1856 **parenchymal and non-parenchymal cell types of the human liver (GSE124395).** **D.** *CLDN1* and
1857 **marker gene expression in lymphoid cells of the human liver (GSE124395).** **E.** Double staining of
1858 ***CLDN1* and CD68 (left panel) or CD34 (right panel) by immunohistochemistry indicates absent**
1859 **expression of *CLDN1* in endothelial cells and macrophages. Scale bars indicate 200 μ m (large**
1860 **image) and 100 μ m (small image), respectively.** **F.** Negative or positive correlation of *CLDN1*
1861 **expression with grade of differentiation ($r= -0.46$, $p < 2.2 \times 10^{-16}$, gene set**
1862 **“AIZARANI_LIVER_C14_HEPATOCYTES_2”, left panel) or de-differentiation ($r= 0.31$,**
1863 **$p=3.75 \times 10^{-8}$, gene set “AIZARANI_LIVER_C39_EPCAM_POS_BILE_DUCT_CELLS_4”,**
1864 **right panel).** The grade of differentiation or de-differentiation corresponds to the extent of
1865 **enrichment of gene sets related to mature hepatocytes or *EPCAM*⁺ bile duct cells(15) as published**
1866 **in MSigDB(49). Only cells with *CLDN1* expression covered were included.** **G.** UMAPs of
1867 ***Mesothelin (MSLN)* and *CLDN1* expression in healthy or fibrotic liver (GSE136103(16)) is shown.**
1868 **H.** Overlap of *CLDN1* co-expressed genes in human fibroblasts (E-MTAB-10324) with gene sets
1869 **related to tissue development and differentiation is shown. Abbreviations: *ACTA2*= alpha smooth**
1870 **muscle actin; *ALB*=Albumin; *CD*= cluster of differentiation; *COL1A1*= Collagen 1A1; *CLDN1*=**
1871 ***Claudin 1*; *KRT19*= Keratin 19; *MSLN*= Mesothelin; NK cells= Natural Killer cells; *PDGFRA*=**
1872 **platelet derived growth factor receptor alpha; *TLR7*= Toll-like receptor 7.**

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Gene Id	ALE.F02-001 interaction detected?
CLDN1	Yes
CLDN2	No
CLDN3	No
CLDN4	No
CLDN5	No
CLDN6	No
CLDN7	No
CLDN8	No
CLDN9	No
CLDN10	No
CLDN11	No
CLDN12	No
CLDN14	No
CLDN15	No
CLDN16	No
CLDN17	No
CLDN18	No
CLDN19	No
CLDN20	No
CLDN22	No
CLDN23	No
CLDN24	No
CLDN25	No
CLDN34	No

1874 **Figure S3, related to Fig. 1. CLDN1 mAbs are highly specific for human CLDN1.**
1875 **Interaction of CLDN1 mAbs (representatively shown for ALE.F02) with human plasma membrane**
1876 **and secreted proteins, as assessed by Retrogenix assay is shown. A strong positive signal was only**
1877 **detected for hCLDN1 and the IgG heavy chain. No cross-reactivity was found for >5000 other**
1878 **proteins tested. Minor non-specific interactions were found for CXCL12 and IGF1. Abbreviations:**
1879 **CLDN=Claudin; CXCL12=C-X-C Motif Chemokine Ligand 12; IGHG3=Immunoglobulin Heavy**
1880 **Constant Gamma 3; IGF=Insulin like growth factor 1; Rep=Replicate.**
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1883 **Figure S4, related to Fig. 1. Non-junctional CLDN1 expression in activated stellate**
 1884 **cells. A. Representative images of CLDN1 mAb H3L3 binding to patient derived HLMFs, as**
 1885 **assessed by immunofluorescence. Scale bars indicate 100 μ m. B. Representative confocal**
 1886 **microscopic images CLDN1 mAb H3L3 binding to patient derived HLMFs. Scale bars indicate**
 1887 **100 μ m. Abbreviations: α -SMA= alpha smooth muscle actin; CLDN1= Claudin 1; aHSC=**
 1888 **activated Hepatic stellate cells.**

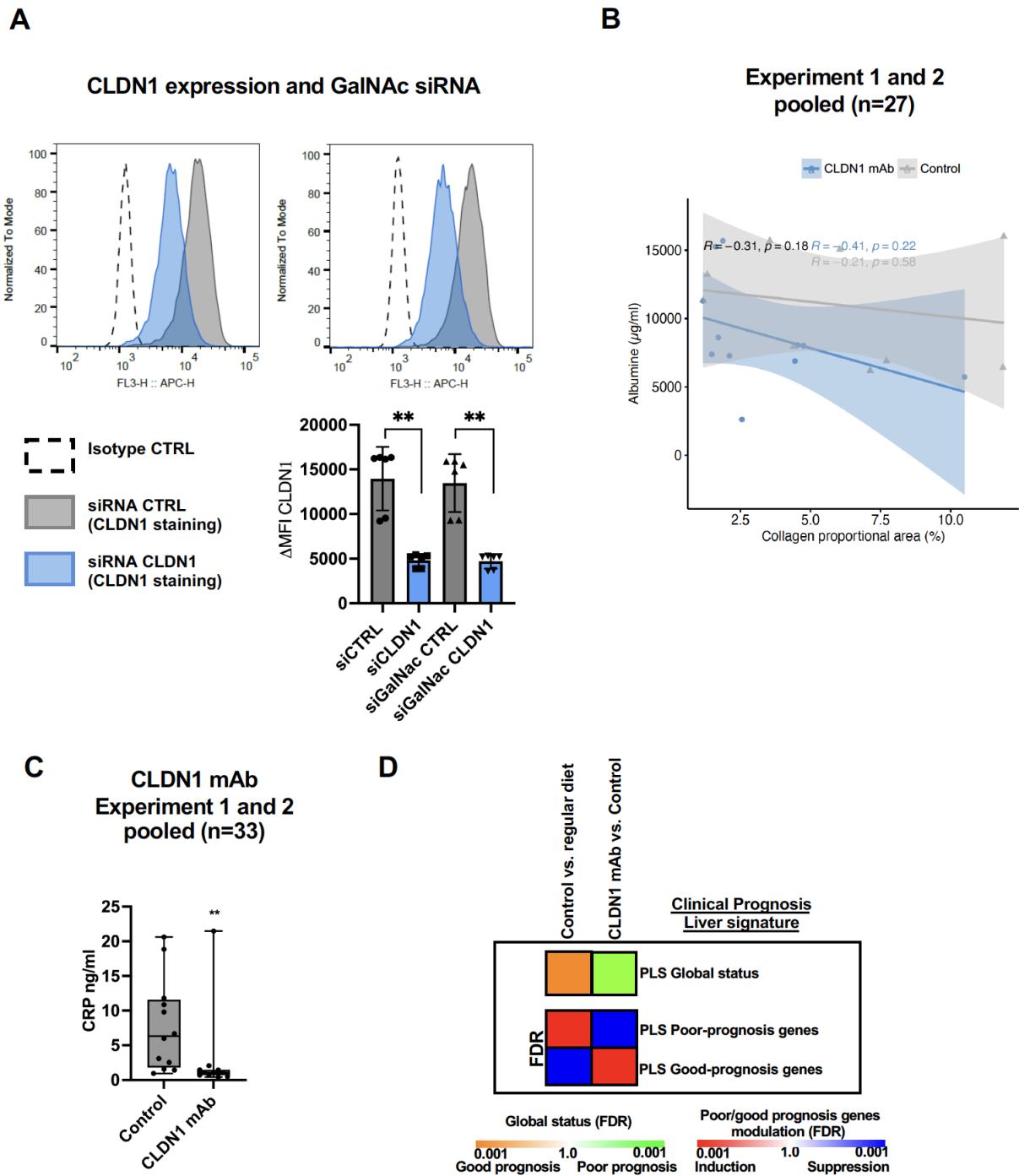


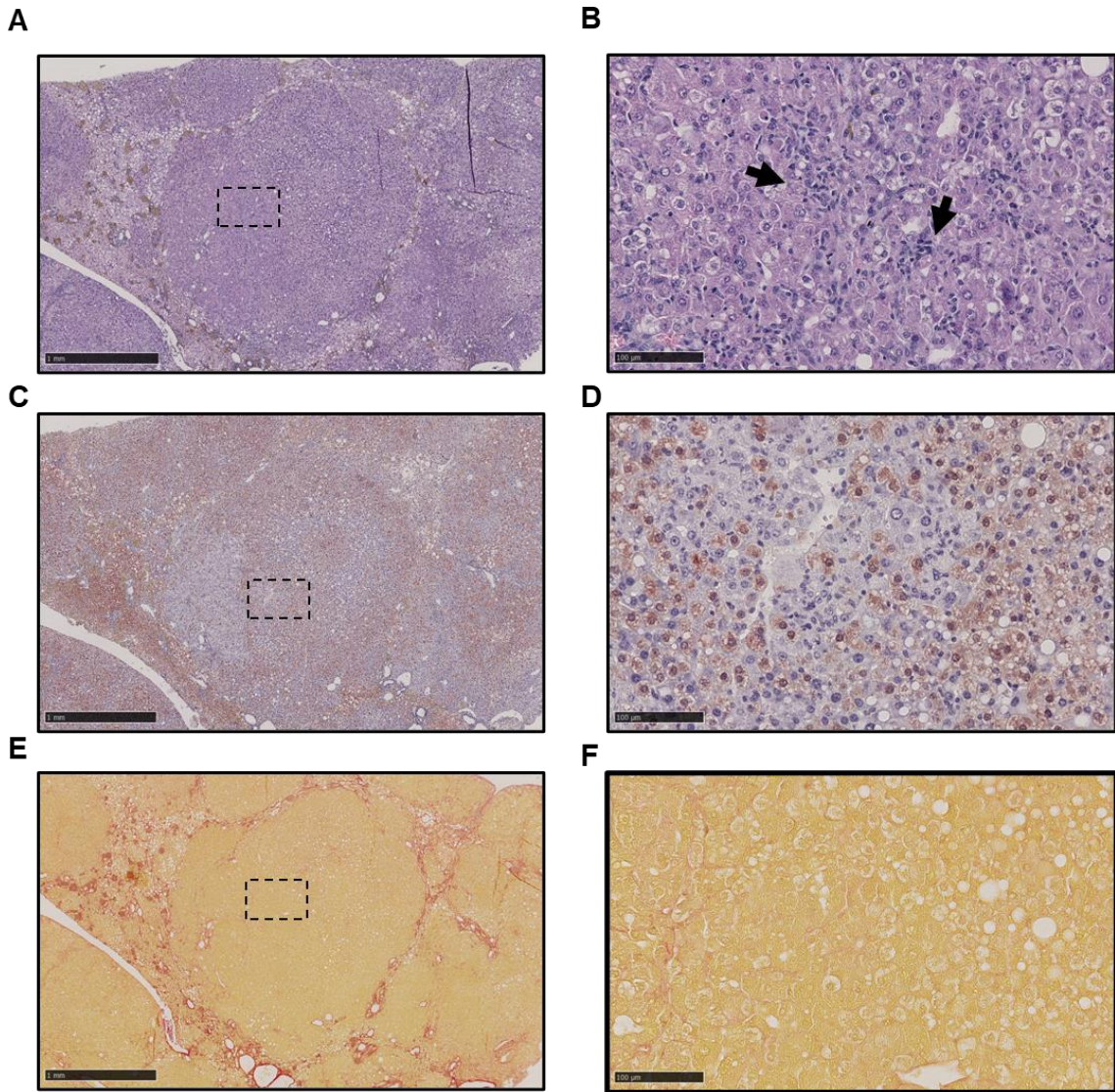
Figure S5 related to Fig. 2. Validation of cell surface CLDN1 knockdown by GalNac

siRNA in Huh7 liver cells. A. Effect of GalNac siRNA mediated suppression of CLDN1

expression and validation in Huh7 cells. CLDN1 protein expression following siCLDN1 or

1894 GalNAc-siCLDN1 mediated knockdown in Huh7 cells was assessed by flow cytometry (n=2
1895 independent experiments with n=3 biological replicates per condition). Graphs shows Δ MFI of
1896 CLDN1 mAb compared to Control mAb binding to the respective cells. ** p<0.01, U-test. **B.**
1897 Humanization and fibrosis in humanized liver fibrosis mice. Similar baseline levels of human
1898 albumin in both treatment groups and absent correlation of humanization with observed
1899 antifibrotic effects (total collagen proportional area) is shown. **C.** Effect of CLDN1 mAb treatment
1900 on serum C-reactive protein. Significant reduction of plasma CRP levels in CLDN1 mAb treated
1901 mice compared to control. Pooled data of experiments 1 and 2 are shown, control n=12, CLDN1
1902 mAb n=11. ** p<0.01, KW test. **D.** Modulation of PLS to good (green) or poor (orange) prognosis
1903 status(25)in liver tissues of humanized NASH fibrosis mice treated with CLDN1 mAb or control.
1904 The significance (FDR, Kolmogorov-Smirnov test) of induction (red) or suppression (blue) of PLS
1905 poor- or good-prognosis genes is illustrated below. Abbreviations: CLDN1= Claudin 1; CRP= C-
1906 reactive protein; FDR= False discovery rate; PLS= prognostic liver signature.

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Figure S6, related to Fig. 2. Human liver chimeric mice fed with CDA-HFD diet show expansive regenerative nodules of hepatocytes with reactive ductules consisting of mouse and human hepatocytes. A-B. H&E staining at low (A) and high (B) magnification showing an expansive nodule consisting of nodular growing mouse hepatocytes (negative for FAH see C and D), reactive ductules (arrow) and human hepatocytes (positive for FAH see C and D). C-D. Human hepatocytes identified by FAH staining in the chimeric liver are shown at low (A) and high (B)

1915 magnification. E-F. Sirius Red staining at low (A) and high (B) magnification showing less
1916 fibrosis in the nodule suggesting a higher proliferation rate of cells in nodules compared to the
1917 surrounding liver. Scale bar in A, C and E =1 mm. Scale bar in B, D and F=100 μm. Rectangles
1918 in the left panels correspond to the area illustrated in the right panels. Abbreviations: CDA-HFD
1919 = choline-deficient, L-amino acid-defined, high fat diet.

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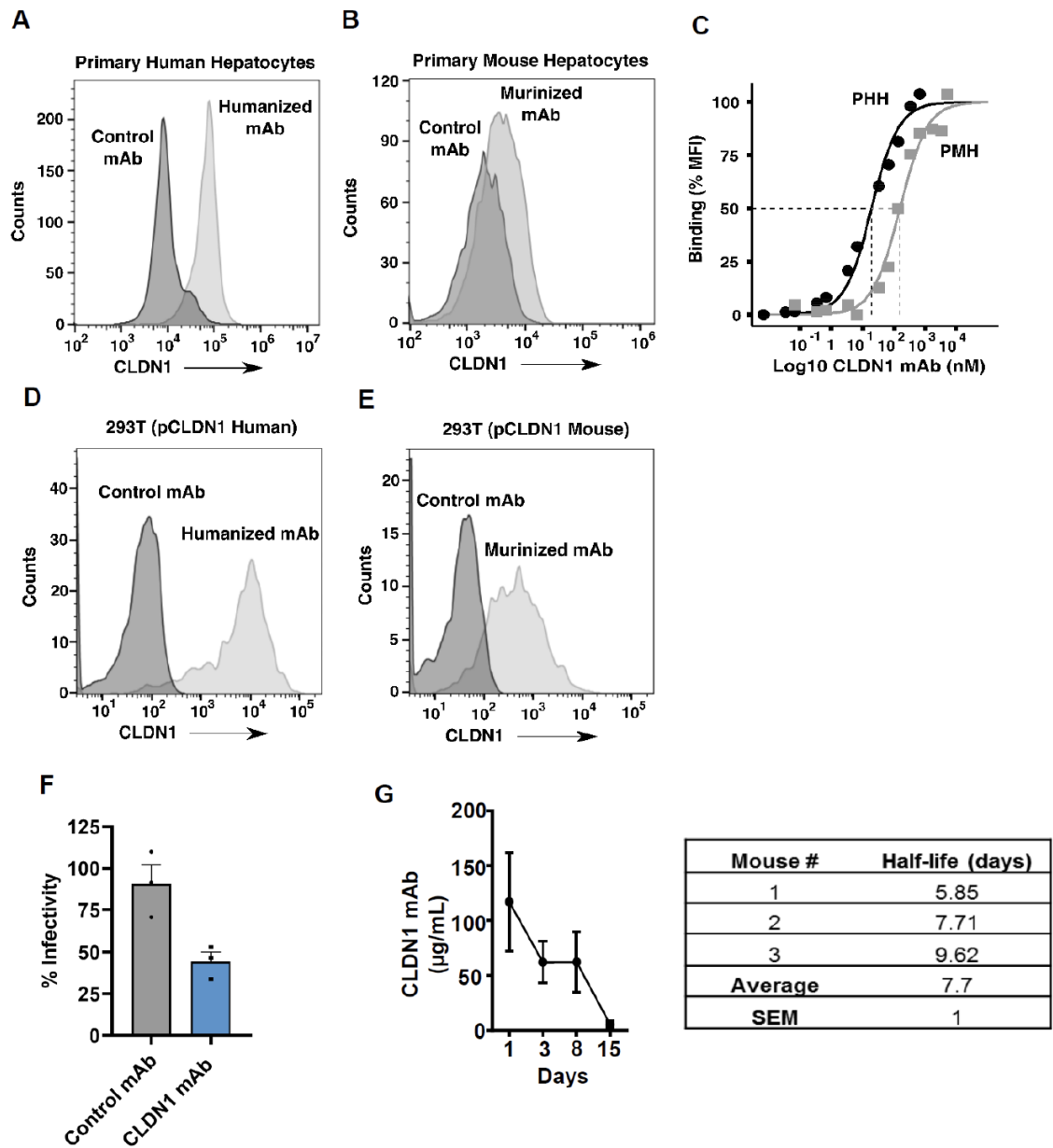
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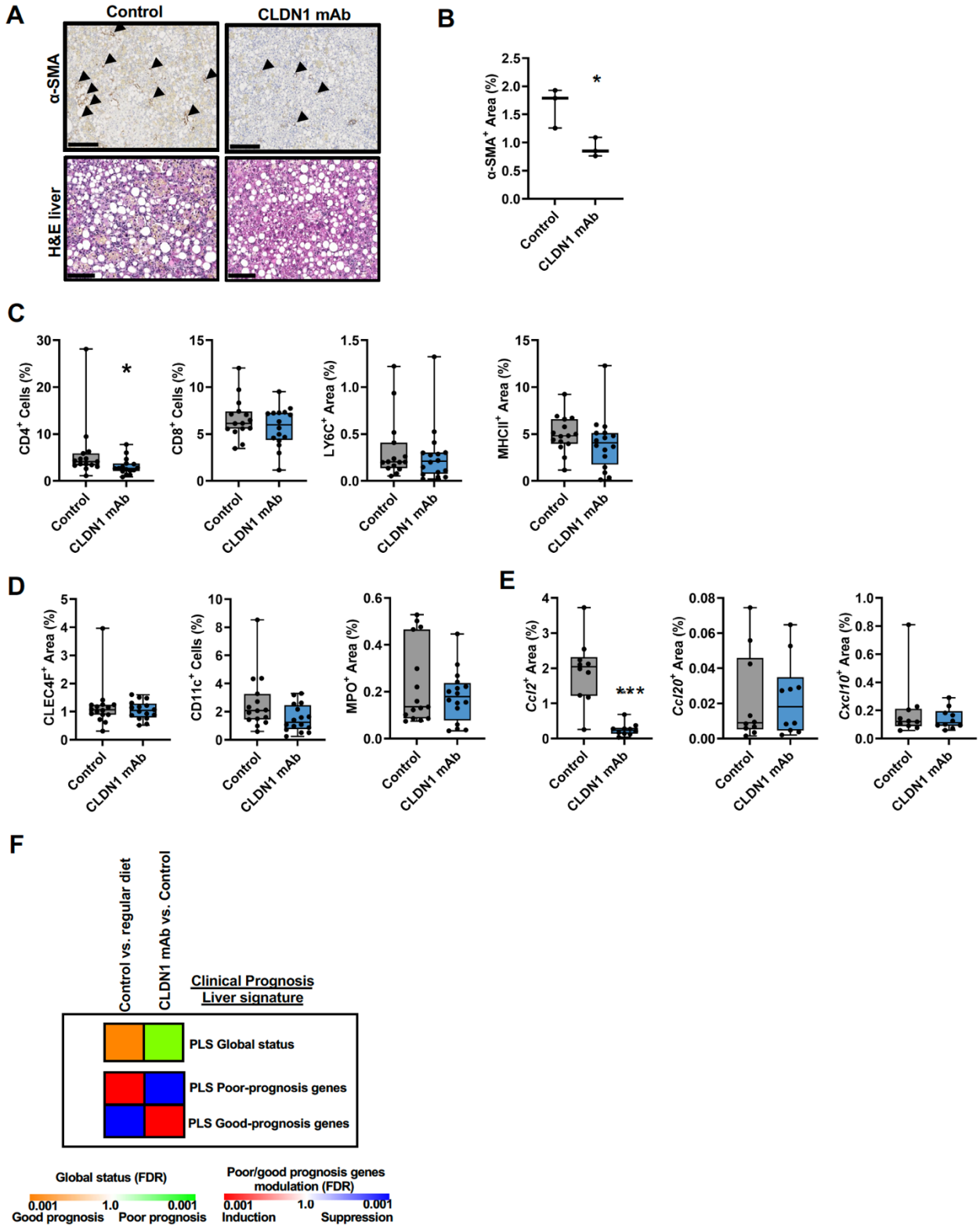
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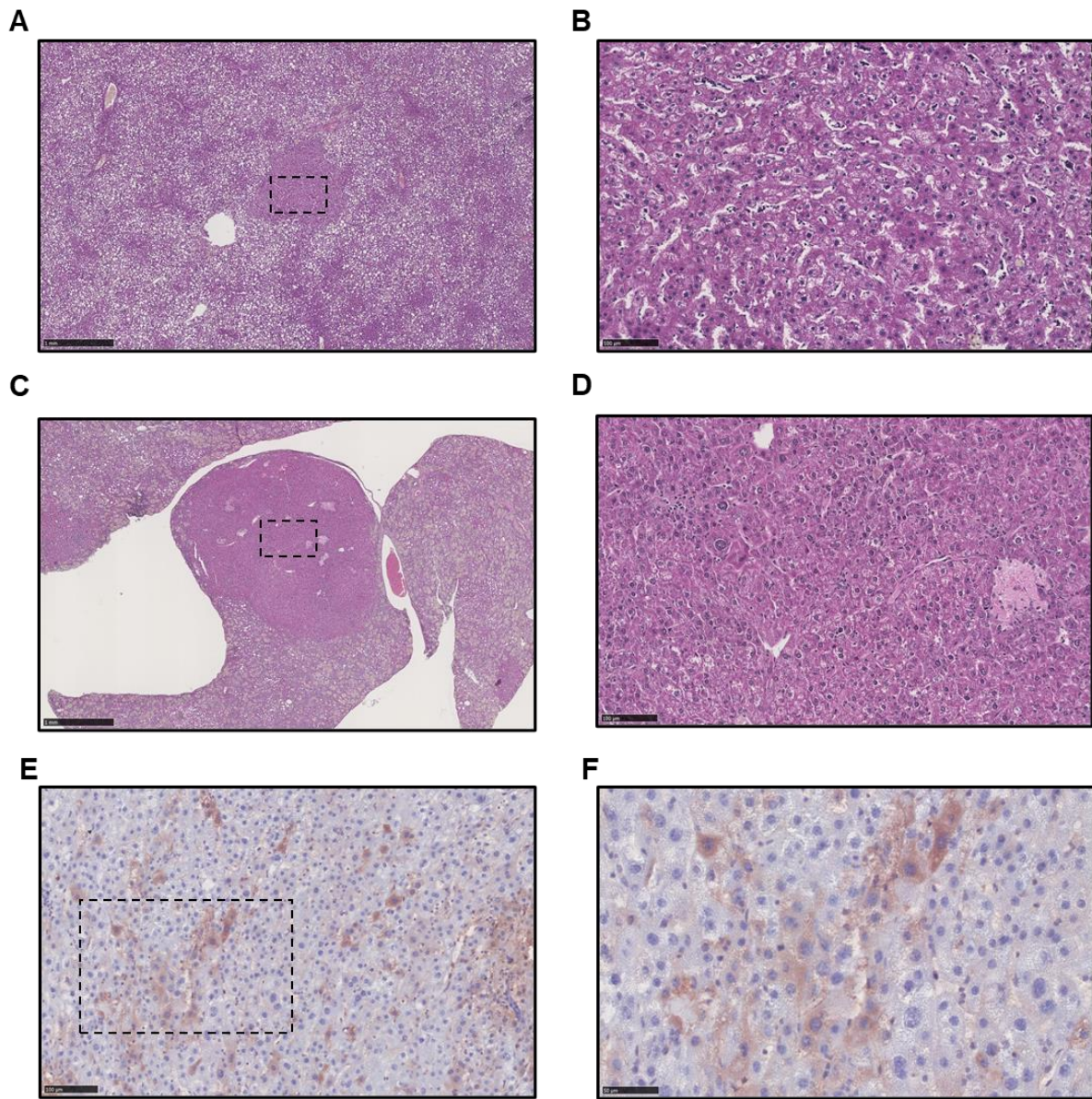
Figure S7, related to Fig. 3. Functional assessment and pharmacokinetics of the murinized and humanized anti-human CLDN1-specific mAb. A-B. Binding of humanized anti-CLDN1 mAb H3L3 or murinized CLDN1 mAb to CLDN1 expressed on primary human (PHH) (A) or mouse hepatocytes (PMH) (B) as assessed by flow cytometry is shown **C**. The binding kinetics of the interaction between humanized or murinized mAb against human or mouse

1932 CLDN1 expressed on PHH and PMH were determined by applying the Michaelis-Menten
1933 mathematical model (PHH: black, apparent Kd of \approx 19 nM; PMH: grey, apparent Kd of \approx 154 nM),
1934 respectively. D-E. The humanized and murinized CLDN1 mAb show robust binding to 293T cells,
1935 engineered to express human or murine CLDN1 (mCLDN1), respectively. F. mCLDN1 expressing
1936 293T cells were incubated with a murinized CLDN1 mAb (100 μ g/mL) for 1 h at 37 $^{\circ}$ C prior to
1937 incubation with HCV pseudoparticles bearing glycoproteins JHF1 genotype 2a of HCV. HCVpp
1938 entry into 293T cells was assessed by measuring luciferase activity after 72 h and is shown as
1939 percentage relative to entry into untreated cells. *p<0.05, Student's t-test. G. Left panel: Serum
1940 concentrations of the murinized CLDN1 mAb were determined at the indicated time points after a
1941 single i.p. injection of 500 μ g (25 mg/kg) of murinized mAb into three C3H mice. Right panel:
1942 The half-life of the murinized CLDN1-specific is shown, as determined using regression curve
1943 analyses. Results in A-E are representative for at least 3 independent experiments with biological
1944 duplicates per condition. Abbreviations: SEM=standard error of the mean; PHH=primary human
1945 hepatocytes; PMH=primary mouse hepatocytes.

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1954 **Figure S8, related to Fig. 3. Analyses of liver inflammatory changes and effects on**
1955 **the prognostic liver signature (PLS) following CLDN1 mAb treatment in DEN-CDAHFD**
1956 **mice. A. Representative histological images of myofibroblast activation (upper panel) and**
1957 **steatosis (bottom panel) in mouse livers. B. Quantitative assessment of α -SMA proportional areas**
1958 **in treatment groups. C. Immunostaining shows significant suppression of CD4+ T cell infiltration**
1959 **in murine livers of mice treated with CLDN1 mAb (p=0.02, U-test, left panel). No effect was**
1960 **observed on presence of CD8+ T cells, LY6C+ macrophages and MHCII+ antigen presenting cells.**
1961 **D. Immunostaining did not reveal any significant changes in the quantity of CLEC4F+ Kupffer**
1962 **cells, CD11c+ dendritic cells and MPO+ neutrophils in CLDN1 mAb versus control treated mice.**
1963 **E. Cytokine in situ hybridization reveals significant suppression of *Ccl2* expression in CLDN1**
1964 **mAb treated DEN-CDAHFD mice (p=0.0003, U-test, left panel), but no effect of *Ccl20* and**
1965 ***Cxcl10*. F. Modulation of PLS to good (green) or poor (orange) prognosis status(25) in liver tissues**
1966 **of DEN-CDAHFD mice treated with CLDN1 mAb or control. The significance (FDR,**
1967 **Kolmogorov-Smirnov test) of induction (red) or suppression (blue) of PLS poor- or good-**
1968 **prognosis genes is illustrated below. Abbreviations: α -SMA= alpha smooth muscle actin; *Ccl2*=**
1969 **chemokine ligand 2; *Ccl20*= chemokine ligand 20; CD= Cluster of differentiation; CLDN1=**
1970 **Claudin 1; *Cxcl10*= C-X-C motif chemokine 10; CLEC4F= C-Type Lectin Domain Family 4**
1971 **Member F; FDR= False discovery rate; H&E= Hematoxylin and eosin; LY6C= lymphocyte**
1972 **antigen 6 complex, locus C; MHCII= major histocompatibility complex II; PLS= Prognostic liver**
1973 **signature.**



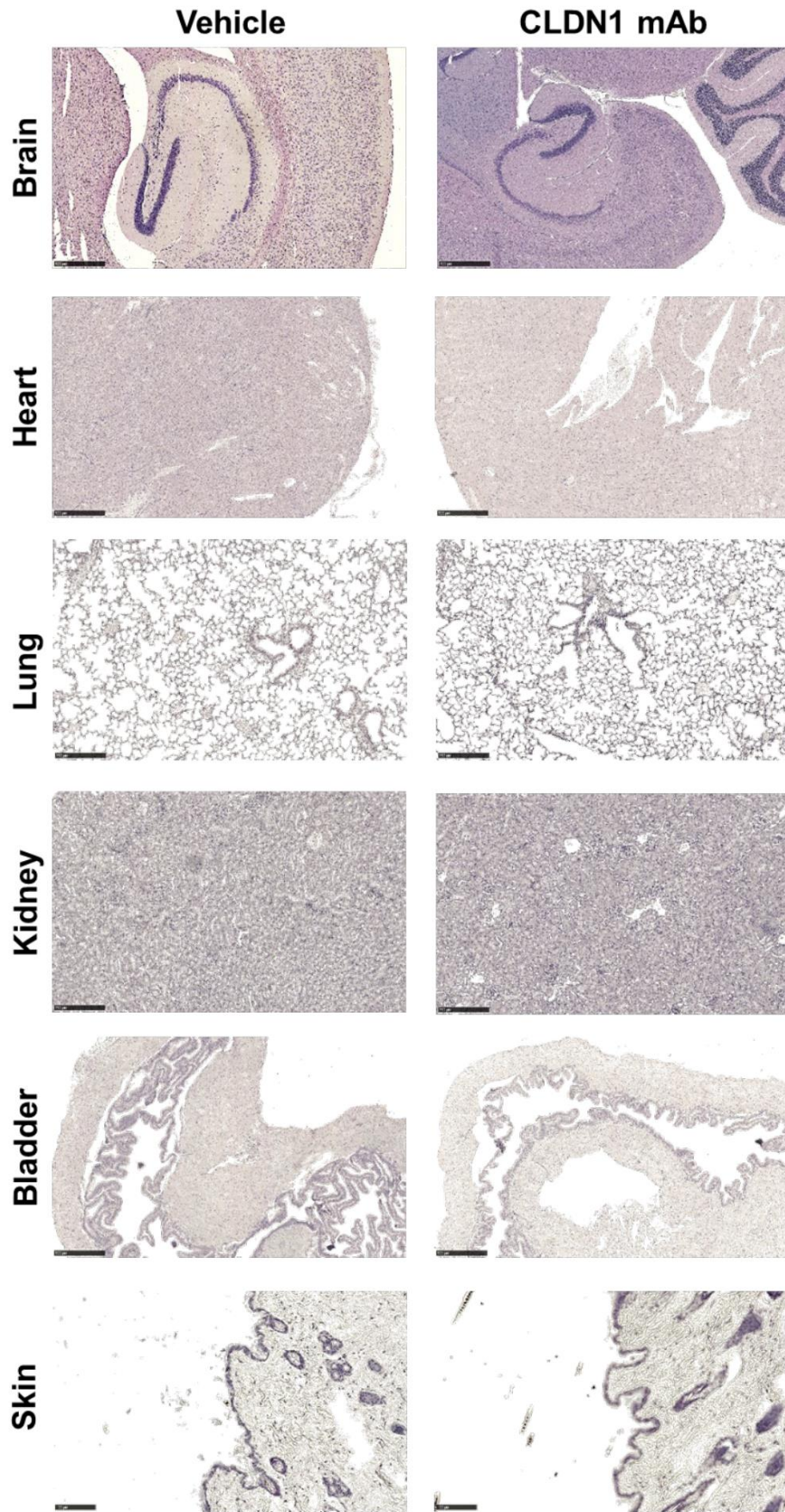
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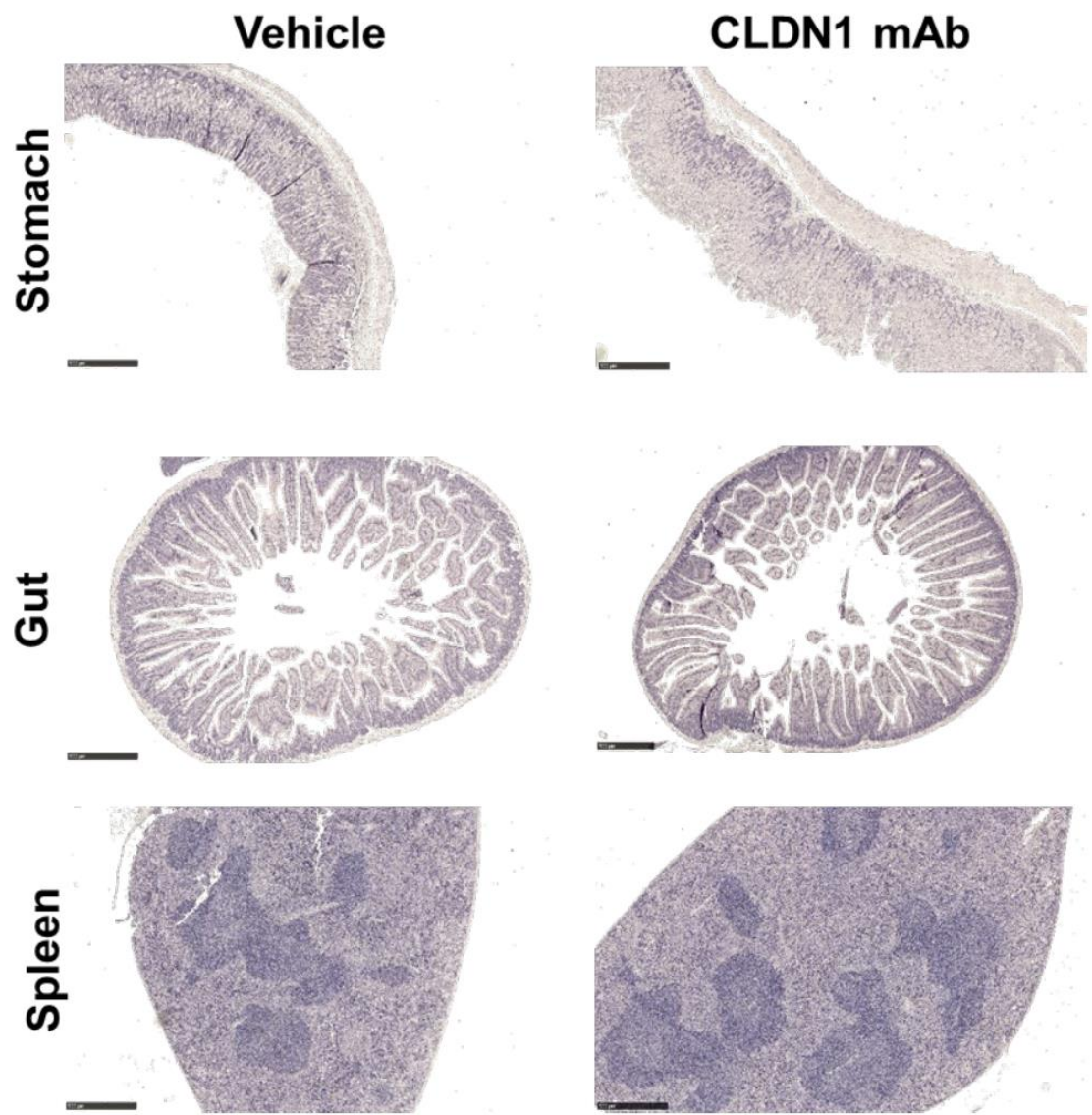
Figure S9, related to Fig. 3. DEN-CDAHFD mice develop nodules consisting of HCC and preneoplastic lesions. A-B. Example of a preneoplastic lesion at low (A) and high (B) magnification. H&E staining shows an irregular nodule with more compact growth pattern and conserved 1-cell thick liver cell plates. Portal tracts are missing in the nodule. C-D. Example of HCC nodule at low (A) and high (B) magnification. H&E staining shows an expansive nodule

1983 with compact growth pattern with thickened liver cell plates, anisokaryosis of hepatocytes and the
1984 absence of portal tracts. E-F. Example of HCC nodule positive for HSP70 by
1985 immunohistochemistry at low (E) and high magnification (F). Scale bar in A and C=1 mm. Scale
1986 bar in B, D and E=100 μ m. Scale bar in F=50 μ m. Rectangles in the left panels correspond to the
1987 area illustrated in the right panels.

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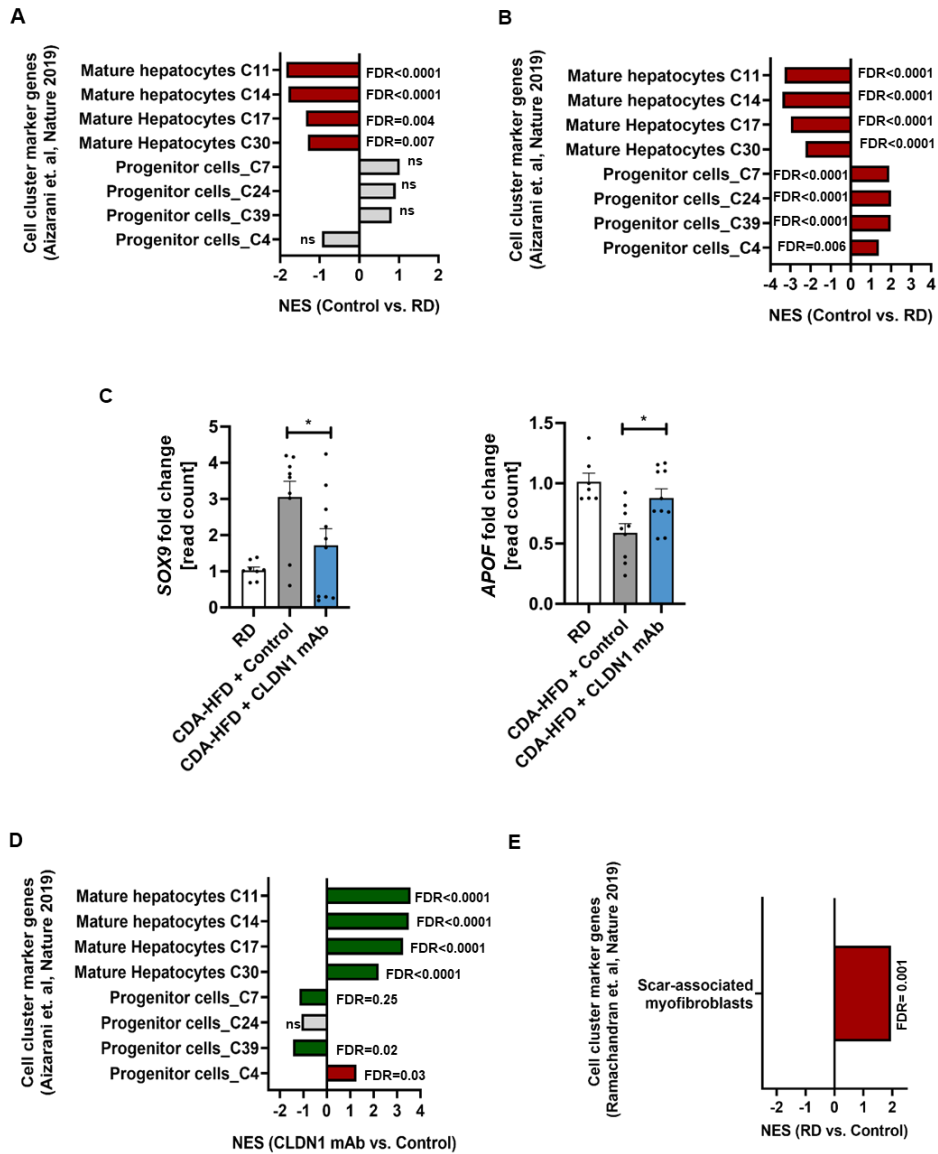
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Figure S10, related to Fig 3. Histopathology of organs in DEN-CDA-HFD mouse model treated with CLDN1-specific mAb or vehicle control for 16 weeks. All the organs were fixed in formalin, embedded in paraffin, stained by hematoxylin and eosin and analyzed by an expert veterinary pathologist from Pheomonin-ICS, Illkirch, France. One-hundred-twenty-eight

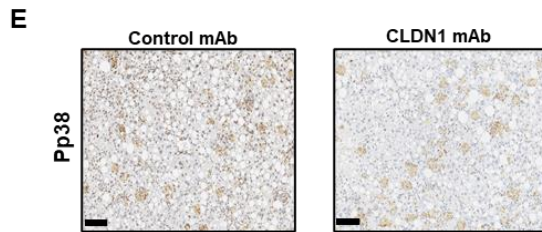
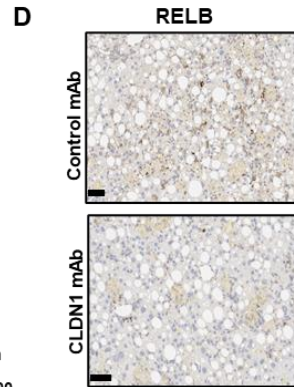
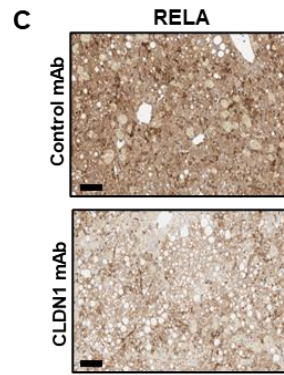
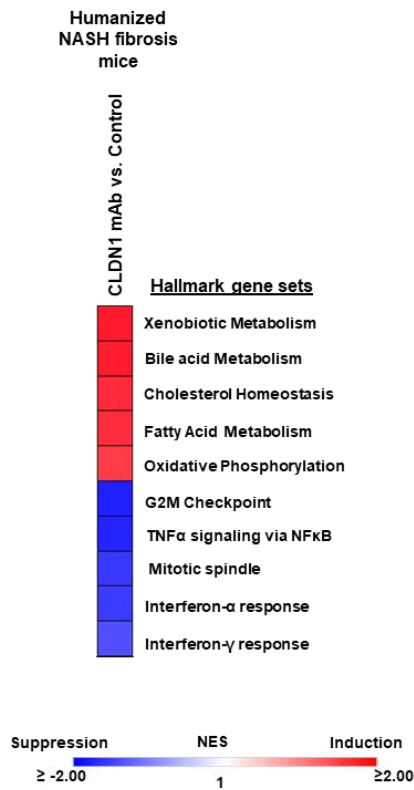
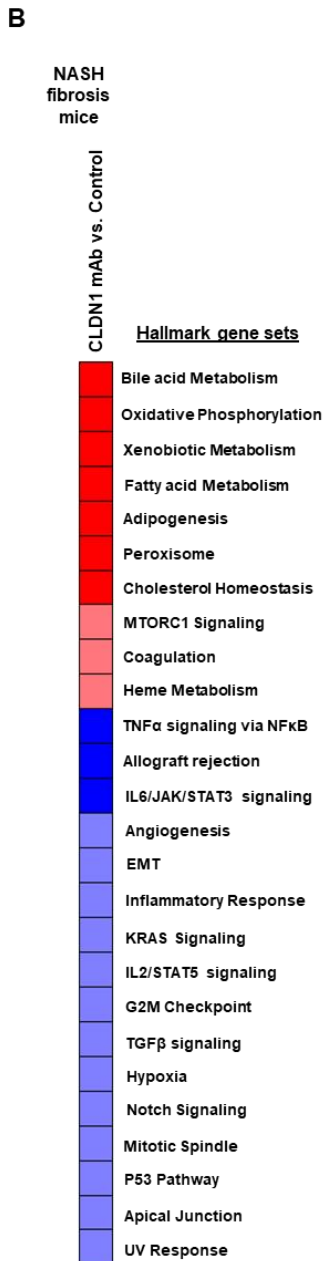
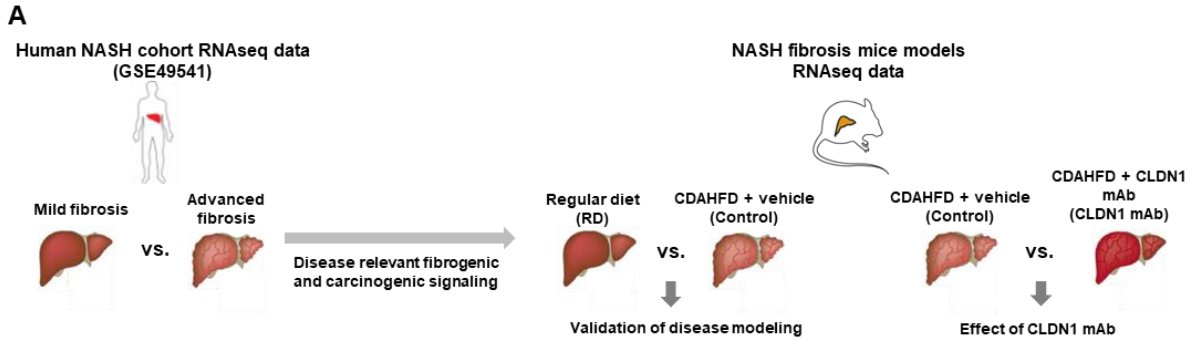
1996 histological slides were analyzed. Eosin was weak on some sections (as shown in the brain image
 1997 of the vehicle control group) without affecting the quality of the analysis.



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1999 Figure S11, related to Fig. 5. CLDN1 mAb affects cell plasticity *in vivo* and in cell-
 2000 based model systems. A-B. Differential expression of gene sets characterizing mature hepatocytes
 2001 ((15) and MSigDB: AIZARANI LIVER C11/C14/C17C30 HEPATOCYTES) and immature
 2002 progenitor cells ((15) and

2003 MSigDB:AIZARANI LIVER C4/C7/C24/C39 EPCAM POS BILE DUCT CELLS) in
2004 healthy (RD) versus fibrotic livers in the humanized (A) and classical NASH fibrosis mouse model
2005 (B) is shown. C. Effect of CLDN1 mAb on progenitor/stem cell marker *SOX9* or *Sox9* and mature
2006 hepatocyte marker *APOF* or *Apof* in humanized and DEN-CDA-HFD mice respectively (pooled
2007 data, humanized mice: RD, n=3; CDA-HFD+Control, n=3, CDA-HFD+ CLDN1 mAb, n=4; DEN-
2008 CDA-HFD mice: RD, n=5, CDA-HFD+Control, n=6, CDA-HFD+ CLDN1 mAb, n=6, p=0.05 and
2009 p=0.04, U-test, respectively). D. Effect of CLDN1 mAb on hepatocyte de-differentiation in NASH
2010 fibrosis mice. E. Modulation of gene sets characterizing scar-associated myofibroblasts (table S9)
2011 in healthy (RD) versus fibrotic livers in the classical NASH fibrosis mouse model. Colored
2012 horizontal bars indicate NES of significantly (FDR<0.25, Kolmogorov-Smirnov test, respectively)
2013 altered gene sets. Vertical bars show mean \pm SEM. *p<0.05, t-test, respectively. Abbreviations:
2014 CLDN1= Claudin 1; FDR= False discovery rate; NASH= Non-alcoholic steatohepatitis; RD=
2015 Regular diet; NES= Normalized enrichment score.



2025 Figure S12, related to Fig. 5. CLDN1 mAb affects cell signaling *in vivo*. A. Graphical
2026 illustration of methodological approach to assess fibrosis associated signaling in NASH mouse
2027 models. B. Unbiased assessment of Hallmark gene sets by GSEA in two NASH fibrosis mouse
2028 models. Heatmaps indicate NES of significantly (FDR <0.05) altered gene sets. C-E.
2029 Representative immunohistochemistry of RELA, RELB and phospho-p38 in CLDN1 or Control
2030 mAb treated NASH fibrosis mice.

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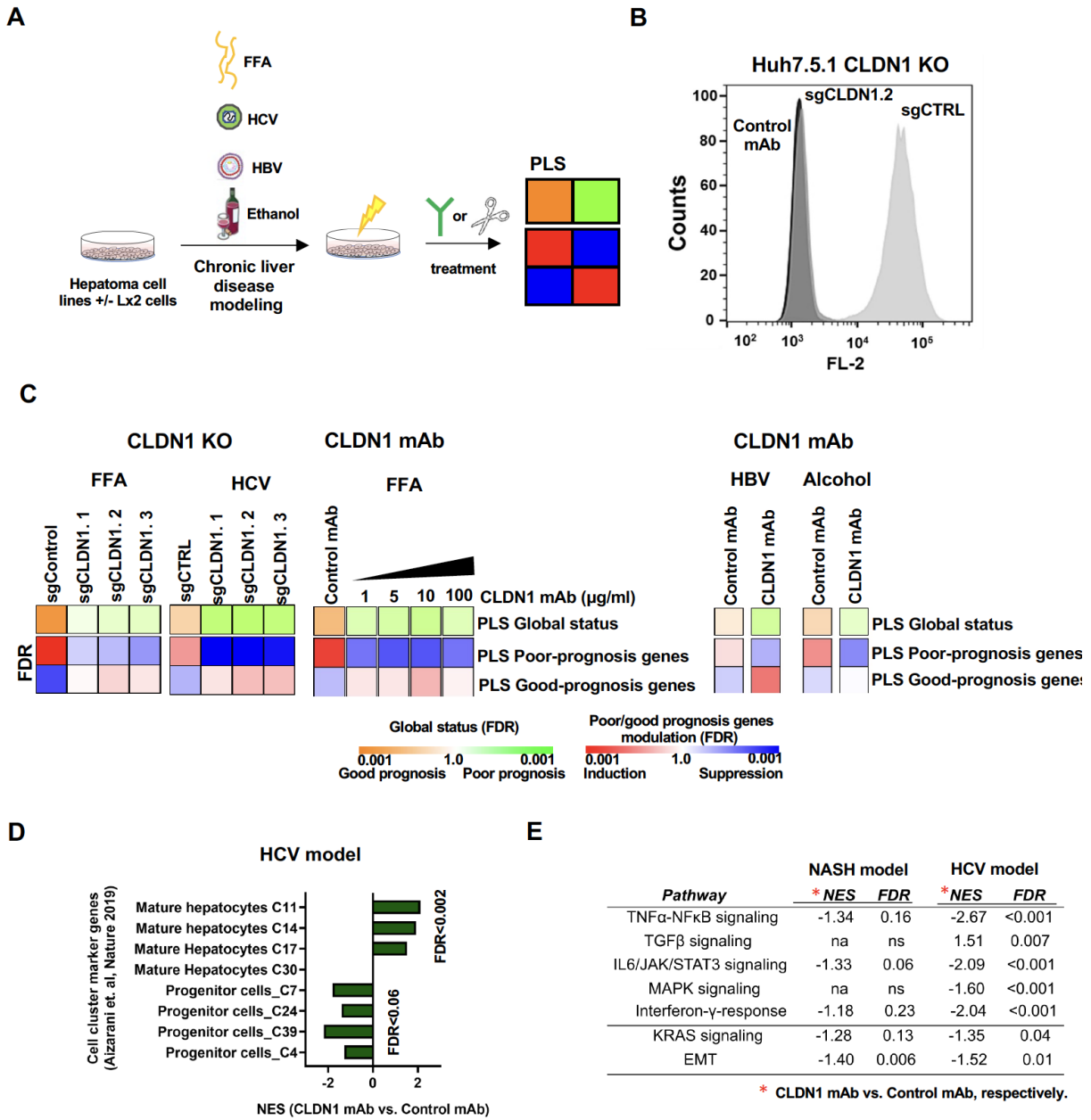


Figure S13, related to Fig. 5. CLDN1 mAb affects cell circuits relevant for patient prognosis in cell-based model systems. A. Graphical illustration of PLS assessment in *in vitro* models of all major etiologies of chronic liver disease. **B.** Absent binding of humanized CLDN1

2052 mAb to Huh7.5.1-Cas9 cells expressing single guide RNAs (sgRNAs), assessed by flow cytometry
2053 is shown. C. Modulation of PLS to good (green) or poor (orange) prognosis status in sgCLDN1 or
2054 sgCTRL transfected- as well as CLDN1 mAb or control mAb-treated *in vitro* models of NASH,
2055 alcoholic liver disease, HBV and HCV infection compared to Mock cells. The significance (FDR,
2056 Kolmogorov-Smirnov test) of induction (red) or suppression (blue) of PLS poor- or good-
2057 prognosis genes is illustrated below. D. Modulation of liver progenitor and mature hepatocyte
2058 related gene sets in Huh7.5.1^{dif} infected with HCV and treated with CLDN1 mAb or control mAb.
2059 E. Modulation of fibrosis- and carcinogenesis-associated signaling pathways by CLDN1 mAb in
2060 the HCV and NASH *in vitro* model. Abbreviations: HBV= Hepatitis B Virus; HCV= Hepatitis C
2061 virus; EMT= epithelial-mesenchymal transition; FDR= False discovery rate; FFA= Free fatty
2062 acids; KO= Knockout; NES= Normalized enrichment score; PLS= Prognostic Liver Signature;
2063 SEM= Standard error of the mean; sg= single guides.

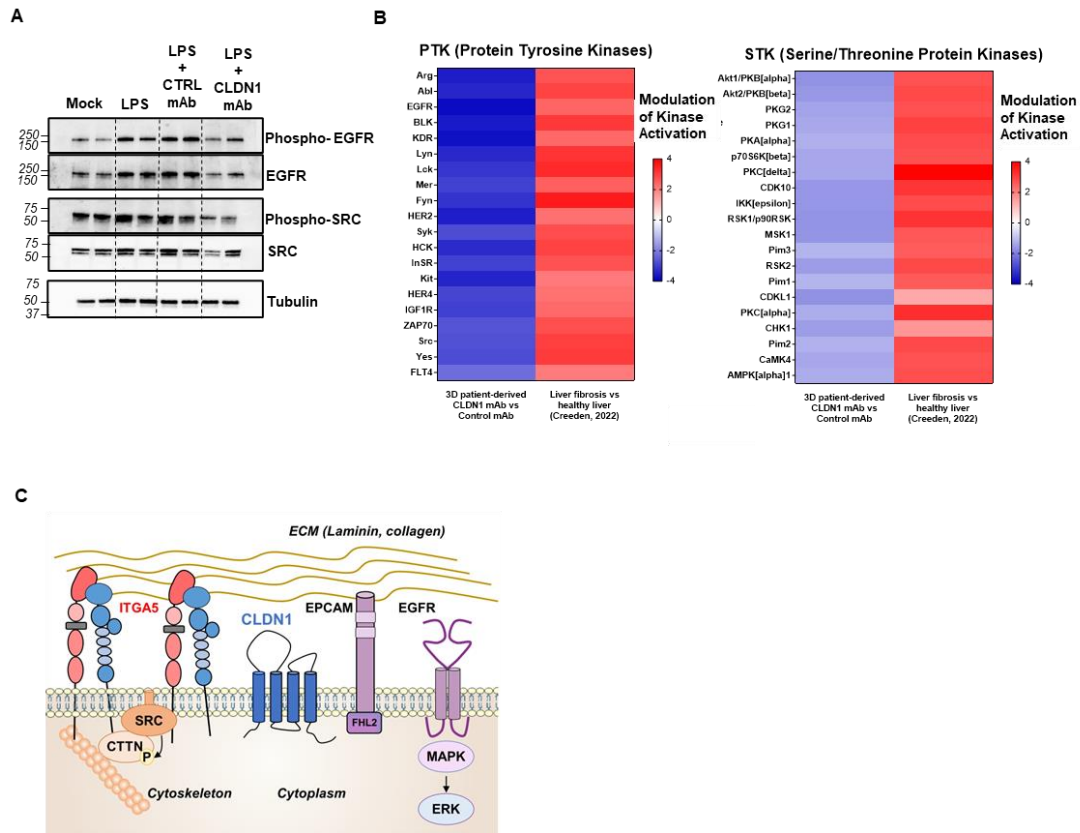
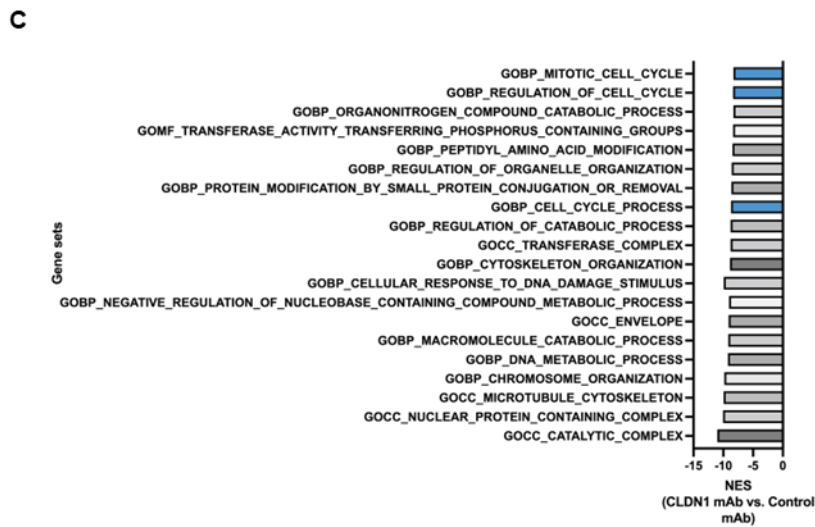
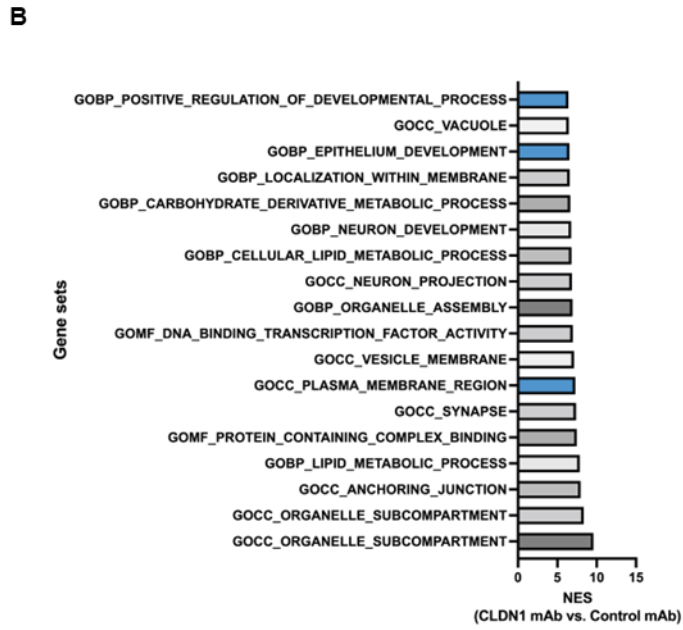
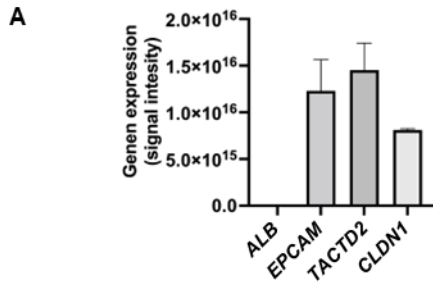


Figure S14, related to Fig. 5-6. CLDN1 mAb modulates membrane receptor signaling

in precision cut liver slices and spheroids. A. CLDN1 mAb suppresses ERK and Src phosphorylation in precision cut liver slices. One representative experiment out of two is shown.

B. Heatmap showing the 20 most strongly modulated Protein Tyrosine kinases (PTK) and Serin/Threonine Protein Kinases (STK) in CLDN1 mAb vs. Control mAb treated spheroids (left panels). The modulation of the activities of the same kinases in fibrotic vs. healthy liver as described in a recently published kinome atlas of the fibrotic liver(34) is shown in the right panels.

C. Model of CLDN1 receptor membrane biology. CLDN1 interacts with EGFR, ITGA5 and EPCAM mediating SRC and EGFR/ERK signaling. Abbreviations: CLDN1 = Claudin 1; EGFR= Epithelial growth factor receptor; LPS= Lipopolysaccharide, PTK= Protein Tyrosine Kinase; STK= Serin/threonine Protein Kinase.



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2088 Figure S15, related to Fig. 5. CLDN1mAb modulates gene expression related to cell
2089 proliferation and epithelial cell development in cirrhotic liver derived organoids. A. Gene
2090 expression of ALB, EPCAM, TACSTD2 and CLDN1 by RNAseq in cirrhosis-derived liver
2091 organoids. B-C. RNAseq data of CLDN1 mAb or control mAb-treated cirrhotic liver derived
2092 organoids were assessed by GSEA. Bars indicate normalized enrichment score of all significantly
2093 (FDR< 0.05, Kolmogorov-Smirnov test) enriched gene ontology pathways in CLDN1 mAb (A) or
2094 control mAb-(B) treated organoids. Blue color highlight most relevant pathways in liver organoids
2095 shown in Fig. 7D. Abbreviations: CLDN1= Claudin 1.

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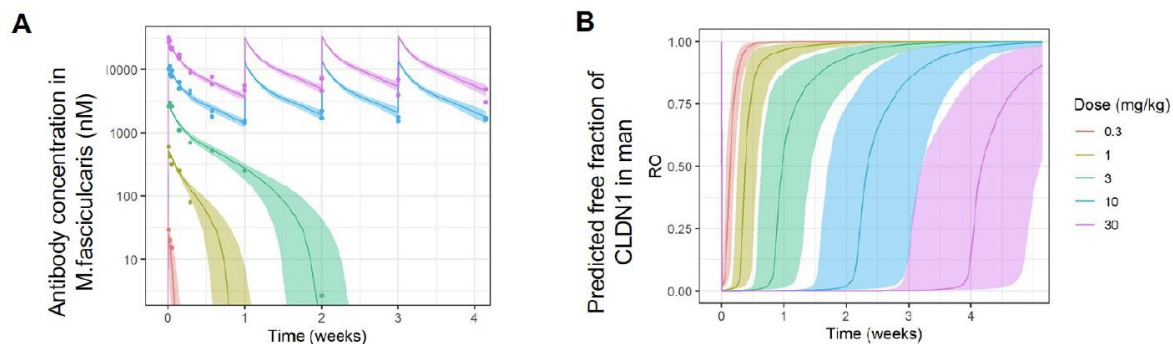


Figure S16. Anti-CLDN1 mAb pharmacokinetics in non-human primates. A.

Predicted exposure of the anti-CLDN1 antibody ALE.F02 in macaques (median, 5th and 95th percentiles from 200 simulated profiles). Dots are the observed serum concentrations. All data in nmol/L, color correspond to the dose levels. B. Predicted receptor occupancy in human, indicating the total systemic free accessible CLDN1 not occupied by the administered antibody as a function of time. Shown are median, 5th and 95th percentiles from 100 simulated PK/PD profiles.

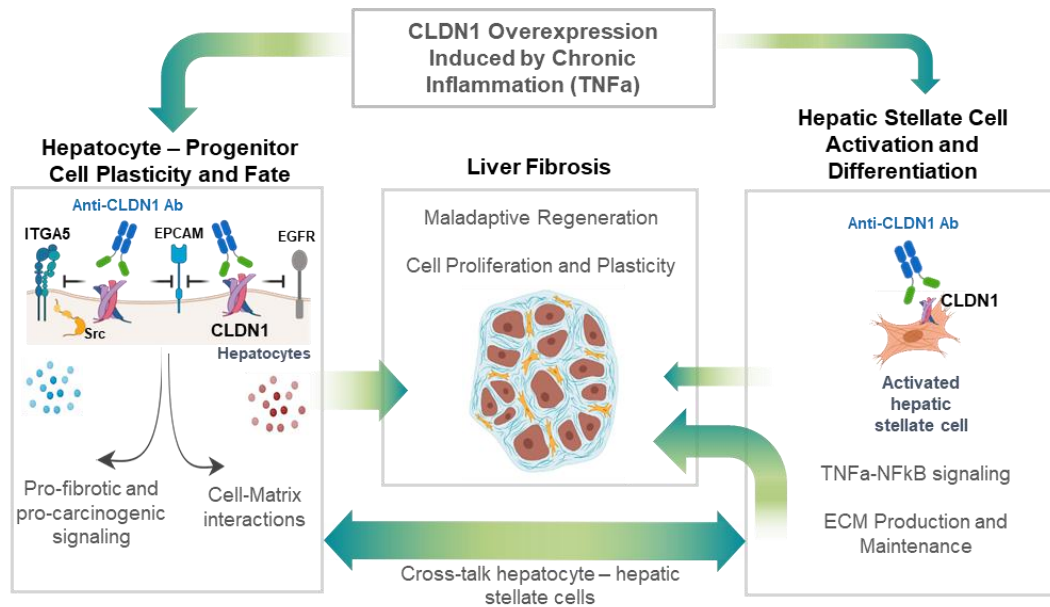


Figure S17. Mechanistic model of the anti-fibrotic effect of CLDN1 mAb in the liver.

CLDN1 expression is regulated by the TNF- α -NF κ B pathway and upregulated upon chronic inflammation. Liver epithelial cells including hepatocytes and its progenitors are the primary target cells of CLDN1 mAb mediating its anti-fibrotic effect. CLDN1 binds to EPCAM, EGFR and ITGA5 in the epithelial cell membrane and perturbation of these interactions by CLDN1 mAb inhibits downstream SRC and MAPK pathway, playing a key role in cell plasticity, cell-matrix interactions as well as fibrogenesis and carcinogenesis. The second cell type mediating the anti-fibrotic properties of the mAb are hepatic stellate cells/myofibroblasts. CLDN1 mAb inhibits TNF- α -NF κ B signaling resulting in robust inhibition of myofibroblast activation and differentiation. Cross-talk between hepatocyte epithelial cells and fibroblasts ultimately results in suppression of maladaptive regeneration, cell proliferation and plasticity leading to ECM accumulation and fibrosis.

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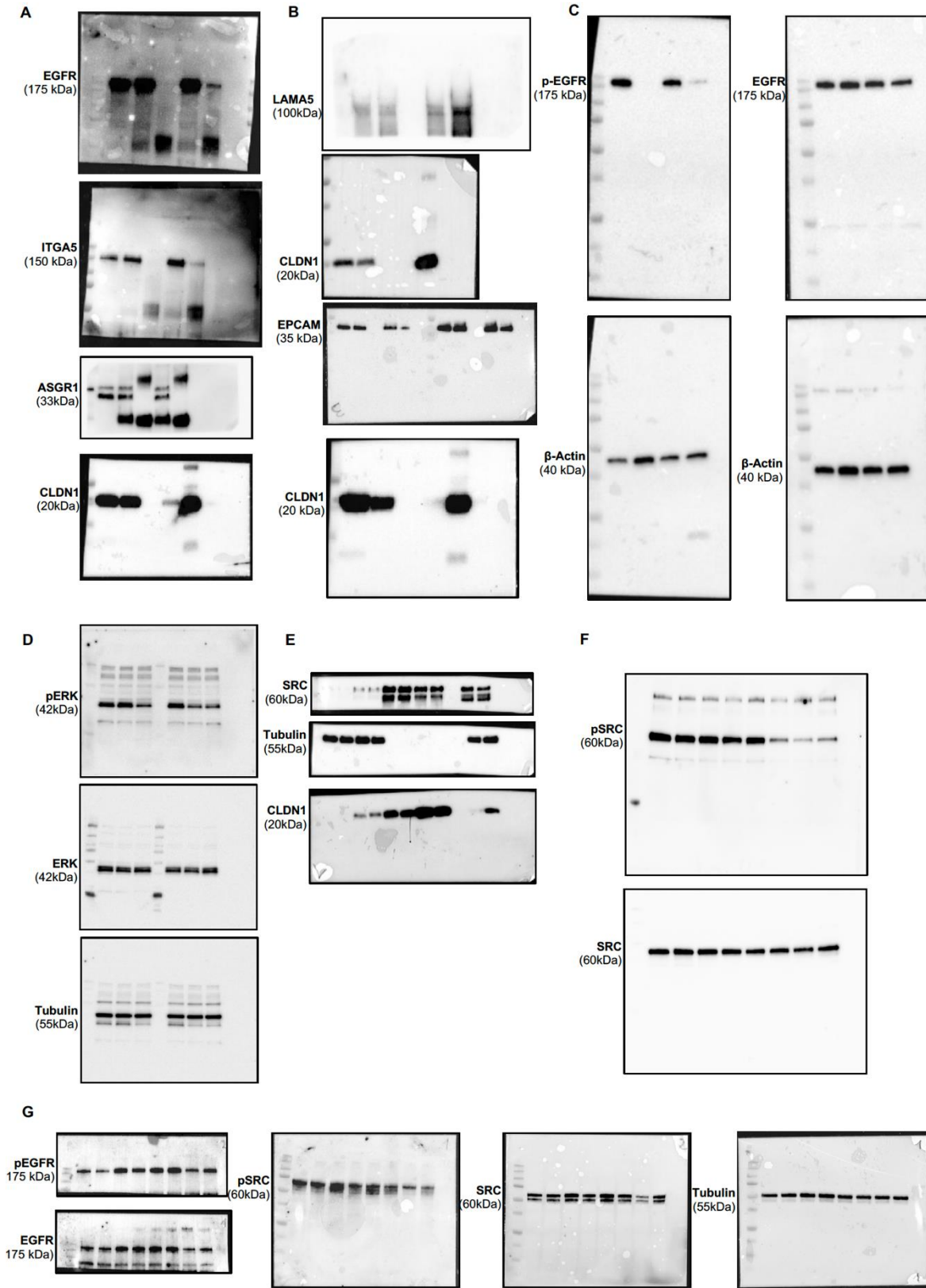


Figure S18. Original blots shown in Figure 6 and figure S14.

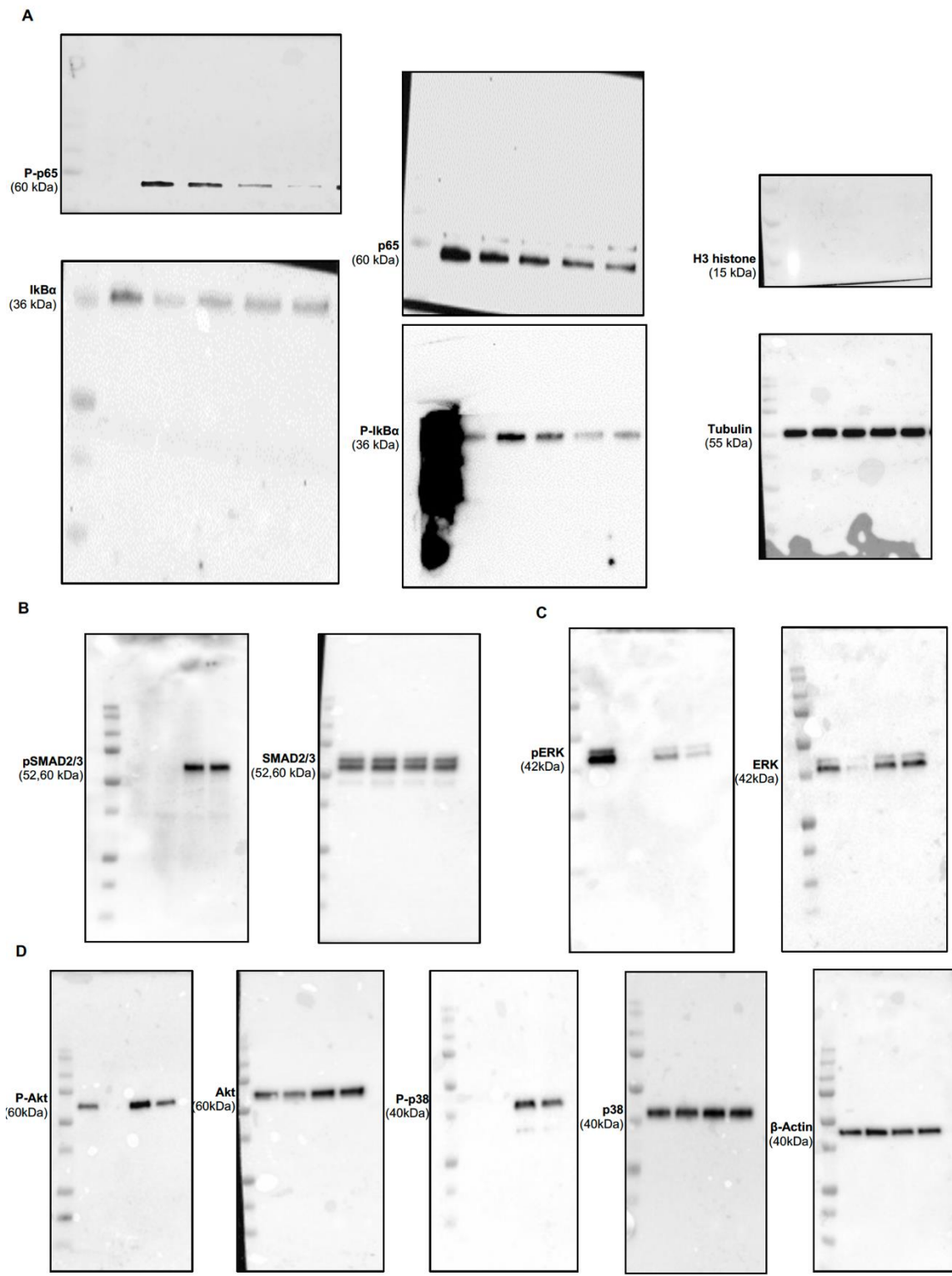


Figure S19. Original blots shown in Figure 7.

2166 **SUPPLEMENTARY TABLES**

2167 **Table S1, related to Fig. 1A. Demographic and clinical characteristics of the University of**
 2168 **Strasbourg NASH cohort.**

	<u>Control</u> <u>(n=10)</u>	<u>NASH</u> <u>(n=10)</u>
<u>Age (years)</u>	<u>43 (23-73)</u>	<u>39 (25-54)</u>
<u>Female (%)</u>	<u>18 (90)</u>	<u>4 (40)</u>
<u>Waist circumference (cm)</u>	<u>98.5 (75-149)</u>	<u>136 (100-170)</u>
<u>BMI (kg/m²)</u>	<u>31.9 (22.4-50.0)</u>	<u>46.9 (40.5-60.5)</u>
<u>Blood fasting glucose (mg/dL)</u>	<u>84 (66-130)</u>	<u>114 (83-162)</u>
<u>Insulin (μUI/mL)</u>	<u>6.1 (1.7-29.5)</u>	<u>9.2 (4.8-83.5)</u>
<u>HOMA-IR index</u>	<u>1.14 (0.33-5.90)</u>	<u>3.31 (0.99-33.40)</u>
<u>Total cholesterol (mg/dL)</u>	<u>166 (113-288)</u>	<u>151 (93-181)</u>
<u>Triglycerides (mg/dL)</u>	<u>122 (60-209)</u>	<u>194 (93-273)</u>
<u>FFA (mg/dL)</u>	<u>26 (8-36)</u>	<u>24 (13-35)</u>
<u>LDL cholesterol (mg/dL)</u>	<u>106 (59-217)</u>	<u>81 (46-101)</u>
<u>HDL cholesterol (mg/dL)</u>	<u>41 (31-67)</u>	<u>31 (18-42)</u>
<u>AST (UI/L)</u>	<u>21.5 (12-85)</u>	<u>48.5 (20-176)</u>
<u>ALT (UI/L)</u>	<u>18 (5-122)</u>	<u>56.5 (27-229)</u>
<u>ALP (UI/L)</u>	<u>60 (36-122)</u>	<u>54.5 (35-97)</u>
<u>GGT (UI/L)</u>	<u>20.5 (5-221)</u>	<u>35.5 (19-114)</u>
<u>Total bilirubin (mg/dL)</u>	<u>0.6 (0.3-1.01)</u>	<u>0.6 (0.3-0.9)</u>
<u>Iron (μg/dL)</u>	<u>76 (30-197)</u>	<u>66 (30-146)</u>
<u>Ferritin (ng/mL)</u>	<u>70.5 (14-399)</u>	<u>155 (10-2380)</u>
<u>Transferrin saturation %</u>	<u>27.5 (9-81)</u>	<u>23.5 (10-49)</u>
<u>CRP (mg/L)</u>	<u>2.84 (0.18-9.59)</u>	<u>6.27 (1.39-19.40)</u>

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 2170 **Continuous variables are indicated as median and range. Abbreviations: ALP=alkaline phosphate,**
 2171 **ALT=alanine aminotransferase, AST=aspartate aminotransferase, BMI=Body Mass Index,**
 2172 **CRP=C-reactive protein, FFA=free fatty acid, GGT=gamma-glutamyl transferase, HDL=high**
 2173 **density lipoprotein, HOMA-IR=homeostatic model assessment of insulin resistance, LDL=low**
 2174 **density lipoprotein, NAFL=non-alcoholic fatty liver, NASH=non-alcoholic steatohepatitis.**

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Table S2, related to Fig. 2. Individual data of the main efficacy endpoints of the humanized NASH mice treated GalNac siCLDN1 and siCTRL.

<u>Group</u>	<u>Mouse ID</u>	<u>Fibrosis in humanized area %</u>	<u>Tumor Number</u>
<u>siCTRL</u>	<u>1512</u>	<u>4.874</u>	<u>83</u>
	<u>1513</u>	<u>3.738</u>	<u>31</u>
	<u>1515</u>	<u>3.782</u>	<u>25</u>
	<u>1528</u>	<u>3.819</u>	<u>28</u>
	<u>1555</u>	<u>5.94</u>	<u>25</u>
	<u>1558</u>	<u>4.33</u>	<u>45</u>
	<u>Median</u>	<u>4.07</u>	<u>29.5</u>
<u>Mean</u>	<u>4.41</u>	<u>39.5</u>	
<u>s.e.m.</u>	<u>0.35</u>	<u>9.2</u>	
<u>siCLDN1</u>	<u>1505</u>	<u>2.743</u>	<u>23</u>
	<u>1506</u>	<u>0.596</u>	<u>6</u>
	<u>1509</u>	<u>3.295</u>	<u>17</u>
	<u>1511</u>	<u>4.79</u>	<u>4</u>
	<u>1722</u>	<u>2.633</u>	<u>34</u>
	<u>Median</u>	<u>2.74</u>	<u>17.0</u>
<u>Mean</u>	<u>2.81</u>	<u>16.8</u>	
<u>s.e.m.</u>	<u>0.67</u>	<u>5.5</u>	
<u>Test</u>	<u>KW</u>	<u>KW</u>	
<u>p-value</u>	<u>0.045</u>	<u>0.044</u>	

Abbreviations: KW= Kruskal-Wallis test, s.e.m.= standard error of the mean.

2210 Table S3, related to Fig. 2. Individual data of the main efficacy endpoints of the humanized
 2211 NASH mice treated with vehicle control or humanized CLDN1 mAb.

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2213 Experiment #1

<u>Group</u>	<u>Mouse ID</u>	<u>Total fibrosis %</u>	<u>Fibrosis in humanized area %</u>	<u>Tumor Number</u>
<u>Vehicle</u>	<u>4409</u>	<u>10.495</u>	<u>6.30</u>	<u>24</u>
	<u>4411</u>	<u>6.589</u>	<u>4.66</u>	<u>30</u>
	<u>4412</u>	<u>6.261</u>	<u>3.35</u>	<u>17</u>
<u>Median</u>		<u>6.59</u>	<u>4.66</u>	<u>24.00</u>
<u>Mean</u>		<u>7.78</u>	<u>4.77</u>	<u>23.67</u>
<u>s.e.m.</u>		<u>1.36</u>	<u>0.86</u>	<u>3.76</u>
<u>CLDN1 mAb</u>	<u>4405</u>	<u>1.101</u>	<u>0.51</u>	<u>17</u>
	<u>4407</u>	<u>5.843</u>	<u>1.80</u>	<u>5</u>
	<u>4408</u>	<u>3.168</u>	<u>1.51</u>	<u>12</u>
	<u>4424</u>	<u>1.516</u>	<u>0.68</u>	<u>11</u>
<u>Median</u>		<u>2.34</u>	<u>1.09</u>	<u>11.50</u>
<u>Mean</u>		<u>2.91</u>	<u>1.12</u>	<u>11.25</u>
<u>s.e.m.</u>		<u>1.08</u>	<u>0.31</u>	<u>2.46</u>
<u>Test</u>		<u>MW</u>	<u>MW</u>	<u>MW</u>
<u>p-value</u>		<u>0.0339</u>	<u>0.0339</u>	<u>0.0498</u>

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2215 Abbreviations: MW= Mann Whitney U test, s.e.m.= standard error of the mean.

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Experiment #2

<u>Group</u>	<u>Mouse ID</u>	<u>Total fibrosis %</u>	<u>Fibrosis in humanized area %</u>	<u>Tumor Number</u>
<u>Vehicle</u>	<u>1005</u>	<u>7.708</u>	<u>7.708</u>	<u>9</u>
	<u>1006</u>	<u>11.862</u>	<u>12.531</u>	<u>10</u>
	<u>4472</u>	<u>11.8925</u>	<u>11.8925</u>	<u>NA</u>
	<u>4477</u>	<u>6.048</u>	<u>4.438</u>	<u>22</u>
	<u>4478</u>	<u>1.167</u>	<u>0.886</u>	<u>13</u>
	<u>4479</u>	<u>10.4915</u>	<u>9.34</u>	<u>14</u>
	<u>4490</u>	<u>4.751</u>	<u>4.046</u>	<u>7</u>
	<u>4491</u>	<u>1.881</u>	<u>2.017</u>	<u>7</u>
	<u>4492</u>	<u>7.13</u>	<u>7.13</u>	<u>7</u>
	<u>4493</u>	<u>4.401</u>	<u>6.152</u>	<u>5</u>
<u>Median</u>	<u>6.59</u>	<u>6.64</u>	<u>9.00</u>	
<u>Mean</u>	<u>6.73</u>	<u>6.61</u>	<u>10.44</u>	
<u>s.e.m.</u>	<u>1.21</u>	<u>0.84</u>	<u>1.75</u>	

<u>CLDN1 mAb</u>	<u>1001</u>	<u>2.11</u>	<u>2.667</u>	<u>5</u>
	<u>1002</u>	<u>4.55</u>	<u>5.352</u>	<u>5</u>
	<u>1008</u>	<u>1.48</u>	<u>1.48</u>	<u>4</u>
	<u>1009</u>	<u>4.446</u>	<u>4.5225</u>	<u>4</u>
	<u>1010</u>	<u>1.716</u>	<u>1.716</u>	<u>4</u>
	<u>4470</u>	<u>1.618</u>	<u>1.864</u>	<u>2</u>
	<u>4471</u>	<u>1.1705</u>	<u>1.5995</u>	<u>4</u>
	<u>4483</u>	<u>2.556</u>	<u>2.308</u>	<u>5</u>
	<u>4484</u>	<u>3.552</u>	<u>0.96</u>	<u>13</u>
	<u>4485</u>	<u>1.322</u>	<u>0.583</u>	<u>10</u>
<u>Median</u>	<u>1.91</u>	<u>1.79</u>	<u>4.50</u>	
<u>Mean</u>	<u>2.45</u>	<u>2.31</u>	<u>5.60</u>	
<u>s.e.m.</u>	<u>0.41</u>	<u>0.48</u>	<u>1.05</u>	
<u>Test</u>	<u>MW</u>	<u>MW</u>	<u>MW</u>	
<u>p-value</u>	<u>0.013</u>	<u>0.013</u>	<u>0.0093</u>	

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Abbreviations: MW= Mann Whitney U test, s.e.m.= standard error of the mean.

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Table S4, related to Fig. 3. Individual data of the main efficacy endpoints of DEN-CDA-HFD

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mice treated with vehicle control or CLDN1 mAb.

<u>Group</u>	<u>Mouse ID</u>	<u>Fibrosis %</u>	<u>Tumor macroscopy (Y=1/N=0)</u>	<u>Tumor N at histology</u>	<u>Max tumor size (mm)</u>	<u>Tumor HSP70+ (Y=1/N=0)</u>
<u>Vehicle</u>	621	11.25	1	9	5.8	1
	622	8.59	1	7	2.2	1
	623	14.19	1	8	7.0	1
	624	9.95	1	3	1.5	0
	625	11.07	1	1	1.2	0
	631	9.53	0	0	NA	0
	632	18.69	1	3	1.5	0
	633	6.83	1	2	0.9	0
	634	7.69	1	1	2.1	0
	635	11.46	1	2	1.6	0
	641	8.28	1	11	1.2	1
	642	11.89	1	4	0.6	0
	643	8.24	1	3	1.0	0
	644	8.01	1	13	1.6	0
	645	8.79	1	10	8.1	1
	652	9.52	1	11	9.3	1
	653	6.67	1	17	13.0	1
654	9.26	1	4	1.6	1	
<u>Median</u>		<u>9.39</u>	<u>NA</u>	<u>4.00</u>	<u>1.62</u>	<u>NA</u>
<u>Mean</u>		<u>10.00</u>	<u>0.94</u>	<u>6.06</u>	<u>3.55</u>	<u>0.44</u>
<u>s.e.m.</u>		<u>0.68</u>	<u>0.06</u>	<u>1.15</u>	<u>0.89</u>	<u>0.12</u>
<u>CLDN1 mAb</u>	626	9.92	0	1	1.4	0
	627	11.76	1	7	6.0	1
	628	8.29	0	0	NA	0
	629	8.93	0	0	NA	0
	630	8.81	0	1	0.9	0
	636	5.16	0	2	0.5	0
	637	4.64	0	3	0.8	0
	638	6.57	1	2	1.3	0
	639	4.76	1	2	1.3	0
	640	6.87	0	1	1.1	0
	646	6.05	0	0	NA	0
	647	6.29	0	1	0.7	0
	648	13.55	1	2	1.1	0
	649	4.33	0	1	0.6	0

	<u>650</u>	<u>6.06</u>	<u>0</u>	<u>1</u>	<u>0.6</u>	<u>0</u>
	<u>656</u>	<u>4.39</u>	<u>1</u>	<u>6</u>	<u>1.5</u>	<u>0</u>
	<u>657</u>	<u>7.91</u>	<u>0</u>	<u>0</u>	<u>NA</u>	<u>0</u>
	<u>658</u>	<u>8.60</u>	<u>0</u>	<u>2</u>	<u>0.4</u>	<u>0</u>
	<u>659</u>	<u>4.94</u>	<u>0</u>	<u>2</u>	<u>0.8</u>	<u>0</u>
	<u>660</u>	<u>7.97</u>	<u>1</u>	<u>3</u>	<u>1.1</u>	<u>0</u>
<u>Median</u>	<u>6.72</u>	<u>N/A</u>	<u>1.50</u>	<u>1.00</u>	<u>N/A</u>	<u>N/A</u>
<u>Mean</u>	<u>7.29</u>	<u>0.30</u>	<u>1.85</u>	<u>1.25</u>	<u>0.05</u>	<u>0.05</u>
<u>s.e.m.</u>	<u>0.56</u>	<u>0.11</u>	<u>0.41</u>	<u>0.33</u>	<u>0.05</u>	<u>0.05</u>
<u>Test</u>	<u>MW</u>	<u>FT</u>	<u>MW</u>	<u>MW</u>	<u>FT</u>	<u>FT</u>

	<u>Per mouse</u>				
<u>p-value</u>	<u>0.003</u>	<u><0.001</u>	<u>0.001</u>	<u>0.001</u>	<u>0.007</u>
	<u>Per image</u>				
	<u><0.001</u>				

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2228 Abbreviations: FT= Fisher test, HSP70= heat shock protein 70, MW= Mann Whitney U test, N/A=2229 not applicable.. s.e.m.= Standard error of the mean.

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2242 **Table S5, related to Fig. 3. Metabolic parameters and CLDN1 mAb concentrations in DEN-CDA-**
 2243 **HFD mice treated with vehicle control or murinized CLDN1 mAb.**

	<u>Vehicle</u> <u>(mean ± s.e.m.)</u>	<u>CLDN1 mAb</u> <u>(mean ± s.e.m.)</u>	<u>p-value</u> <u>(MW test)</u>
<u>ALT (UI/L)</u>	<u>244 ± 12</u>	<u>217 ± 14</u>	<u>0.033</u>
<u>AST (UI/L)</u>	<u>276 ± 16</u>	<u>271 ± 20</u>	<u>0.664</u>
<u>ALP (UI/L)</u>	<u>129 ± 30.2</u>	<u>103.1 ± 3.3</u>	<u>0.584</u>
<u>Total bilirubin (µmol/L)</u>	<u>3.89 ± 0.40</u>	<u>4.57 ± 0.62</u>	<u>0.511</u>
<u>Total proteins (g/L)</u>	<u>46.2 ± 0.8</u>	<u>49.1 ± 0.4</u>	<u>0.013</u>
<u>Albumin (g/L)</u>	<u>22.4 ± 0.7</u>	<u>23.8 ± 0.5</u>	<u>0.275</u>
<u>Creatinine (µmol/L)</u>	<u>8.79 ± 0.50</u>	<u>7.98 ± 0.39</u>	<u>0.316</u>
<u>Urea (mmol/L)</u>	<u>9.01 ± 0.18</u>	<u>8.75 ± 0.39</u>	<u>0.371</u>
<u>Sodium (mmol/L)</u>	<u>145.0 ± 1.7</u>	<u>147.7 ± 0.7</u>	<u>0.059</u>
<u>Potassium (mmol/L)</u>	<u>5.33 ± 0.14</u>	<u>5.04 ± 0.13</u>	<u>0.152</u>
<u>Calcium (mmol/L)</u>	<u>2.05 ± 0.04</u>	<u>2.09 ± 0.02</u>	<u>0.602</u>
<u>Glucose (mmol/L)*</u>	<u>8.25 ± 0.51</u>	<u>9.12 ± 0.31</u>	<u>0.179</u>
<u>Total cholesterol (mmol/L)*</u>	<u>1.08 ± 0.06</u>	<u>1.12 ± 0.05</u>	<u>0.784</u>
<u>CLDN1 mAb (µg/mL)</u>	<u>--</u>	<u>125.8 ± 8.5</u>	<u>--</u>

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 2245 ***Mice not fasted.**

2246 **Abbreviations: ALT= alanine aminotransferase, AST= aspartate aminotransferase, ALP= alkaline**
 2247 **phosphatases, MW= Mann Whitney U test, s.e.m.= standard error of the mean.**

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2252 **Table S6, related to Fig. 3. Individual data of the main efficacy endpoints of DDC mice treated**
 2253 **with vehicle control or anti-CLDN1 mAb.**

<u>Group</u>	<u>Mouse ID</u>	<u>CPA %</u>	<u>Ishak score</u>
<u>Vehicle</u>	<u>1746</u>	<u>9.505</u>	<u>3</u>
	<u>1747</u>	<u>8.77</u>	<u>3</u>
	<u>1748</u>	<u>10.46</u>	<u>2</u>
	<u>1749</u>	<u>11.456</u>	<u>4</u>
	<u>1750</u>	<u>12.03</u>	<u>2</u>
	<u>1756</u>	<u>8.542</u>	<u>2</u>
	<u>1757</u>	<u>12.4</u>	<u>4</u>
	<u>1758</u>	<u>8.902</u>	<u>3</u>
	<u>1759</u>	<u>12.488</u>	<u>3</u>
	<u>1760</u>	<u>8.505</u>	<u>3</u>
	<u>1766</u>	<u>10.725</u>	<u>3</u>
	<u>1767</u>	<u>11.172</u>	<u>4</u>
	<u>1768</u>	<u>10.103</u>	<u>3</u>
	<u>1769</u>	<u>10.774</u>	<u>3</u>
	<u>1770</u>	<u>12.057</u>	<u>3</u>
	<u>1776</u>	<u>11.539</u>	<u>4</u>
	<u>1777</u>	<u>10.485</u>	<u>3</u>
	<u>1778</u>	<u>11.498</u>	<u>3</u>
<u>1779</u>	<u>9.644</u>	<u>4</u>	
<u>1780</u>	<u>12.207</u>	<u>4</u>	
<u>Median</u>		<u>10.749</u>	
<u>IQR</u>		<u>2.053</u>	
<u>Mean</u>		<u>10.663</u>	
<u>sd</u>		<u>1.328203</u>	
<u>CLDN1 mAb</u>	<u>1741</u>	<u>6.917</u>	<u>2</u>
	<u>1742</u>	<u>10.031</u>	<u>3</u>
	<u>1743</u>	<u>6.557</u>	<u>2</u>
	<u>1744</u>	<u>8.872</u>	<u>3</u>
	<u>1745</u>	<u>8.099</u>	<u>2</u>
	<u>1751</u>	<u>7.08</u>	<u>2</u>
	<u>1752</u>	<u>7.487</u>	<u>3</u>
	<u>1753</u>	<u>8.559</u>	<u>3</u>
	<u>1754</u>	<u>8.314</u>	<u>4</u>
	<u>1755</u>	<u>7.207</u>	<u>2</u>
	<u>1761</u>	<u>8.167</u>	<u>2</u>
	<u>1762</u>	<u>8.723</u>	<u>2</u>

	<u>1763</u>	<u>8.792</u>	<u>3</u>
	<u>1764</u>	<u>8.366</u>	<u>3</u>
	<u>1765</u>	<u>8.925</u>	<u>3</u>
	<u>1771</u>	<u>7.227</u>	<u>3</u>
	<u>1772</u>	<u>7.07</u>	<u>3</u>
	<u>1773</u>	<u>7.327</u>	<u>2</u>
	<u>1774</u>	<u>6.595</u>	<u>3</u>
	<u>1775</u>	<u>5.54</u>	<u>2</u>

Median 7.793
IQR 1.523
Mean 7.793
sd 1.064973

<u>Test</u>	<u>T</u>
<u>95% C.I.</u>	<u>-3.64 to -2.09</u>
<u>p-value</u>	<u><0.0001</u>

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Abbreviations: IQR = Interquartile range; sd = standard deviation; T = Student's T-test; 95% C.I.
= 95% Confidence Interval.

2269 **Table S7, related to Fig. 4.** Demographic and clinical characteristics of patients shown in **Fig.**

2270 **4C-G.**

	<u>Age(y)</u>	<u>Sex</u>	<u>Chronic liver disease</u>	<u>Indication for liver resection</u>	<u>Fibrosis stage</u>	<u>Applied type of tissue</u>
<u>353</u>	<u>83</u>	<u>M</u>	<u>NAFLD</u>	<u>HCC</u>	<u>F2</u>	<u>diseased, non-tumorous</u>
<u>351</u>	<u>78</u>	<u>M</u>	<u>NAFLD</u>	<u>CCM</u>	<u>F0</u>	<u>diseased, non-tumorous</u>
<u>410</u>	<u>70</u>	<u>M</u>	<u>-</u>	<u>CCM</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>471</u>	<u>70</u>	<u>M</u>	<u>-</u>	<u>GBC</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>525</u>	<u>50</u>	<u>F</u>	<u>-</u>	<u>BCM</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>452</u>	<u>75</u>	<u>M</u>	<u>-</u>	<u>NETM</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>506</u>	<u>48</u>	<u>F</u>	<u>-</u>	<u>CCM</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>514</u>	<u>31</u>	<u>F</u>	<u>-</u>	<u>CCM</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>515</u>	<u>44</u>	<u>M</u>	<u>-</u>	<u>CCM</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>480</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>CCM</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>481</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>CCM</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>484</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>CCM</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>488</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>CCM</u>	<u>F0</u>	<u>healthy, non-tumorous</u>
<u>489</u>	<u>-</u>	<u>-</u>	<u>-</u>	<u>HCC</u>	<u>F4</u>	<u>diseased, non-tumorous</u>

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 2272 Abbreviations: BCM= breast cancer metastasis, CCM= colon cancer liver metastasis, F= female,

2273 GBC= Gallbladder adenocarcinoma, HCC= Hepatocellular carcinoma, M= male, NAFLD= Non-
 2274 alcoholic fatty liver disease, NASH=Non-alcoholic steatohepatitis, NETM: pancreatic
 2275 neuroendocrine tumor metastasis; y= years.

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Table S8, related to Fig. 4. Demographic and clinical characteristics of patients shown in Fig.4H.

	<u>NASH #1</u>	<u>NASH #2</u>	<u>NASH #3</u>	<u>NASH #4</u>	<u>NASH #5</u>
<u>Age (years)</u>	<u>74</u>	<u>60</u>	<u>74</u>	<u>81</u>	<u>75</u>
<u>Sex (Male/Female)</u>	<u>Male</u>	<u>Male</u>	<u>Male</u>	<u>Male</u>	<u>Male</u>
<u>Fibrosis stage(26)</u>	<u>4</u>	<u>3</u>	<u>3</u>	<u>4</u>	<u>2</u>
<u>Obesity (Yes/No)</u>	<u>Yes</u>	<u>No</u>	<u>No</u>	<u>No</u>	<u>No</u>
<u>Diabetes (Yes/No)</u>	<u>Yes</u>	<u>Yes</u>	<u>No</u>	<u>No</u>	<u>Yes</u>
<u>Hypertension (Yes/No)</u>	<u>Yes</u>	<u>Yes</u>	<u>No</u>	<u>No</u>	<u>No</u>

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Abbreviations: NASH= Non-alcoholic steatohepatitis.

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Table S9, related to Fig. 5. Genes used to define the scar-associated myofibroblast*.

<u>DCN</u>	<u>TMSB10</u>	<u>FBLN1</u>	<u>RPL36</u>	<u>CYR61</u>	<u>MARCKSL1</u>
<u>C1R</u>	<u>EFEMP1</u>	<u>CD81</u>	<u>PRSS23</u>	<u>CCL2</u>	<u>THBS2</u>
<u>LUM</u>	<u>BGN</u>	<u>MMP2</u>	<u>IL32</u>	<u>RPS12</u>	<u>CTSC</u>
<u>COL3A1</u>	<u>MMP23B</u>	<u>PDGFRA</u>	<u>ANXA1</u>	<u>ADAMTSL2</u>	<u>TCEAL4</u>
<u>C1S</u>	<u>IFITM3</u>	<u>FBLN5</u>	<u>NR2F1</u>	<u>IGFBP7</u>	<u>EMP3</u>
<u>C7</u>	<u>PPIB</u>	<u>COLEC11</u>	<u>TSPAN4</u>	<u>CTSD</u>	<u>WBP5</u>
<u>COL1A2</u>	<u>NNMT</u>	<u>CD74</u>	<u>COL5A1</u>	<u>ITGBL1</u>	<u>RPLP1</u>
<u>COL1A1</u>	<u>NPC2</u>	<u>SPON2</u>	<u>ENG</u>	<u>IGFBP3</u>	<u>RPS17</u>
<u>CFH</u>	<u>COL6A1</u>	<u>COL6A3</u>	<u>RPL37</u>	<u>FSTL1</u>	<u>RPS23</u>
<u>TIMP1</u>	<u>MARCKS</u>	<u>COL14A1</u>	<u>ISLR</u>	<u>PPIC</u>	<u>RPS15</u>
<u>PCOLCE</u>	<u>AEBP1</u>	<u>G0S2</u>	<u>RPS15A</u>	<u>FCGRT</u>	<u>HLA-DPA1</u>
<u>CST3</u>	<u>THY1</u>	<u>LTBP4</u>	<u>PTGDS</u>	<u>PLTP</u>	<u>COL4A2</u>
<u>OLFML3</u>	<u>HLA-DRB1</u>	<u>RCN3</u>	<u>RRBP1</u>	<u>SSR2</u>	<u>TFPI</u>
<u>CXCL12</u>	<u>SRPX</u>	<u>IGFBP4</u>	<u>EFEMP2</u>	<u>RPS18</u>	<u>HLA-DRA</u>
<u>CLEC11A</u>	<u>COL6A2</u>	<u>LY6E</u>	<u>INMT</u>	<u>CLEC2B</u>	<u>IGF2</u>
<u>GGT5</u>	<u>S100A10</u>	<u>MGP</u>	<u>SPARC</u>	<u>IGFBP6</u>	<u>LAMB1</u>
<u>CD63</u>	<u>MEG3</u>	<u>RPL13</u>	<u>ECM1</u>	<u>TIMP2</u>	<u>CCL21</u>
<u>FTL</u>	<u>EMILIN1</u>	<u>LGALS3BP</u>	<u>CCDC80</u>	<u>VCAN</u>	<u>CEBPD</u>
<u>RARRES2</u>	<u>RPL12</u>	<u>TMEM176A</u>	<u>SERPING1</u>	<u>ALDH1A1</u>	<u>RARRES1</u>
<u>ASPN</u>	<u>S100A11</u>	<u>PRELP</u>	<u>FN1</u>	<u>TPT1</u>	<u>DAAMI</u>
<u>S100A13</u>	<u>LRP1</u>	<u>TYROBP</u>	<u>LXN</u>	<u>OSOX1</u>	
<u>RBP1</u>	<u>ADH1B</u>	<u>TMEM176B</u>	<u>MFAP4</u>	<u>RPS24</u>	
<u>SERPINF1</u>	<u>CYBA</u>	<u>IFITM1</u>	<u>RPL39</u>	<u>RAMP1</u>	
<u>DPT</u>	<u>RPL28</u>	<u>RPS28</u>	<u>VKORC1</u>	<u>F2R</u>	

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2293 *derived from(16).

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Table S10, related to Fig. 7. Genes used to define the scar-associated myofibroblast type A*.

<u><i>COLEC11</i></u>	<u><i>HLA-A</i></u>	<u><i>EDNRB</i></u>	<u><i>CALM2</i></u>	<u><i>MASP1</i></u>	<u><i>PTGIR</i></u>
<u><i>IGFBP7</i></u>	<u><i>HLA-DRB1</i></u>	<u><i>HGF</i></u>	<u><i>CITED2</i></u>	<u><i>ALDH1A1</i></u>	<u><i>HLA-DRB5</i></u>
<u><i>PPP1R14A</i></u>	<u><i>HLA-B</i></u>	<u><i>HLA-C</i></u>	<u><i>TMEM204</i></u>	<u><i>TMSB4X</i></u>	<u><i>ITM2C</i></u>
<u><i>GGT5</i></u>	<u><i>C11orf96</i></u>	<u><i>TPM1</i></u>	<u><i>COX7A1</i></u>	<u><i>CTSD</i></u>	<u><i>SGCA</i></u>
<u><i>CALD1</i></u>	<u><i>LTBP4</i></u>	<u><i>ENG</i></u>	<u><i>BST2</i></u>	<u><i>HLA-DPA1</i></u>	<u><i>ARHGAP15</i></u>
<u><i>TYROBP</i></u>	<u><i>4-Sep</i></u>	<u><i>COL4A2</i></u>	<u><i>CCL21</i></u>	<u><i>MARCKS</i></u>	<u><i>RGS16</i></u>
<u><i>B2M</i></u>	<u><i>MYL9</i></u>	<u><i>RAMP1</i></u>	<u><i>RBPM5</i></u>	<u><i>ASPN</i></u>	<u><i>COL4A1</i></u>
<u><i>ADAMTSL2</i></u>	<u><i>C8orf4</i></u>	<u><i>IGFBP3</i></u>	<u><i>RBP1</i></u>	<u><i>GPX3</i></u>	

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*derived from(16).

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2311 **Table S11, related to Fig. 7. Genes used to define the scar-associated myofibroblast type B*.**

<u>COL1A2</u>	<u>IGF1</u>	<u>NNMT</u>	<u>RPLP0</u>	<u>YBX3</u>	<u>LXN</u>
<u>S100A6</u>	<u>RARRES1</u>	<u>TSHZ2</u>	<u>VIM</u>	<u>MMP2</u>	<u>CCND2</u>
<u>C3</u>	<u>SERPINF1</u>	<u>ADIRF</u>	<u>ANXA1</u>	<u>RPSA</u>	<u>SFRP2</u>
<u>FBLN1</u>	<u>MDK</u>	<u>STEAP1</u>	<u>RPS3</u>	<u>PTGIS</u>	<u>IGFBP4</u>
<u>CCDC80</u>	<u>CLU</u>	<u>CTHRC1</u>	<u>S100A16</u>	<u>IGFBP2</u>	<u>DNAJB1</u>
<u>COL1A1</u>	<u>FSTL1</u>	<u>COL6A3</u>	<u>DHRS3</u>	<u>NR4A2</u>	<u>PLP2</u>
<u>OGN</u>	<u>SLIT3</u>	<u>LGALS1</u>	<u>VCAN</u>	<u>SVIL</u>	<u>CAV1</u>
<u>SPARCL1</u>	<u>ANXA2</u>	<u>OSR1</u>	<u>COL6A1</u>	<u>BOC</u>	<u>MGP</u>
<u>S100A4</u>	<u>IGFBP6</u>	<u>OAF</u>	<u>MMP23B</u>	<u>GPRC5A</u>	<u>CAPZB</u>
<u>NBL1</u>	<u>COL3A1</u>	<u>S100A10</u>	<u>CRABP2</u>	<u>PCOLCE</u>	

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2313 *derived from(16).

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2315 **Table S12, related to Fig. 7. Demographic and clinical characteristics of patients recruited for**
2316 **isolation of HSCs.**

<u>ID</u>	<u>Age(y)</u>	<u>Sex</u>	<u>Chronic liver disease</u>	<u>Indication for liver resection</u>	<u>Isolated cell type</u>
<u>352</u>	<u>48</u>	<u>M</u>	<u>No</u>	<u>CCM</u>	<u>HSCs</u>
<u>372</u>	<u>51</u>	<u>M</u>	<u>No</u>	<u>CCM</u>	<u>HSCs</u>
<u>374</u>	<u>59</u>	<u>F</u>	<u>No</u>	<u>CCM</u>	<u>HSCs</u>
<u>383</u>	<u>71</u>	<u>F</u>	<u>No</u>	<u>CCM</u>	<u>HSCs</u>
<u>389</u>	<u>82</u>	<u>F</u>	<u>NAFLD (F2)*</u>	<u>CCA</u>	<u>HSCs</u>
<u>397</u>	<u>23</u>	<u>F</u>	<u>No</u>	<u>PHL</u>	<u>HSCs</u>
<u>401</u>	<u>36</u>	<u>F</u>	<u>No</u>	<u>CCM</u>	<u>HSCs</u>

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2318 *Fibrosis stage(26)

2319 Abbreviations: CCA= Cholangiocellular Carcinoma, CCM= Colon cancer liver metastasis;
2320 HSC=Hepatic stellate cells, NAFLD= Non-alcoholic fatty liver disease, PHL= Primary hepatic
2321 leiomyosarcoma, y= years.

2323 **Table S13, related to Fig. 8. Individual Sirius-red positive areas in renal fibrosis UO mice**
 2324 **treated with vehicle control or CLDN1 mAb.**

Control						CLDN1-specific mAb					
Mouse ID	Photo No.	Total area (pixel)	Positive area (pixel)	Positive area (%)	Positive area (%)	Mouse ID	Photo No.	Total area (pixel)	Positive area (pixel)	Positive area (%)	Positive area (%)
101	1	3145728	344415	10.95	8.88	201	1	2942661	91823	3.12	3.14
	2	3145728	277600	8.82			2	3145728	9667	0.31	
	3	3145728	616310	19.59			3	3145728	178982	5.69	
	4	3145728	106844	3.40			4	3145728	105012	3.34	
	5	2525427	41941	1.66			5	3145728	102614	3.26	
102	1	3145728	54042	1.72	6.88	202	1	2955677	149707	5.07	4.26
	2	1852892	282606	15.25			2	3145728	157964	5.02	
	3	2901737	105846	3.65			3	3145728	158958	5.05	
	4	2680358	270561	10.09			4	3145728	137962	4.39	
	5	1677944	61894	3.69			5	3145728	55942	1.78	
103	1	2613185	45756	1.75	12.74	203	1	3145728	106839	3.40	3.01
	2	3145728	79596	2.53			2	3145728	96460	3.07	
	3	3145728	105531	3.35			3	3145728	104407	3.32	
	4	2128925	256408	12.04			4	3145728	108886	3.46	
	5	1805725	795103	44.03			5	3145728	56876	1.81	
104	1	1907636	96743	5.07	5.62	204	1	2053687	221370	10.78	7.07
	2	3145728	116189	3.69			2	2922969	175727	6.01	
	3	2498956	300009	12.01			3	3145728	280234	8.91	
	4	3145728	197408	6.28			4	3145728	95235	3.03	
	5	3145728	32587	1.04			5	3145728	208497	6.63	
105	1	3145728	107920	3.43	5.12	205	1	3145728	26479	0.84	1.70
	2	3145728	228570	7.27			2	3145728	113376	3.60	
	3	3145728	57914	1.84			3	3145728	31501	1.00	
	4	3145728	163861	5.21			4	3145728	24949	0.79	
	5	3145728	247323	7.86			5	3145728	71784	2.28	
106	1	2391160	25941	1.08	5.31	206	1	3145728	56743	1.80	1.77
	2	2481193	192484	7.76			2	3145728	84916	2.70	
	3	2409692	128920	5.35			3	3145728	52829	1.68	
	4	3145728	46287	1.47			4	3145728	61369	1.95	
	5	3145728	343353	10.91			5	3145728	22164	0.70	
107	1	2952140	36770	1.25	9.96	207	1	3145728	44821	1.42	0.79
	2	1283270	262341	20.44			2	3145728	33427	1.06	
	3	1882451	145227	7.71			3	3145728	33393	1.06	
	4	3145728	140876	4.48			4	3145728	5123	0.16	
	5	2652902	421933	15.90			5	3145728	7575	0.24	
108	1	3145728	225836	7.18	5.42	208	1	3054515	39178	1.28	1.41
	2	3145728	302298	9.61			2	3016949	51887	1.72	
	3	3145728	207383	6.59			3	2771583	44394	1.60	
	4	3145728	88954	2.83			4	2615483	39127	1.50	
	5	3145728	28495	0.91			5	2568128	23833	0.93	

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Table S14, related to Fig. 8. Individual Ashcroft scores in bleomycin pulmonary fibrosis mice treated with vehicle control or anti-CLDN1 mAb.

Group	Mouse ID	Photo No.																			Mean	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		20
Control	101																					
	102	5	3	3	3	3	3	1	2	2	2	1	2	1	2	1	2	2	2	2	1	2.2
	103	4	6	7	7	3	6	7	7	6	6	7	5	4	3	5	3	2	1	3	1	4.7
	104	5	3	3	3	3	3	2	3	3	4	3	3	3	3	3	3	2	3	1	1	2.9
	105	5	6	6	6	6	3	4	8	7	3	3	4	6	5	6	7	5	3	2	1	4.8
	106	4	5	3	4	5	5	3	3	5	6	6	3	3	2	2	4	3	4	3	3	3.8
	107	3	3	3	3	5	2	3	5	6	8	8	7	6	7	6	3	4	3	8	8	5.1
	108																					
	109	3	6	8	8	5	2	5	3	4	3	7	8	8	8	5	4	5	8	6	6	5.6
	110	2	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	2	1	1.2
	111	2	2	2	2	1	2	2	2	1	3	3	2	1	1	1	2	2	2	2	2	1.9
	112																					
	113																					
	114	4	5	4	4	2	2	2	3	3	4	3	2	1	2	2	2	3	3	3	3	2.9
	115	4	4	6	3	5	3	3	3	3	3	3	3	3	4	4	3	2	3	2	3	3.4
	116	7	7	7	7	7	7	7	7	7	7	7	8	7	7	6	6	5	4	3	3	6.3
	117	5	5	3	5	7	7	3	8	5	7	7	7	7	5	4	5	3	3	3	5	5.2
	118	5	7	6	5	7	6	6	3	4	5	6	3	3	8	5	5	4	3	4	3	4.9
CLDN1-specific mAb	201	2	3	3	2	2	3	1	3	1	1	1	3	1	3	4	1	3	2	3	2	2.2
	202																					
	203	1	1	1	3	0	2	3	3	1	2	0	0	1	1	0	2	2	2	4	3	1.6
	204	1	1	2	3	2	1	0	0	1	1	0	0	2	1	2	5	4	3	2	3	1.7
	205	4	3	3	3	3	3	3	4	3	3	2	4	3	2	3	3	3	3	3	5	3.2
	206	3	4	2	3	4	4	2	3	3	3	3	2	1	2	3	3	3	3	3	2	2.8
	207	3	4	3	3	3	3	3	3	2	2	2	2	3	3	3	4	3	3	2	3	2.9
	208	2	1	3	1	2	2	2	2	3	3	1	1	1	4	3	4	2	3	3	2	2.3
	209	3	1	3	3	3	3	1	3	3	3	3	1	2	4	2	3	3	3	3	2	2.6
	210																					
	211																					
	212	1	1	3	4	3	3	3	4	3	4	2	3	3	2	3	5	2	4	3	2	2.9
	213	2	3	3	3	3	3	3	3	3	4	5	7	8	8	8	8	7	3	7	5	4.8
	214	3	3	3	3	3	2	3	2	3	3	2	3	4	3	4	2	3	3	3	2	2.9
	215	3	5	3	6	4	5	3	3	4	2	3	3	4	6	6	8	2	4	3	3	4.0
	216	2	3	3	2	3	3	3	3	3	2	3	3	3	3	2	3	3	3	3	3	2.8
	217																					
	218																					

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Table S15, related to Fig. 8. *HAS*^{high} fibroblast marker genes*.

<u>AC09049</u> <u>8.1</u>	<u>ETF1</u>	<u>GNPTAB</u>	<u>CSTB</u>	<u>MESDC1</u>	<u>ANKRD37</u>	<u>ATPIF1</u>	<u>HLA-C</u>
<u>MT-CYB</u>	<u>KIAA132</u> <u>4L</u>	<u>LITAF</u>	<u>RCAN1</u>	<u>RAB3A</u>	<u>TLE1</u>	<u>ZFAND3</u>	<u>METAP2</u>
<u>A2M</u>	<u>PGM3</u>	<u>MYL9</u>	<u>MID1</u>	<u>SLC25A3</u> <u>3</u>	<u>RCN3</u>	<u>CD70</u>	<u>PPP4R2</u>
<u>CDKN2A</u>	<u>PTGES3</u>	<u>ISG20L2</u>	<u>PKM</u>	<u>CEBPZ</u>	<u>OSER1</u>	<u>PFKFB3</u>	<u>NR2F2</u>
<u>GALNT13</u>	<u>FAT1</u>	<u>CDKN1A</u>	<u>MAP1LC3</u> <u>B</u>	<u>CACNA2</u> <u>D1</u>	<u>IGF2</u>	<u>VCAN</u>	<u>ADD3</u>
<u>HSP90AA</u> <u>1</u>	<u>CXorf40</u> <u>B</u>	<u>SLC16A1</u>	<u>MTRNR2L</u> <u>12</u>	<u>SMARCA</u> <u>1</u>	<u>CD276</u>	<u>EDNRA</u>	<u>WTAP</u>
<u>HSPE1</u>	<u>PTP4A1</u>	<u>GPRC5A</u>	<u>COL6A2</u>	<u>SEMA3C</u>	<u>YPEL2</u>	<u>EMP1</u>	<u>EFEMP1</u>
<u>LINC016</u> <u>05</u>	<u>CD9</u>	<u>PRRC2C</u>	<u>GXYLT2</u>	<u>SKIL</u>	<u>GOLM1</u>	<u>CDC42E</u> <u>P2</u>	<u>MT1M</u>
<u>HSPD1</u>	<u>CERCAM</u>	<u>MGP</u>	<u>ATXN7</u>	<u>UCK2</u>	<u>HINT1</u>	<u>SPCS1</u>	<u>ARPC5L</u>
<u>FHL2</u>	<u>ARF4</u>	<u>PNPLA8</u>	<u>CTSL</u>	<u>HBEGF</u>	<u>CREM</u>	<u>QSOX1</u>	<u>PDLIM4</u>
<u>KIAA121</u> <u>7</u>	<u>COL1A1</u>	<u>HAS1</u>	<u>RALGPS2</u>	<u>S1PR3</u>	<u>PPIC</u>	<u>ARMCX3</u>	<u>WT1</u>
<u>PDLIM3</u>	<u>EIF5A</u>	<u>PLK2</u>	<u>THBS3</u>	<u>FGF2</u>	<u>OAF</u>	<u>JARID2</u>	<u>FRZB</u>
<u>NAF1</u>	<u>FAM180</u> <u>A</u>	<u>FKBP4</u>	<u>MT-ND5</u>	<u>TOB1</u>	<u>BAG2</u>	<u>TFG</u>	<u>KDELR2</u>
<u>TNFSF9</u>	<u>MORF4L</u> <u>2</u>	<u>BTAFL1</u>	<u>ARC</u>	<u>SFTPC</u>	<u>CCDC71L</u>	<u>SOCS3</u>	<u>IFITM3</u>
<u>LINC001</u> <u>52</u>	<u>ISLR</u>	<u>RPL17</u>	<u>GAS7</u>	<u>FLNA</u>	<u>THBS2</u>	<u>CXCL14</u>	<u>XBPI</u>
<u>MT-ATP8</u>	<u>INSIG1</u>	<u>ARHGAP5</u>	<u>CYB5A</u>	<u>RHOC</u>	<u>PCDH7</u>	<u>MAP2K3</u>	<u>CYP11B1</u>
<u>SELK</u>	<u>MEDAG</u>	<u>VEGFA</u>	<u>PITPNB</u>	<u>ATP5G2</u>	<u>ERVK3-1</u>	<u>ARSI</u>	<u>ADGRD1</u>
<u>SLC12A8</u>	<u>ZBTB21</u>	<u>IFITM2</u>	<u>CMTM3</u>	<u>PPRC1</u>	<u>NAV1</u>	<u>MYH9</u>	<u>LIMCH1</u>
<u>MT-CO2</u>	<u>MAP4K5</u>	<u>SLC4A7</u>	<u>TAF13</u>	<u>MT-ND4</u>	<u>CPNE8</u>	<u>WWTR1</u>	<u>H3F3A</u>
<u>TLL1</u>	<u>BLOC1S</u> <u>6</u>	<u>C16orf45</u>	<u>TCF21</u>	<u>SCGB1A1</u>	<u>ARHGDIB</u>	<u>MINOS1</u>	<u>SFPQ</u>
<u>AC11340</u> <u>4.1</u>	<u>STIP1</u>	<u>KLHL21</u>	<u>IPMK</u>	<u>PTRH2</u>	<u>MAP3K4</u>	<u>SCG2</u>	<u>PSAP</u>
<u>MT-CO3</u>	<u>TPM2</u>	<u>FERMT2</u>	<u>BAZ1A</u>	<u>ECM1</u>	<u>ALDH2</u>	<u>HLA-</u> <u>DPB1</u>	<u>CHSY1</u>
<u>HSP90AB</u> <u>1</u>	<u>RP11-</u> <u>210L7.3</u>	<u>ROR1</u>	<u>HSPB8</u>	<u>ADAMTS</u> <u>16</u>	<u>YWHAQ</u>	<u>GLA</u>	<u>KDM5B</u>
<u>CDKN2B</u>	<u>AHSA1</u>	<u>PAMR1</u>	<u>VAT1</u>	<u>TEX10</u>	<u>COX4I2</u>	<u>PGAP1</u>	<u>PTGES</u>

<u>HSPA4L</u>	<u>GEM</u>	<u>TXN</u>	<u>JOSD1</u>	<u>CLDN11</u>	<u>EBF1</u>	<u>MAGED1</u>	<u>LAMC1</u>
<u>FEM1C</u>	<u>ITGB1</u>	<u>UBE2B</u>	<u>CTC-444N24.11</u>	<u>MICAL2</u>	<u>CALM1</u>	<u>LY96</u>	<u>USP2</u>
<u>ABL2</u>	<u>OSMR-ASI</u>	<u>ZNF460</u>	<u>VIM</u>	<u>TFB2M</u>	<u>IGF1</u>	<u>SRGN</u>	<u>ATP5L</u>
<u>HSPA8</u>	<u>MEG3</u>	<u>BAIAP2</u>	<u>NDEL1</u>	<u>CTSD</u>	<u>EIF3J</u>	<u>GOPC</u>	<u>PCBP2</u>
<u>MXRA5</u>	<u>KDM6B</u>	<u>ASB1</u>	<u>MCC</u>	<u>TUBB2A</u>	<u>EIF2S1</u>	<u>CHIC2</u>	<u>PDGFRL</u>
<u>MT-CO1</u>	<u>PTHLH</u>	<u>IQCJ-SCHIP1</u>	<u>UBAP1</u>	<u>NGF</u>	<u>SEPW1</u>	<u>RSL1D1</u>	<u>ZFC3H1</u>
<u>GPX3</u>	<u>SULF1</u>	<u>MYC</u>	<u>MIR22HG</u>	<u>JAG1</u>	<u>SAMD9</u>	<u>CTSK</u>	<u>BNC2</u>
<u>RP11-474O21.5</u>	<u>ACLY</u>	<u>COL3A1</u>	<u>TUBB3</u>	<u>HTRA3</u>	<u>COX4I1</u>	<u>PNO1</u>	<u>HNRNPA B</u>
<u>RABGEF1</u>	<u>MIR4435-2HG</u>	<u>HSD3B7</u>	<u>HMG2</u>	<u>MAPRE1</u>	<u>GART</u>	<u>ANGPT1</u>	<u>SPG20</u>
<u>LINC01060</u>	<u>CCT2</u>	<u>HSPA1A</u>	<u>RUNX2</u>	<u>HMG3</u>	<u>RCN1</u>	<u>CSNK1A1</u>	<u>MAPK11L</u>
<u>UGDH</u>	<u>PDK4</u>	<u>GNG12</u>	<u>LRRC59</u>	<u>MLF1</u>	<u>STAG1</u>	<u>ANXA5</u>	<u>KRT18</u>
<u>GCLM</u>	<u>TES</u>	<u>ARL5B</u>	<u>BMP1</u>	<u>GTPBP4</u>	<u>LSM12</u>	<u>NNMT</u>	<u>SRP14</u>
<u>USP12</u>	<u>FAM3C</u>	<u>CEBPD</u>	<u>SDC2</u>	<u>DOT1L</u>	<u>DENND4A</u>	<u>LOX</u>	<u>LRRC17</u>
<u>TAGLN</u>	<u>CHMP1B</u>	<u>PLIN3</u>	<u>OSMR</u>	<u>CCNK</u>	<u>PXDN</u>	<u>TGIF1</u>	<u>PHGDH</u>
<u>NR1P1</u>	<u>NR4A3</u>	<u>PRKCI</u>	<u>MSC</u>	<u>3-Mar</u>	<u>CD248</u>	<u>EPHX1</u>	<u>CLIP1</u>
<u>DNAJA1</u>	<u>EIF1</u>	<u>ANKRD28</u>	<u>TSR1</u>	<u>TMEM263</u>	<u>SMS</u>	<u>ANK2</u>	<u>HNRNPF</u>
<u>DCBLD2</u>	<u>NR4A2</u>	<u>PRRX1</u>	<u>HBP1</u>	<u>RRM1</u>	<u>ANGPTL4</u>	<u>C1QTNF3</u>	<u>IGFBP7</u>
<u>CRABP2</u>	<u>ELOVL5</u>	<u>FBN1</u>	<u>NOTCH2</u>	<u>ADAMTS1L</u>	<u>SESN3</u>	<u>CDC42SE1</u>	<u>AMD1</u>
<u>ZBTB38</u>	<u>STX4</u>	<u>RARG</u>	<u>KIAA1462</u>	<u>CRIP2</u>	<u>RGS2</u>	<u>FKBP14</u>	<u>TMEM59</u>
<u>RLF</u>	<u>FNIP2</u>	<u>VAPB</u>	<u>TGM2</u>	<u>NDFIP2</u>	<u>SSR4</u>	<u>SH3D19</u>	<u>LOXL1</u>
<u>BAG3</u>	<u>SCGB3A1</u>	<u>CDK17</u>	<u>KPNA4</u>	<u>AC05879.1.1</u>	<u>TPBG</u>	<u>FLNB</u>	<u>TOR1AIP2</u>
<u>CHORDC1</u>	<u>SERTAD1</u>	<u>SLC19A2</u>	<u>SERTAD2</u>	<u>KTNI</u>	<u>UBQLN1</u>	<u>ARPC3</u>	<u>ANKLE2</u>
<u>SPARC</u>	<u>SGK1</u>	<u>RND3</u>	<u>ADAMTS2</u>	<u>NOP58</u>	<u>MYL6</u>	<u>FKBP5</u>	<u>CTTN</u>
<u>DYRK3</u>	<u>SLC30A1</u>	<u>BZW2</u>	<u>EGR3</u>	<u>KLF4</u>	<u>BDNF</u>	<u>NUP58</u>	<u>PER3</u>
<u>ZSWIM6</u>	<u>HERC4</u>	<u>TXNRD1</u>	<u>FOSB</u>	<u>ZMAT3</u>	<u>GALNT2</u>	<u>UOCR10</u>	<u>GLUD1</u>
<u>PLA2G4A</u>	<u>HAS2</u>	<u>HIVEP2</u>	<u>TUBA1C</u>	<u>FSCN1</u>	<u>RBPI</u>	<u>CXCL6</u>	<u>CCDC80</u>
<u>CPXM1</u>	<u>ZDBF2</u>	<u>RAB7A</u>	<u>NFE2L2</u>	<u>CCNT1</u>	<u>ZC3HAV1</u>	<u>CMSS1</u>	<u>SMDT1</u>

<u>SERPINH1</u>	<u>DPYSL3</u>	<u>PANX1</u>	<u>SMIM3</u>	<u>SLFN11</u>	<u>PSME1</u>	<u>ITPRIP</u>	<u>KPNA2</u>
<u>DOK5</u>	<u>PRRG3</u>	<u>SELM</u>	<u>RNF149</u>	<u>UBE2D3</u>	<u>RNASEK</u>	<u>HSPB1</u>	<u>PEG10</u>
<u>MT-ND4L</u>	<u>HDLBP</u>	<u>ALDH1A3</u>	<u>AKAP12</u>	<u>FNDC1</u>	<u>AGO2</u>	<u>OTUD4</u>	<u>COX6A1</u>
<u>HSPA4</u>	<u>COQ10B</u>	<u>CPZ</u>	<u>EGFL6</u>	<u>SEPP1</u>	<u>CLMP</u>	<u>TUBB2B</u>	<u>CHCHD10</u>
<u>ANXA1</u>	<u>ASCC3</u>	<u>CADPS2</u>	<u>CTNNA1</u>	<u>TIMP2</u>	<u>H3F3B</u>	<u>RC3H1</u>	<u>GPC1</u>
<u>ARL4C</u>	<u>DNAJB6</u>	<u>ATP13A3</u>	<u>HMOX1</u>	<u>TCEB1</u>	<u>S100A4</u>	<u>NUPR1</u>	<u>MYL12A</u>
<u>COL1A2</u>	<u>MT-ND2</u>	<u>FBXO34</u>	<u>C10orf10</u>	<u>RPS6KA3</u>	<u>TAF1D</u>	<u>RARRES1</u>	<u>ARHGAP21</u>
<u>SQSTM1</u>	<u>TIPARP</u>	<u>IPO7</u>	<u>ITGAV</u>	<u>CIRBP</u>	<u>AFG3L2</u>	<u>SERINC5</u>	<u>EPB41L2</u>
<u>TCP1</u>	<u>MTRNR2L8</u>	<u>HRH1</u>	<u>MAPK6</u>	<u>DDX21</u>	<u>SH3PXD2B</u>	<u>EDIL3</u>	<u>LY6E</u>
<u>PHLDA1</u>	<u>TSC22D2</u>	<u>INPP1</u>	<u>RGMB</u>	<u>ID1</u>	<u>NIFK</u>	<u>ADH1C</u>	<u>ADAMTS15</u>
<u>NT5E</u>	<u>P4HA1</u>	<u>TNFRSF10B</u>	<u>MMP2</u>	<u>CCDC109B</u>	<u>CD44</u>	<u>PTPN1</u>	<u>CXCL12</u>
<u>ARID5B</u>	<u>MT-ND3</u>	<u>TNFAIP6</u>	<u>METRNL</u>	<u>MYOF</u>	<u>FAM110B</u>	<u>TRAF4</u>	<u>ETNK1</u>
<u>ROR1-ASI</u>	<u>JAM3</u>	<u>UAP1</u>	<u>SPON2</u>	<u>BZW1</u>	<u>DDX3X</u>	<u>ABHD2</u>	<u>PLA2G5</u>
<u>SNAI2</u>	<u>TWIST1</u>	<u>PLAUR</u>	<u>TIMP3</u>	<u>NTM</u>	<u>SEMA4A</u>	<u>DSTN</u>	<u>GRPEL1</u>
<u>GJA1</u>	<u>PPP1R14A</u>	<u>UCHL3</u>	<u>MAP4K4</u>	<u>GPSM2</u>	<u>UHRF1BP1L</u>	<u>DSEL</u>	<u>CILP2</u>
<u>LIMA1</u>	<u>PTGIS</u>	<u>JUN</u>	<u>CNTN4</u>	<u>EMILIN2</u>	<u>TRIO</u>	<u>NFATC1</u>	<u>FBLN2</u>
<u>LRRC8C</u>	<u>LMCD1</u>	<u>CD74</u>	<u>UTP4</u>	<u>AFF4</u>	<u>KCNE4</u>	<u>FKBP10</u>	<u>SUMO2</u>
<u>ZFAND2A</u>	<u>TPM4</u>	<u>FGFR1</u>	<u>CLEC11A</u>	<u>NOP16</u>	<u>USP15</u>	<u>SPSB1</u>	<u>HMGB1</u>
<u>SPAG9</u>	<u>IL1R1</u>	<u>ERF1</u>	<u>AFAP1</u>	<u>TSPO</u>	<u>MIR222HG</u>	<u>UBE3A</u>	<u>NOV</u>
<u>HSPH1</u>	<u>ANGPTL2</u>	<u>PLOD2</u>	<u>NOLC1</u>	<u>CADM3</u>	<u>GABPB1</u>	<u>TMEM2</u>	<u>ALDH1A1</u>
<u>RPS26</u>	<u>RPL41</u>	<u>GABARAPL1</u>	<u>TNFRSF12A</u>	<u>CTNNB1</u>	<u>AOC3</u>	<u>SOX4</u>	<u>CA12</u>
<u>CHD1</u>	<u>REL</u>	<u>GNAI3</u>	<u>LRIF1</u>	<u>TTC3</u>	<u>RGS5</u>	<u>SRGAP1</u>	<u>HIGD1B</u>
<u>EIF4E</u>	<u>KCTD9</u>	<u>YWHAZ</u>	<u>ZFP36L1</u>	<u>AKIRIN1</u>	<u>GNL2</u>	<u>IL33</u>	<u>PERP</u>
<u>YWHAG</u>	<u>ELL2</u>	<u>SACS</u>	<u>CLIC4</u>	<u>FRS2</u>	<u>MDM2</u>	<u>FABP5</u>	<u>KLF2</u>
<u>CBLB</u>	<u>DDX3Y</u>	<u>FRMD6</u>	<u>IFI16</u>	<u>GSTP1</u>	<u>EMP2</u>	<u>SEC23A</u>	<u>PABPC1</u>
<u>DNAJB4</u>	<u>FAM198B</u>	<u>PPTC7</u>	<u>NXT1</u>	<u>GNL3</u>	<u>UFM1</u>	<u>COX8A</u>	<u>NFAT5</u>

<u>MSX2</u>	<u>C1QTNF6</u>	<u>COL4A1</u>	<u>MRPL18</u>	<u>HIST3H2A</u>	<u>SLC40A1</u>	<u>CCT3</u>	<u>C14orf2</u>
<u>RAB23</u>	<u>PPDPF</u>	<u>COL4A2</u>	<u>DNTTIP2</u>	<u>ITGA11</u>	<u>IL6R</u>	<u>PEBP1</u>	<u>BCAP31</u>
<u>PEA15</u>	<u>TICAM1</u>	<u>MAFF</u>	<u>DNAJB9</u>	<u>SLC20A1</u>	<u>SEMA3B</u>	<u>NUFIP2</u>	<u>HOTAIRM1</u>
<u>DRAM1</u>	<u>TOP1</u>	<u>NBPF14</u>	<u>ALG13</u>	<u>PRMT9</u>	<u>ELN</u>	<u>RGCC</u>	<u>SEMA6A</u>
<u>EIF4A3</u>	<u>COL6A1</u>	<u>WDR43</u>	<u>FKBP9</u>	<u>NDUFA4</u>	<u>HLA-DPA1</u>	<u>DES</u>	<u>NDUFA3</u>
<u>LHFPL2</u>	<u>ETV3</u>	<u>MLLT11</u>	<u>MED13</u>	<u>CREB3L1</u>	<u>COL5A1</u>	<u>PIM1</u>	<u>ACSL3</u>
<u>DNAJB1</u>	<u>WBP5</u>	<u>HNRNPA2B1</u>	<u>LATS2</u>	<u>CCT4</u>	<u>PFDN2</u>	<u>DDX5</u>	<u>OLFML3</u>
<u>IL6ST</u>	<u>SOD3</u>	<u>DNMBP</u>	<u>NUP153</u>	<u>ESYT2</u>	<u>HIVEP1</u>	<u>AHNAK</u>	<u>EIF5</u>
<u>ACTA2</u>	<u>SLPI</u>	<u>SDCBP</u>	<u>SERBP1</u>	<u>TPT1</u>	<u>HIPK3</u>	<u>FLRT2</u>	<u>PHLDB2</u>
<u>YES1</u>	<u>ABI3BP</u>	<u>CD55</u>	<u>C3orf58</u>	<u>CRY1</u>	<u>APP</u>	<u>IGFBP6</u>	<u>CD68</u>
<u>ADH1B</u>	<u>CREB5</u>	<u>NAA50</u>	<u>FOSL1</u>	<u>TRIM69</u>	<u>EIF1AX</u>	<u>PAICS</u>	<u>MAP3K8</u>
<u>GLIS3</u>	<u>MMP14</u>	<u>MAT2A</u>	<u>KLF3</u>	<u>HEG1</u>	<u>FLNC</u>	<u>SSC5D</u>	<u>PCDHGC3</u>
<u>MT-ND1</u>	<u>DPT</u>	<u>CAMSAP2</u>	<u>FAM114A1</u>	<u>CYP51A1</u>	<u>ANKH</u>	<u>KDEL3</u>	<u>ANXA2</u>
<u>HSPA9</u>	<u>PTGFRN</u>	<u>RCOR1</u>	<u>PCBP1</u>	<u>HNRNPH3</u>	<u>ARL4D</u>	<u>IER3</u>	<u>KRTCAP2</u>
<u>C1orf21</u>	<u>SYAP1</u>	<u>HSPA1B</u>	<u>USP36</u>	<u>MRC2</u>	<u>UBL3</u>	<u>CKAP4</u>	<u>COL5A2</u>
<u>SNX9</u>	<u>APOD</u>	<u>GPC6</u>	<u>ACSL4</u>	<u>TSC22D1</u>	<u>PHLDB1</u>	<u>RAB1A</u>	<u>INHBA</u>
<u>RYBP</u>	<u>GFPT2</u>	<u>HNRNPA0</u>	<u>TSPAN5</u>	<u>NDUFA4L2</u>	<u>EDF1</u>	<u>ASAHI</u>	<u>NPM1</u>
<u>TXNIP</u>	<u>PRSS23</u>	<u>CYCS</u>	<u>SAMD8</u>	<u>PXDC1</u>	<u>CD200</u>	<u>SNRPB</u>	<u>CD81</u>
<u>CACYBP</u>	<u>RUNX1</u>	<u>HECTD2</u>	<u>COX5B</u>	<u>FAP</u>	<u>DCLK1</u>	<u>NIP7</u>	<u>FILIP1</u>
<u>FSTL1</u>	<u>BIN1</u>	<u>WDR45B</u>	<u>SARIA</u>	<u>AHNAK2</u>	<u>PPP1R15B</u>	<u>ATP1B3</u>	<u>HLA-A</u>
<u>GNPNAT1</u>	<u>MT-ND6</u>	<u>THBS1</u>	<u>HLA-DRB1</u>	<u>CSRNP1</u>	<u>EIF4G2</u>	<u>HSPA5</u>	<u>EEA1</u>
<u>RANBP2</u>	<u>FAM46A</u>	<u>STK17A</u>	<u>MEST</u>	<u>MEIS2</u>	<u>PPP2R2A</u>	<u>POLR1C</u>	<u>RP11-14N7.2</u>
<u>KLF6</u>	<u>PTGS2</u>	<u>SPRY2</u>	<u>SLC38A2</u>	<u>TSPAN3</u>	<u>KRAS</u>	<u>NR4A1</u>	<u>SLC39A14</u>
<u>ZFAND5</u>	<u>CALM2</u>	<u>IPO5</u>	<u>NRBF2</u>	<u>THAP2</u>	<u>NUP98</u>	<u>LHFP</u>	<u>FOSL2</u>
<u>U2AF1L5</u>	<u>DDX27</u>	<u>CMBL</u>	<u>TUBB6</u>	<u>SLC39A6</u>	<u>IFI27</u>	<u>HMGAI</u>	<u>LAMA4</u>
<u>ITIH5</u>	<u>NSUN2</u>	<u>DUSP5</u>	<u>SPHK1</u>	<u>CLIC2</u>	<u>ANTXR2</u>	<u>SEC31A</u>	<u>OPTN</u>
<u>JMJD1C</u>	<u>HNRNPU</u>	<u>H2AFJ</u>	<u>HLA-DRA</u>	<u>TOB2</u>	<u>COPS2</u>	<u>NR1D2</u>	<u>PAFAH1B1</u>

<u>HOMER1</u>	<u>COL14A1</u>	<u>APBB3</u>	<u>MMP23B</u>	<u>KLF9</u>	<u>MRPS6</u>	<u>PDGFD</u>	<u>NOTCH3</u>
<u>EPHB2</u>	<u>RASAL2</u>	<u>BACH1</u>	<u>S100A16</u>	<u>TMED5</u>	<u>B4GALT1</u>	<u>MYLK</u>	<u>DAZAP2</u>
<u>DCUN1D3</u>	<u>ZNF703</u>	<u>RAP1B</u>	<u>TSHZ2</u>	<u>PFKP</u>	<u>C2</u>	<u>ATF4</u>	<u>ATF3</u>
<u>PLXDC1</u>	<u>KLHL4</u>	<u>CLCF1</u>	<u>APOE</u>	<u>MCL1</u>	<u>MYO1E</u>	<u>PLAU</u>	<u>RASL11A</u>
<u>AES</u>	<u>GPM6B</u>	<u>KCTD20</u>	<u>LDHB</u>	<u>ZBTB16</u>	<u>SCGB3A2</u>	<u>LMOD1</u>	<u>PDPN</u>
<u>RBBP6</u>	<u>UGCG</u>	<u>HMCN1</u>	<u>C8orf4</u>	<u>ATP5D</u>	<u>PNP</u>	<u>SNHG12</u>	<u>TPI1</u>
<u>RELB</u>	<u>FTH1</u>	<u>MFAP5</u>	<u>ABCA1</u>	<u>ABCA9</u>	<u>TINAGL1</u>	<u>TRIB1</u>	<u>ARL6IP4</u>
<u>TNXB</u>	<u>PTP4A3</u>	<u>KITLG</u>	<u>F10</u>	<u>DBN1</u>	<u>GOLGA4</u>	<u>CAST</u>	<u>MIR155HG</u>
<u>HLA-DRB5</u>	<u>TALDO1</u>	<u>DCXR</u>	<u>PFDN5</u>	<u>LRRN4CL</u>	<u>CALU</u>	<u>OLFML2B</u>	<u>CFD</u>
<u>SLC3A2</u>	<u>PTRF</u>	<u>NDUFS5</u>	<u>SPATS2L</u>	<u>NUDT4</u>	<u>SAMHD1</u>	<u>LYZ</u>	<u>RORA</u>
<u>PLAGL1</u>	<u>PPP1R10</u>	<u>NEU1</u>	<u>CFI</u>	<u>WDR83OS</u>	<u>SGCE</u>	<u>EIF4G1</u>	<u>DUSP4</u>
<u>FAM126A</u>	<u>PTN</u>	<u>GUK1</u>	<u>ANTXR1</u>	<u>ACKR3</u>	<u>DBNDD2</u>	<u>CNN1</u>	<u>HIF1A</u>
<u>SFRP1</u>	<u>PTGDS</u>	<u>COL15A1</u>	<u>UGP2</u>	<u>CD82</u>	<u>FGF7</u>	<u>MGST3</u>	<u>PRR13</u>
<u>RPS19</u>	<u>NDUFB10</u>	<u>G3BP1</u>	<u>CAPN2</u>	<u>ARFGAP3</u>	<u>CD4</u>	<u>FNDC3B</u>	<u>UQCRI1</u>
<u>RPL22L1</u>	<u>ATP6V0E1</u>	<u>BRD2</u>	<u>FKBP1A</u>	<u>EIF3A</u>	<u>NFIL3</u>	<u>LGALS3BP</u>	<u>CIB1</u>
<u>NDUFV2</u>	<u>CLEC2B</u>	<u>CHN1</u>	<u>NDUFA13</u>	<u>VPS28</u>	<u>S100A6</u>	<u>ZEB2</u>	<u>CD34</u>
<u>MBNL2</u>	<u>GPC3</u>	<u>RRBP1</u>	<u>USMG5</u>	<u>DNAJA4</u>	<u>ANAPC16</u>	<u>LDHA</u>	<u>S100A11</u>
<u>SCPEP1</u>	<u>GGT5</u>	<u>COX5A</u>	<u>EIF3K</u>	<u>NGFRAP1</u>	<u>TCEB2</u>	<u>LINC00657</u>	<u>GDF15</u>
<u>KRT8</u>	<u>MARCKS</u>	<u>SELENBP1</u>	<u>GPCPD1</u>	<u>EDNRB</u>	<u>LXN</u>	<u>ABLIM1</u>	<u>NCL</u>
<u>UQCRB</u>	<u>MFAP2</u>	<u>IL1RL1</u>	<u>CHPF</u>	<u>RDH10</u>	<u>ADIRF</u>	<u>SPTAN1</u>	<u>ADAM12</u>
<u>EGFR</u>	<u>TNS1</u>	<u>TACC1</u>	<u>GPNMB</u>	<u>RGN</u>	<u>GRINA</u>	<u>SYNCRIP</u>	<u>MACF1</u>
<u>SLC7A5</u>	<u>C2orf40</u>	<u>LPL</u>	<u>CLEC3B</u>	<u>NDUFB7</u>	<u>TGFB1I1</u>	<u>COX6B1</u>	<u>VASN</u>
<u>PCOLCE</u>	<u>STAT3</u>	<u>CTSS</u>	<u>LEPR</u>	<u>DKK3</u>	<u>SFRP4</u>	<u>ITGA1</u>	<u>CXCL8</u>
<u>ATP5J</u>	<u>ADGRF5</u>	<u>COMP</u>	<u>MT1A</u>	<u>ROBO2</u>	<u>NME3</u>	<u>MEF2C</u>	<u>LSP1</u>
<u>CCL26</u>	<u>SCN7A</u>	<u>DKK1</u>	<u>RNASE1</u>	<u>SGCA</u>	<u>S100A10</u>	<u>OSR1</u>	<u>AURKAIP1</u>
<u>MXRA8</u>	<u>RSRP1</u>	<u>NPNT</u>	<u>ARL6IP5</u>	<u>PIEZO2</u>	<u>CHRD1</u>	<u>SLC16A7</u>	<u>SLC2A3</u>
<u>SYPL1</u>	<u>MT1X</u>	<u>HILPDA</u>	<u>TGFBR3</u>	<u>PSME2</u>	<u>CD302</u>	<u>MARCKSL1</u>	<u>TUBA1A</u>

<u>COMT</u>	<u>KCNMA1</u>	<u>CXCL2</u>	<u>TBX2</u>	<u>PODN</u>	<u>BCL3</u>	<u>NBL1</u>
<u>HGF</u>	<u>DDIT4</u>	<u>HOPX</u>	<u>NDFIP1</u>	<u>04-sept</u>	<u>THY1</u>	<u>CES1</u>
<u>CYBA</u>	<u>SFTPBP</u>	<u>ABHD5</u>	<u>TNFRSF1</u> <u>A</u>	<u>CSRPI</u>	<u>C7</u>	<u>ITGA8</u>
<u>RBM39</u>	<u>TYROBP</u>	<u>AKRIC1</u>	<u>SCARA5</u>	<u>IGFBP4</u>	<u>ATP6V1F</u>	<u>SH3BGR</u> <u>L</u>

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* Derived from(41).

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Table S16, related to Fig.8. ACTA2⁺ myofibroblast marker genes*.

<u>FN1</u>	<u>TFPI2</u>	<u>FAP</u>	<u>CD34</u>	<u>MGST1</u>	<u>SMPDL3A</u>	<u>TNFRSF1A</u>	<u>LPP</u>
<u>LTBP2</u>	<u>FGFR4</u>	<u>CD302</u>	<u>LGALS3</u>	<u>SOD3</u>	<u>CFH</u>	<u>ATP13A3</u>	<u>ID3</u>
<u>LIMCH1</u>	<u>SLC40A1</u>	<u>CD248</u>	<u>EFEMP1</u>	<u>PMP22</u>	<u>CPXM1</u>	<u>PLN</u>	<u>FHL1</u>
<u>CDH11</u>	<u>ASPN</u>	<u>ARC</u>	<u>CTGF</u>	<u>ANTXR1</u>	<u>SELENBP1</u>	<u>MAFF</u>	<u>SERPINA3</u>
<u>ADIRF</u>	<u>DNAJB1</u>	<u>CD55</u>	<u>SLC25A4</u>	<u>NOLC1</u>	<u>HNRNPF</u>	<u>EIF4A1</u>	<u>CILP</u>
<u>PLA2G2A</u>	<u>COL5A2</u>	<u>CHD1</u>	<u>NCL</u>	<u>TCF12</u>	<u>FAM162B</u>	<u>ARL4D</u>	<u>FIBIN</u>
<u>A2M</u>	<u>PALLD</u>	<u>MDK</u>	<u>MYH11</u>	<u>ADGRD1</u>	<u>IGFBP7</u>	<u>MYOC</u>	<u>FABP4</u>
<u>MACF1</u>	<u>EIF4A3</u>	<u>GJA4</u>	<u>NR4A2</u>	<u>WDR43</u>	<u>EZR</u>	<u>EGR3</u>	<u>C1R</u>
<u>ITGBL1</u>	<u>TCF21</u>	<u>CALM1</u>	<u>COL1A1</u>	<u>NAMPT</u>	<u>CLCF1</u>	<u>RARRES1</u>	<u>MAP1B</u>
<u>CES1</u>	<u>PLIN2</u>	<u>EFHD1</u>	<u>ESAM</u>	<u>KCTD12</u>	<u>MYL6</u>	<u>PDK4</u>	<u>C2orf40</u>
<u>HAS1</u>	<u>MT1M</u>	<u>HMGNI</u>	<u>NDNF</u>	<u>PCBP1</u>	<u>PDLIM5</u>	<u>SFPQ</u>	<u>KIAA1217</u>
<u>MYC</u>	<u>G0S2</u>	<u>PTP4A1</u>	<u>MMP19</u>	<u>PRSS23</u>	<u>SLC4A7</u>	<u>PIK3R1</u>	<u>HTRA1</u>
<u>TM4SF1</u>	<u>NPNT</u>	<u>ITM2A</u>	<u>RPL41</u>	<u>ABLIM1</u>	<u>MIR22HG</u>	<u>SPTBN1</u>	<u>SRGN</u>
<u>MOXD1</u>	<u>HSP90AB1</u>	<u>FOSL1</u>	<u>HNRNPAB</u>	<u>FKBP4</u>	<u>WISP2</u>	<u>SEMA3C</u>	<u>THBS1</u>
<u>COL6A3</u>	<u>TMEM119</u>	<u>DNAJA1</u>	<u>NT5E</u>	<u>CXCL12</u>	<u>CHMP1B</u>	<u>AEBP1</u>	<u>TGM2</u>
<u>MAMDC2</u>	<u>NR4A3</u>	<u>BCAM</u>	<u>WT1</u>	<u>INPP4B</u>	<u>CFB</u>	<u>SRSF3</u>	<u>SLC2A3</u>
<u>ROBO2</u>	<u>ANGPT1</u>	<u>ENC1</u>	<u>LMO4</u>	<u>CLEC3B</u>	<u>ACTG2</u>	<u>UBC</u>	<u>CEBPB</u>
<u>ERRFI1</u>	<u>SPINT2</u>	<u>TPM2</u>	<u>TNC</u>	<u>PII6</u>	<u>ADAMTS9</u>	<u>UGP2</u>	<u>SERPINA3.1</u>
<u>COL8A1</u>	<u>SLC38A5</u>	<u>LSP1</u>	<u>MTRNR2L12</u>	<u>KRT8</u>	<u>ZNF331</u>	<u>PHLDA2</u>	<u>MGP</u>
<u>UGDH</u>	<u>CTHRC1</u>	<u>HMOX1</u>	<u>ATF3</u>	<u>MXRA8</u>	<u>LGALS1</u>	<u>LMOD1</u>	<u>IFI6</u>
<u>MFAP2</u>	<u>SELK</u>	<u>ISYNA1</u>	<u>KLF9</u>	<u>PFDN2</u>	<u>CREB5</u>	<u>CRISPLD2</u>	<u>SERPINE1</u>
<u>CCDC80</u>	<u>PTGIS</u>	<u>OSR1</u>	<u>NOTCH3</u>	<u>FLNC</u>	<u>GABARAPL1</u>	<u>LGALS3BP</u>	<u>TXNIP</u>
<u>MEDAG1</u>	<u>ADAMTS1</u>	<u>NDRG1</u>	<u>PLAU</u>	<u>11-sept</u>	<u>MEF2C</u>	<u>PIM3</u>	<u>CXCL1</u>
<u>CDKN1A</u>	<u>RGS5</u>	<u>3-Mar</u>	<u>PCOLCE2</u>	<u>ZYX</u>	<u>FAM46A</u>	<u>IL32</u>	<u>FHL2</u>
<u>EMILIN1</u>	<u>SFRP1</u>	<u>NRP2</u>	<u>SRSF2</u>	<u>RABGEF1</u>	<u>RANBP2</u>	<u>ITGA5</u>	<u>HIF1A</u>

<u>GPRC5A</u>	<u>SMOC2</u>	<u>ITGA2</u>	<u>MTHFD2</u>	<u>PPP1R12 A</u>	<u>ENO1</u>	<u>NUDT4</u>	<u>BGN</u>
<u>UAP1</u>	<u>DES</u>	<u>EPS8</u>	<u>ELL2</u>	<u>FIGF</u>	<u>YBX3</u>	<u>MT-CYB</u>	<u>FBN1</u>
<u>PLXDC2</u>	<u>LDHA</u>	<u>FKBP1A</u>	<u>FBLN5</u>	<u>OSTC</u>	<u>RP11- 14N7.2</u>	<u>NRIP1</u>	<u>PDPN</u>
<u>MT1A</u>	<u>DIO2</u>	<u>SEPP1</u>	<u>MMP2</u>	<u>JUNB</u>	<u>GNL3</u>	<u>ARID5B</u>	<u>THBD</u>
<u>PLEKH H2</u>	<u>NOP16</u>	<u>NDUFA4 L2</u>	<u>COL10A1</u>	<u>ITM2C</u>	<u>CCL11</u>	<u>S100A13</u>	<u>RGS16</u>
<u>QSOX1</u>	<u>CRIP1</u>	<u>HSD11B1</u>	<u>EGFL6</u>	<u>ENAH</u>	<u>HNRNPU</u>	<u>MMP14</u>	<u>BTG2</u>
<u>IGF2</u>	<u>TDO2</u>	<u>ETV1</u>	<u>GNG11</u>	<u>WDR1</u>	<u>UACA</u>	<u>HEYL</u>	<u>INHBA</u>
<u>C3</u>	<u>HIGD1B</u>	<u>PTP4A3</u>	<u>CTSB</u>	<u>YWHAG</u>	<u>INSIG1</u>	<u>EDNRA</u>	<u>RASD1</u>
<u>RHOB</u>	<u>LUM</u>	<u>RSPO3</u>	<u>LSAMP</u>	<u>ATP1A1</u>	<u>TUBB3</u>	<u>AKR1C1</u>	<u>CHRD1</u>
<u>RARRES 2</u>	<u>HSPH1</u>	<u>ELN</u>	<u>COL1A2</u>	<u>ACTA2</u>	<u>PLK2</u>	<u>TCF4</u>	<u>KLF2</u>
<u>GPC3</u>	<u>COL16A1</u>	<u>CIQTNF1</u>	<u>SPARC</u>	<u>TPT1</u>	<u>STMN1</u>	<u>TIMP3</u>	<u>C10orf10</u>
<u>SCN7A</u>	<u>DDX21</u>	<u>CNN2</u>	<u>LBH</u>	<u>TUBB2A</u>	<u>RSL1D1</u>	<u>CBLB</u>	<u>TSC22D3</u>
<u>DKK3</u>	<u>TUBB4B</u>	<u>SCARB2</u>	<u>LHFP</u>	<u>PA2G4</u>	<u>HSPB6</u>	<u>MINOS1</u>	<u>FGF7</u>
<u>ITGA8</u>	<u>SGK1</u>	<u>MFGE8</u>	<u>ZNF106</u>	<u>BTG3</u>	<u>ANGPTL4</u>	<u>FMO2</u>	<u>HSPB8</u>
<u>HSPD1</u>	<u>CDC42E P2</u>	<u>TAGLN2</u>	<u>ADGRF5</u>	<u>NPC2</u>	<u>XBP1</u>	<u>HSPA1A</u>	<u>TNFRSF1 2A</u>
<u>MT2A</u>	<u>CPE</u>	<u>PIIB</u>	<u>NAP1L1</u>	<u>LINC011 33</u>	<u>PTMA</u>	<u>DDX3X</u>	<u>MEG3</u>
<u>POSTN</u>	<u>KRT18</u>	<u>HSPA8</u>	<u>SCARA5</u>	<u>CADM3</u>	<u>SNRPB</u>	<u>TXN</u>	<u>EGR1</u>
<u>CD82</u>	<u>TSPAN13</u>	<u>COL5A1</u>	<u>VEGFA</u>	<u>DSTN</u>	<u>F2R</u>	<u>SOCS3</u>	<u>COL12A1</u>
<u>COMP</u>	<u>GDF10</u>	<u>F3</u>	<u>TSPAN8</u>	<u>DNTTIP2</u>	<u>ARL6IP5</u>	<u>FAT1</u>	<u>AC090498 .1</u>
<u>IGFBP6</u>	<u>HSP90AA 1</u>	<u>C16orf45</u>	<u>ZFAND5</u>	<u>ABL2</u>	<u>MLLT11</u>	<u>SPSB1</u>	<u>PTGDS</u>
<u>EIF1</u>	<u>NKD2</u>	<u>EIF5A</u>	<u>HES4</u>	<u>RPS27</u>	<u>TUBA1B</u>	<u>MT1G</u>	<u>LINC0015 2</u>
<u>NBL1</u>	<u>IFITM1</u>	<u>ID4</u>	<u>NXT1</u>	<u>FOSL2</u>	<u>HSPB1</u>	<u>VASN</u>	<u>FOSB</u>
<u>MAT2A</u>	<u>DST</u>	<u>CIQTNF7</u>	<u>TIPARP</u>	<u>BAZ1A</u>	<u>HSPA1B</u>	<u>CST3</u>	<u>ZFP36</u>
<u>FST</u>	<u>SLPI</u>	<u>PLPP1</u>	<u>LRRN4CL</u>	<u>SLC16A1</u>	<u>EMP2</u>	<u>IGF1</u>	<u>CFD</u>
<u>COX4I2</u>	<u>ACKR3</u>	<u>FAT4</u>	<u>FHL5</u>	<u>CIQTNF 3</u>	<u>RAB31</u>	<u>CYSTM1</u>	<u>CXCL2</u>
<u>HSPE1</u>	<u>ALDH1A 3</u>	<u>TUBA1C</u>	<u>GNPNAT1</u>	<u>NR2F1</u>	<u>SH3BP5</u>	<u>PIM1</u>	<u>IGFBP3</u>
<u>ENPP2</u>	<u>SNHG15</u>	<u>PTK7</u>	<u>ALDH2</u>	<u>MMP23B</u>	<u>PDLIM4</u>	<u>EPAS1</u>	<u>SOD2</u>
<u>BAG3</u>	<u>ROBO1</u>	<u>COL15A1</u>	<u>MFAP4</u>	<u>FMO3</u>	<u>HSPA9</u>	<u>HMGAI</u>	<u>PTGS2</u>

<u>LTBP1</u>	<u>BDKRB1</u>	<u>CYP7B1</u>	<u>DBNDD2</u>	<u>ADAMTS</u> <u>4</u>	<u>KLF3</u>	<u>CYR61</u>	<u>SFRP4</u>
<u>ATP1B3</u>	<u>AKAP12</u>	<u>STEAP4</u>	<u>PDGFRL</u>	<u>CEBPZ</u>	<u>EIF4E</u>	<u>MYO1B</u>	<u>CXCL3</u>
<u>CYCS</u>	<u>TNFRSF1</u> <u>9</u>	<u>SRSF7</u>	<u>ALDH1A1</u>	<u>KLF4</u>	<u>CSRNP1</u>	<u>IFI16</u>	<u>DPT</u>
<u>BMP5</u>	<u>MCAM</u>	<u>SORBS2</u>	<u>SH3PXD2</u> <u>A</u>	<u>EDNRB</u>	<u>ACTN4</u>	<u>MT1X</u>	<u>DCN</u>
<u>CTSL</u>	<u>CREM</u>	<u>NR4A1</u>	<u>TOB1</u>	<u>NPM1</u>	<u>LAMB1</u>	<u>RRBP1</u>	<u>CRABP2</u>
<u>GFPT2</u>	<u>COL3A1</u>	<u>EIF1B</u>	<u>ETF1</u>	<u>KDM6B</u>	<u>ISG15</u>	<u>MARCKS</u>	<u>GPX3</u>
<u>HMCN1</u>	<u>SNCG</u>	<u>SLC20A1</u>	<u>PHLDA1</u>	<u>SNU13</u>	<u>CSRP2</u>	<u>SRPX</u>	<u>SFRP2</u>
<u>MYL9</u>	<u>PNRC1</u>	<u>AMD1</u>	<u>RGS3</u>	<u>SLIT2</u>	<u>PDGFRB</u>	<u>TPM1</u>	<u>ADH1B</u>
<u>MFAP5</u>	<u>LAMA2</u>	<u>LITAF</u>	<u>APOLD1</u>	<u>TCP1</u>	<u>NREP</u>	<u>TAGLN</u>	<u>ADM</u>
<u>TINAGL</u> <u>1</u>	<u>GSN</u>	<u>TCEB1</u>	<u>UBA2</u>	<u>CRYAB</u>	<u>ZFP36L1</u>	<u>COL6A1</u>	<u>ICAM1</u>
<u>RGCC</u>	<u>EBF1</u>	<u>RAN</u>	<u>DKK1</u>	<u>NOP58</u>	<u>PLAUR</u>	<u>GPNMB</u>	<u>CH25H</u>
<u>SPON1</u>	<u>COL13A1</u>	<u>H3F3B</u>	<u>SH3D19</u>	<u>ACTB</u>	<u>TXNRD1</u>	<u>PPP1R15</u> <u>A</u>	<u>PPP1R14</u> <u>A</u>
<u>ACSL4</u>	<u>PIEZO2</u>	<u>MAOB</u>	<u>CNN1</u>	<u>CYP1B1</u>	<u>TMSB10</u>	<u>HILPDA</u>	<u>-</u>
<u>VCAN</u>	<u>PROCR</u>	<u>PMEPA1</u>	<u>NRP1</u>	<u>WNT2</u>	<u>SERTAD1</u>	<u>COL18A1</u>	<u>-</u>
<u>IFI27</u>	<u>MYH10</u>	<u>STOM</u>	<u>SFTA1P</u>	<u>MT1E</u>	<u>IGFBP2</u>	<u>CXCL8</u>	<u>-</u>

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2339 * Derived from(41).

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Table S17, related to Fig. 8. *PLIN2*⁺ lipomyofibroblast marker genes*.

<u><i>CTSL</i></u>	<u><i>AOC3</i></u>	<u><i>ANGPT1</i></u>	<u><i>LARP4</i></u>	<u><i>METAP2</i></u>	<u><i>SARAF</i></u>	<u><i>ARF5</i></u>	<u><i>PLN</i></u>
<u><i>MT-CYB</i></u>	<u><i>EIF4A3</i></u>	<u><i>RABGEF1</i></u>	<u><i>KCTD20</i></u>	<u><i>NABP1</i></u>	<u><i>EMP3</i></u>	<u><i>MIR222HG</i></u>	<u><i>MINOS1</i></u>
<u><i>BGN</i></u>	<u><i>MGST1</i></u>	<u><i>PA2G4</i></u>	<u><i>ITGA8</i></u>	<u><i>NFIL3</i></u>	<u><i>ANAPC16</i></u>	<u><i>FAM180A</i></u>	<u><i>HIST1H4C</i></u>
<u><i>MYL9</i></u>	<u><i>DNAJC2</i></u>	<u><i>STK40</i></u>	<u><i>COX5B</i></u>	<u><i>C1S</i></u>	<u><i>PRRC2C</i></u>	<u><i>EIF3M</i></u>	<u><i>INPP4B</i></u>
<u><i>TAGLN</i></u>	<u><i>LIMA1</i></u>	<u><i>ZNF800</i></u>	<u><i>SLC43A3</i></u>	<u><i>OLFML3</i></u>	<u><i>ANXA6</i></u>	<u><i>MT1A</i></u>	<u><i>GJA4</i></u>
<u><i>ACTA2</i></u>	<u><i>MEG3</i></u>	<u><i>LINC00473</i></u>	<u><i>NRIP1</i></u>	<u><i>PHLDB1</i></u>	<u><i>RGN</i></u>	<u><i>NME3</i></u>	<u><i>ADAMTS9</i></u>
<u><i>UAP1</i></u>	<u><i>FBN1</i></u>	<u><i>IL1R1</i></u>	<u><i>THBS1</i></u>	<u><i>SRM</i></u>	<u><i>ARPC5</i></u>	<u><i>FGFR4</i></u>	<u><i>CNN3</i></u>
<u><i>ACSL4</i></u>	<u><i>NR4A3</i></u>	<u><i>CCND2</i></u>	<u><i>CD151</i></u>	<u><i>PRKCDBP</i></u>	<u><i>H3F3A</i></u>	<u><i>CSNK1A1</i></u>	<u><i>SI00A4</i></u>
<u><i>PLIN2</i></u>	<u><i>RNF149</i></u>	<u><i>DDX27</i></u>	<u><i>ESYT2</i></u>	<u><i>NNMT</i></u>	<u><i>PLSCR1</i></u>	<u><i>LGALS3BP</i></u>	<u><i>ADIRF</i></u>
<u><i>TMSB4X</i></u>	<u><i>TSC22D1</i></u>	<u><i>RSPO3</i></u>	<u><i>COLEC12</i></u>	<u><i>PTP4A3</i></u>	<u><i>ILF2</i></u>	<u><i>NEAT1</i></u>	<u><i>LGALS1</i></u>
<u><i>GFPT2</i></u>	<u><i>NDUFA4L2</i></u>	<u><i>FBL</i></u>	<u><i>BAG3</i></u>	<u><i>SRSF1</i></u>	<u><i>DAZAP2</i></u>	<u><i>ARL6IP4</i></u>	<u><i>CNN2</i></u>
<u><i>CRIP2</i></u>	<u><i>MT2A</i></u>	<u><i>SRGAP1</i></u>	<u><i>LMOD1</i></u>	<u><i>TCEB1</i></u>	<i>04-sept</i>	<u><i>CDKN1A</i></u>	<u><i>ALDH2</i></u>
<u><i>IGF2</i></u>	<u><i>CAV2</i></u>	<u><i>TMED5</i></u>	<u><i>SNED1</i></u>	<u><i>IVNS1ABP</i></u>	<u><i>CHCHD10</i></u>	<u><i>APOLD1</i></u>	<u><i>RUNX1</i></u>
<u><i>PPP1R14A</i></u>	<u><i>ARL4D</i></u>	<u><i>KDM6B</i></u>	<u><i>FAM162B</i></u>	<u><i>AKIRIN1</i></u>	<u><i>EDNRB</i></u>	<u><i>NFIA</i></u>	<u><i>LITAF</i></u>
<u><i>DKK3</i></u>	<u><i>SLC4A7</i></u>	<u><i>CLDN11</i></u>	<u><i>FGF2</i></u>	<u><i>CRIP1</i></u>	<u><i>ELL2</i></u>	<u><i>CHURC1</i></u>	<u><i>OGN</i></u>
<u><i>TPM2</i></u>	<u><i>F3</i></u>	<u><i>SH3BGR</i></u>	<u><i>PIM1</i></u>	<u><i>BICC1</i></u>	<u><i>H3F3B</i></u>	<u><i>RTN4</i></u>	<u><i>UGCG</i></u>
<u><i>NOP16</i></u>	<u><i>MYH9</i></u>	<u><i>GPNMB</i></u>	<u><i>PRDM2</i></u>	<u><i>SEMA3B</i></u>	<u><i>TGIF1</i></u>	<u><i>TCF21</i></u>	<u><i>HMOX1</i></u>
<u><i>EIF1B</i></u>	<u><i>CSRP1</i></u>	<u><i>SAT1</i></u>	<u><i>COX6A1</i></u>	<u><i>MAMDC2</i></u>	<u><i>ASAHI</i></u>	<u><i>SOD3</i></u>	<u><i>LTBP1</i></u>
<u><i>GPRC5A</i></u>	<u><i>C1R</i></u>	<u><i>FN1</i></u>	<u><i>FRZB</i></u>	<u><i>ADM</i></u>	<u><i>CTHRC1</i></u>	<u><i>SH3PXD2B</i></u>	<u><i>MFGE8</i></u>
<u><i>CXCL12</i></u>	<u><i>PNO1</i></u>	<u><i>LRRC59</i></u>	<u><i>TIPARP</i></u>	<u><i>INTS6</i></u>	<u><i>CES1</i></u>	<u><i>NUPR1</i></u>	<u><i>SLPI</i></u>
<u><i>MT-ATP8</i></u>	<u><i>SRSF2</i></u>	<u><i>EIF5A</i></u>	<u><i>B2M</i></u>	<u><i>CREM</i></u>	<u><i>CTSF</i></u>	<u><i>FIS1</i></u>	<u><i>ETS2</i></u>
<u><i>FST</i></u>	<u><i>THBS2</i></u>	<u><i>PTP4A1</i></u>	<u><i>PXDC1</i></u>	<u><i>CFD</i></u>	<u><i>PALLD</i></u>	<u><i>COX7C</i></u>	<u><i>CLEC3B</i></u>
<u><i>DDX21</i></u>	<u><i>BDKRB2</i></u>	<u><i>ASPN</i></u>	<u><i>DBNDD2</i></u>	<u><i>LURAP1L</i></u>	<u><i>RNASEK</i></u>	<u><i>JMJD1C</i></u>	<u><i>SRGN</i></u>
<i>3-Mar</i>	<u><i>GNPNAT1</i></u>	<u><i>MCL1</i></u>	<u><i>MTIX</i></u>	<u><i>MYL12A</i></u>	<u><i>GCLM</i></u>	<u><i>TMEM47</i></u>	<u><i>PIK3R1</i></u>
<u><i>MT-CO2</i></u>	<u><i>FLNA</i></u>	<u><i>PLTP</i></u>	<u><i>EIF1AX</i></u>	<u><i>CYCS</i></u>	<u><i>PTMS</i></u>	<u><i>CHMP1B</i></u>	<u><i>HSPA5</i></u>
<u><i>ITM2A</i></u>	<u><i>ATP1B3</i></u>	<u><i>C1orf21</i></u>	<u><i>WTAP</i></u>	<u><i>TWISTNB</i></u>	<u><i>SLC16A7</i></u>	<u><i>FCGRT</i></u>	<u><i>JUND</i></u>
<u><i>SPSB1</i></u>	<u><i>PIM3</i></u>	<u><i>VASH2</i></u>	<u><i>TWIST2</i></u>	<u><i>GABARAPL1</i></u>	<u><i>RPS29</i></u>	<u><i>LPP</i></u>	<u><i>TFPI2</i></u>
<u><i>NCL</i></u>	<u><i>BRIX1</i></u>	<u><i>HLA-C</i></u>	<u><i>TSPAN8</i></u>	<u><i>EIF4E</i></u>	<u><i>SPAG9</i></u>	<u><i>SRSF3</i></u>	<u><i>HSPB8</i></u>
<u><i>DSTN</i></u>	<u><i>HSPD1</i></u>	<u><i>FAM126A</i></u>	<u><i>TXNIP</i></u>	<u><i>MEF2C</i></u>	<u><i>TFRC</i></u>	<u><i>GJA1</i></u>	<u><i>GPC3</i></u>

<u>HNRNPAB</u>	<u>SERPINF1</u>	<u>ALDH1A3</u>	<u>EIF3A</u>	<u>TCP1</u>	<u>RAN</u>	<u>HSPB6</u>	<u>S100A13</u>
<u>MYLK</u>	<u>UBC</u>	<u>NR2F2</u>	<u>TUBB2B</u>	<u>ABLIM1</u>	<u>TIMP3</u>	<u>HSPA2</u>	<u>IFITM1</u>
<u>ERRF1</u>	<u>SFPO</u>	<u>EDIL3</u>	<u>C2orf40</u>	<u>G3BP1</u>	<u>TGM2</u>	<u>TRIB1</u>	<u>CSRP2</u>
<u>IGFBP7</u>	<u>HNRNPF</u>	<u>DKC1</u>	<u>BZW2</u>	<u>CHN1</u>	<u>EPAS1</u>	<u>PEA15</u>	<u>TM4SF1</u>
<u>MEDAG</u>	<u>NDUFA4</u>	<u>SOD2</u>	<u>ABCA9</u>	<u>PPP1R12A</u>	<u>PDLIM7</u>	<u>PLAT</u>	<u>ARID5B</u>
<u>MT-CO1</u>	<u>ZFP36L2</u>	<u>FGFR1</u>	<u>EFEMP1</u>	<u>TUBA4A</u>	<u>EHD2</u>	<u>RPS26</u>	<u>CH25H</u>
<u>HSP90AB1</u>	<u>COX4I2</u>	<u>DAB2</u>	<u>BCCIP</u>	<u>TGFBR3</u>	<u>SPHK1</u>	<u>MACF1</u>	<u>HAS2</u>
<u>CYP1B1</u>	<u>PAMR1</u>	<u>SLC25A33</u>	<u>PCOLCE</u>	<u>H2AFJ</u>	<u>SNHG12</u>	<u>NREP</u>	<u>DUSP1</u>
<u>FGF7</u>	<u>ARHGDI B</u>	<u>NIP7</u>	<u>SRSF5</u>	<u>PEBP1</u>	<u>NEDD9</u>	<u>MFAP5</u>	<u>HOPX</u>
<u>PDLIM4</u>	<u>SRPX</u>	<u>CTGF</u>	<u>ENO1</u>	<u>HEYL</u>	<u>CTSB</u>	<u>MT1M</u>	<u>PTGDS</u>
<u>FKBP1A</u>	<u>FOSL1</u>	<u>PNRC1</u>	<u>EGR1</u>	<u>MT-ND6</u>	<u>LAMB1</u>	<u>COX7A2</u>	<u>KLF2</u>
<u>MYC</u>	<u>MGST3</u>	<u>C7</u>	<u>TRMT10C</u>	<u>REXO2</u>	<u>RP11-14N7.2</u>	<u>MSRB3</u>	<u>CXCL1</u>
<u>EIF1</u>	<u>TUBB2A</u>	<u>BIN1</u>	<u>SVEP1</u>	<u>ADD3</u>	<u>GSN</u>	<u>PTX3</u>	<u>PDGFRA</u>
<u>MT-ND4L</u>	<u>COL4A1</u>	<u>PAICS</u>	<u>HNRNPD L</u>	<u>MOXD1</u>	<u>EDF1</u>	<u>LRP1</u>	<u>BTG1</u>
<u>AC090498.1</u>	<u>MESDC1</u>	<u>PTGIS</u>	<u>PPP3CA</u>	<u>CLCF1</u>	<u>GLT8D2</u>	<u>NDUFB7</u>	<u>COL6A2</u>
<u>NOP58</u>	<u>DCAF13</u>	<u>EGFR</u>	<u>BCAM</u>	<u>ARPC3</u>	<u>COMP</u>	<u>S100A16</u>	<u>REL</u>
<u>VEGFA</u>	<u>IGF1</u>	<u>NAMPT</u>	<u>FUS</u>	<u>TNFAIP6</u>	<u>ADGRF5</u>	<u>PTN</u>	<u>ELN</u>
<u>SRSF7</u>	<u>NT5E</u>	<u>TUBB6</u>	<u>C10orf10</u>	<u>SDCBP</u>	<u>CCNL1</u>	<u>TCEAL4</u>	<u>NR4A2</u>
<u>WT1</u>	<u>PTRH2</u>	<u>CYBRD1</u>	<u>EXOSC4</u>	<u>TGFB1I1</u>	<u>HMG2</u>	<u>RRAD</u>	<u>IGFBP2</u>
<u>CALD1</u>	<u>PLBD1</u>	<u>TXNRD1</u>	<u>TXN</u>	<u>BAIAP2</u>	<u>RASL12</u>	<u>IGSF10</u>	<u>S100A10</u>
<u>BTG3</u>	<u>CD82</u>	<u>B4GALT1</u>	<u>TUBB3</u>	<u>PLEKHH2</u>	<u>SORBS2</u>	<u>TUBA1B</u>	<u>FBLN2</u>
<u>MYL6</u>	<u>SH3BP5</u>	<u>CPXM2</u>	<u>SERTAD1</u>	<u>VAMP8</u>	<u>STRAP</u>	<u>MT-ND3</u>	<u>CRABP2</u>
<u>C16orf45</u>	<u>C16orf89</u>	<u>NOTCH3</u>	<u>TPBG</u>	<u>ISYNA1</u>	<u>TIMP2</u>	<u>C1QTNF3</u>	<u>HSPA1B</u>
<u>PDGFRL</u>	<u>HNRNPU</u>	<u>EMILIN2</u>	<u>CCDC109 B</u>	<u>NEGR1</u>	<u>TGFBI</u>	<u>UQCR10</u>	<u>PLK2</u>
<u>LSP1</u>	<u>CYP26B1</u>	<u>GPX3</u>	<u>ABHD5</u>	<u>PCBP1</u>	<u>TNS1</u>	<u>DDX24</u>	<u>ARC</u>
<u>EGFL6</u>	<u>NFE2L2</u>	<u>TNXB</u>	<u>INPP1</u>	<u>STAT3</u>	<u>TMEM204</u>	<u>C9orf16</u>	<u>SFRP2</u>
<u>ATP13A3</u>	<u>XBP1</u>	<u>KRT18</u>	<u>RANBP2</u>	<u>INSIG1</u>	<u>F2R</u>	<u>PDGFRB</u>	<u>HSPA1A</u>
<u>PLAU</u>	<u>KLF3</u>	<u>TINAGL1</u>	<u>PLPP3</u>	<u>IPO7</u>	<u>CTSD</u>	<u>SLC39A14</u>	<u>QSOX1</u>
<u>CCDC80</u>	<u>MYH11</u>	<u>DES</u>	<u>RPF2</u>	<u>CREB5</u>	<u>BRD2</u>	<u>ACTG1</u>	<u>COL14A1</u>
<u>ZFP36L1</u>	<u>FSTL1</u>	<u>POSTN</u>	<u>RBM25</u>	<u>TBX2</u>	<u>EIF3K</u>	<u>JUNB</u>	<u>GADD45 B</u>

<u>HSPA8</u>	<u>SNU13</u>	<u>ITGBL1</u>	<u>SGCA</u>	<u>CCDC47</u>	<u>ZC3H15</u>	<u>STEAP1</u>	<u>HIGD1B</u>
<u>MAT2A</u>	<u>OSR1</u>	<u>SYNCRIP</u>	<u>OSMR</u>	<u>CTSH</u>	<u>SLC3A2</u>	<u>HLA-DRB1</u>	<u>ID2</u>
<u>CHD1</u>	<u>BZW1</u>	<u>TOP1</u>	<u>PROS1</u>	<u>MIDN</u>	<u>KRT8</u>	<u>PSME1</u>	<u>CXCL3</u>
<u>CEBPZ</u>	<u>CPXM1</u>	<u>PLS3</u>	<u>ST3GAL1</u>	<u>SERTAD2</u>	<u>COX6B1</u>	<u>SCN7A</u>	<u>RGS2</u>
<u>PPDPF</u>	<u>STK17A</u>	<u>POLR1C</u>	<u>HNRNPA0</u>	<u>SNRPB</u>	<u>DDR2</u>	<u>C12orf57</u>	<u>NBL1</u>
<u>NPM1</u>	<u>TNFRSF1A</u>	<u>SLC40A1</u>	<u>TPT1</u>	<u>UGP2</u>	<u>C8orf4</u>	<u>ANK2</u>	<u>CXCL2</u>
<u>ATP5G2</u>	<u>GRPEL1</u>	<u>ABL1</u>	<u>CILP</u>	<u>FAM46A</u>	<u>CFL1</u>	<u>ENAH</u>	<u>RGCC</u>
<u>C3</u>	<u>SEPW1</u>	<u>CD34</u>	<u>GABARAP</u>	<u>FABP5</u>	<u>PRRX1</u>	<u>RARRES1</u>	<u>RHOB</u>
<u>EZR</u>	<u>METRNL</u>	<u>TOMM5</u>	<u>TFAM</u>	<u>ACTN1</u>	<u>UBE2N</u>	<u>ANKRD28</u>	<u>EMP1</u>
<u>AMD1</u>	<u>RFK</u>	<u>SCARA5</u>	<u>DDX3Y</u>	<u>CDH11</u>	<u>RPL22L1</u>	<u>HIF1A</u>	<u>FABP4</u>
<u>LBH</u>	<u>KLF4</u>	<u>TNC</u>	<u>ABCA8</u>	<u>TMEM176B</u>	<u>HLA-DPB1</u>	<u>TSKU</u>	<u>ATF3</u>
<u>NXT1</u>	<u>EDNRA</u>	<u>UCK2</u>	<u>RBM39</u>	<u>COX4I1</u>	<u>PDE5A</u>	<u>TLN1</u>	<u>THBD</u>
<u>CHRD1</u>	<u>ABL2</u>	<u>IGFBP4</u>	<u>SLC16A1</u>	<u>RCAN2</u>	<u>MYL12B</u>	<u>SMOC2</u>	<u>CTSK</u>
<u>LTBP2</u>	<u>EBF2</u>	<u>EIF2S1</u>	<u>H2AFX</u>	<u>ADAMTS16</u>	<u>TSC22D3</u>	<u>GPM6B</u>	<u>G0S2</u>
<u>BDKRB1</u>	<u>NOP56</u>	<u>FBLN1</u>	<u>IGFBP6</u>	<u>SLC19A2</u>	<u>PARK7</u>	<u>COL15A1</u>	<u>PRG4</u>
<u>LRRN4CL</u>	<u>COL8A1</u>	<u>SLC20A1</u>	<u>SOAT1</u>	<u>HLA-DRA</u>	<u>HNRNP H1</u>	<u>FERMT2</u>	<u>SGK1</u>
<u>ETF1</u>	<u>MPZL1</u>	<u>TSPAN3</u>	<u>CYB5R3</u>	<u>MARCKSL1</u>	<u>ATPIF1</u>	<u>PLXDC2</u>	<u>FHL2</u>
<u>CAV1</u>	<u>COTL1</u>	<u>LIMCH1</u>	<u>SEMA6A</u>	<u>HIPK3</u>	<u>ABI3BP</u>	<u>SNAI2</u>	<u>APOE</u>
<u>WDR43</u>	<u>RGS5</u>	<u>SNRPD1</u>	<u>HMGN3</u>	<u>MYH10</u>	<u>TGFBR2</u>	<u>RAB13</u>	<u>BMP5</u>
<u>CBLB</u>	<u>HSP90AA1</u>	<u>CALM1</u>	<u>HSPA9</u>	<u>HAS1</u>	<u>SPCS1</u>	<u>HCFC1R1</u>	<u>NR4A1</u>
<u>IFI16</u>	<u>NIFK</u>	<u>GTPBP4</u>	<u>SYNGR2</u>	<u>CD4</u>	<u>GUCY1A3</u>	<u>MCAM</u>	<u>NFKBIA</u>
<u>ACKR3</u>	<u>CIRBP</u>	<u>DNTTIP2</u>	<u>TAF1D</u>	<u>PLA2G2A</u>	<u>RBBP6</u>	<u>ESAM</u>	<u>JUN</u>
<u>LDHA</u>	<u>ARPC5L</u>	<u>GLIS3</u>	<u>SPARC</u>	<u>FLNB</u>	<u>COX7A1</u>	<u>TACC1</u>	<u>CTSC</u>
<u>EGR3</u>	<u>BAZ1A</u>	<u>DDX5</u>	<u>EIF5</u>	<u>PITPNB</u>	<u>IL33</u>	<u>MRC2</u>	<u>GEM</u>
<u>KLF9</u>	<u>SERBP1</u>	<u>CFI</u>	<u>CCT2</u>	<u>FOSB</u>	<u>WDR83OS</u>	<u>MSN</u>	<u>PTGS2</u>
<u>ITGA1</u>	<u>A2M</u>	<u>PERP</u>	<u>HMGAI</u>	<u>RDH10</u>	<u>ATP5D</u>	<u>NDUFB10</u>	<u>CFB</u>
<u>PLAUR</u>	<u>TSHZ2</u>	<u>EIF3J</u>	<u>CRIM1</u>	<u>ACTN4</u>	<u>PODN</u>	<u>TMEM70</u>	<u>C11orf96</u>
<u>RPL41</u>	<u>TUBB4B</u>	<u>GSPT1</u>	<u>NME1</u>	<u>APP</u>	<u>PAG1</u>	<u>WFDC1</u>	<u>CNN1</u>
<u>ATP1A1</u>	<u>TUBA1C</u>	<u>STEAP2</u>	<u>PNPLA8</u>	<u>COX8A</u>	<u>SPINT2</u>	<u>ILK</u>	<u>TNFAIP3</u>
<u>CALM2</u>	<u>CD248</u>	<u>NAA50</u>	<u>EIF5B</u>	<u>VAT1</u>	<u>VIMP</u>	<u>NEXN</u>	<u>VCAN</u>

<u>DCN</u>	<u>DNAJB1</u>	<u>IL6ST</u>	<u>USP36</u>	<u>EIF4A1</u>	<u>ACTG2</u>	<u>HMGNI</u>	<u>ADAMTS</u> <u>4</u>
<u>SFRP1</u>	<u>COL4A2</u>	<u>FAM198</u> <u>B</u>	<u>SPTSSA</u>	<u>UOCRB</u>	<u>SOSTM1</u>	<u>LXN</u>	<u>LMCD1</u>
<u>GNL3</u>	<u>TMEM17</u> <u>6A</u>	<u>RIOK1</u>	<u>VASN</u>	<u>MT-ND2</u>	<u>HTRA3</u>	<u>MIR22H</u> <u>G</u>	<u>SERPIN</u> <u>A3</u>
<u>RSL1D1</u>	<u>MLLT11</u>	<u>TRIO</u>	<u>UOCR11</u>	<u>SF1</u>	<u>TRAPPC</u> <u>1</u>	<u>LMNA</u>	<u>WISP2</u>
<u>YWHAG</u>	<u>MT-ND1</u>	<u>NUFIP2</u>	<u>ELOVL5</u>	<u>KPNA2</u>	<u>SNX9</u>	<u>TNFAIP2</u>	<u>IFI27</u>
<u>PFDN2</u>	<u>CPZ</u>	<u>ANGPTL</u> <u>4</u>	<u>DUSP6</u>	<u>CDC42EP</u> <u>2</u>	<u>GNG11</u>	<u>GAS7</u>	<u>COL3A1</u>
<u>ZNF593</u>	<u>SEMA3C</u>	<u>HSPH1</u>	<u>TNFRSF1</u> <u>2A</u>	<u>WWTR1</u>	<u>HLA-B</u>	<u>SOCS3</u>	
<u>UGDH</u>	<u>RBMS1</u>	<u>OAF</u>	<u>ANXA1</u>	<u>ROBO2</u>	<u>FOSL2</u>	<u>MAFF</u>	
<u>MT-CO3</u>	<u>CD55</u>	<u>TXLNG</u>	<u>DDX3X</u>	<u>AKAP12</u>	<u>GUCY1B</u> <u>3</u>	<u>CCDC71</u> <u>L</u>	
<u>ADAMTS1</u> <u>5</u>	<u>MT-ND5</u>	<u>SELM</u>	<u>SELK</u>	<u>LPL</u>	<u>ZFAS1</u>	<u>PDK4</u>	
<u>NOLC1</u>	<u>HRH1</u>	<u>MRT04</u>	<u>RERG</u>	<u>CTNNAL1</u>	<u>NPNT</u>	<u>MALAT1</u>	
<u>DNAJA1</u>	<u>ITGA5</u>	<u>TPM1</u>	<u>DCLK1</u>	<u>CD9</u>	<u>PDPN</u>	<u>HSPE1</u>	
<u>MTRNR2L</u> <u>12</u>	<u>MT1G</u>	<u>CXCL8</u>	<u>DKK1</u>	<u>RGS16</u>	<u>CCL2</u>	<u>AKR1C1</u>	

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2343 * Derived from(41).

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2346 **Table S18, related to Suppl. Fig. 8. Overview of Non-GLP Study CRL 20229915 performed in**
2347 **cynomolgus monkeys with ALE.F02.**

<u>Study type and duration of dosing</u>	<u>Species, origin, number of animals</u>	<u>Animal ID</u>	<u>Doses (mg/kg/day)</u>	<u>Administration</u>
<u>Single dose, 42 days</u>	<u>Cynomolgus monkey (Vietnam)</u> <u>1 male/group</u>	<u>2001</u>	<u>0.3mg/kg</u>	<u>IV bolus</u>
		<u>2002</u>	<u>3mg/kg</u>	<u>IV bolus</u>
		<u>2003</u>	<u>15mg/kg</u>	<u>IV bolus</u>
<u>Repeat dose, 28 days, 4 doses applied weekly</u>	<u>Cynomolgus monkey (Vietnam)</u> <u>2 males/group</u>	<u>2004</u>	<u>60mg/kg</u>	<u>IV infusion (30 min)</u>
		<u>2005</u>		
		<u>2006</u> <u>2007</u>	<u>150mg/kg</u>	<u>IV infusion (30 min)</u>

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2353 **The following Excel files are available online:**

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2355 **Data file S1. Raw data from figures.**

2356 **Data file S2, related to Figure 6. CLDN1 interaction partners identified by mass spectrometry in**

2357 **anti-CLDN1 mAb- or Control mAb-treated Huh7 cells.**

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