

Gut microbiota-derived imidazole propionate promotes primary sclerosing cholangitis via p38 signalling

Received: 17 October 2025

Accepted: 29 July 2026

Published online: 4 September 2026

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A list of authors and their affiliations appears at the end of the paper

Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease of the bile ducts that can lead to biliary cancer and end-stage liver disease. PSC is associated with inflammatory bowel disease and an altered gut microbiota. However, the molecular mechanisms underlying gut–liver interactions in PSC remain poorly characterized. Here we show that the gut microbiota-derived metabolite imidazole propionate (ImP) is a disease driver in PSC. Individuals with PSC have higher circulating ImP levels than individuals with related conditions, and high ImP levels predict reduced survival in PSC. Cholangiocytes exposed to ImP show activated mammalian target of rapamycin complex 1 (mTORC1) signalling and secrete pro-inflammatory and pro-fibrogenic factors. Chronic administration of ImP to mice induces liver inflammation and fibrosis through a p38-dependent mechanism, upstream of mTORC1. We propose that chronic exposure to ImP induces cholangiocyte injury, which alone or in concert with other factors causes clinical worsening of PSC. Therefore, targeting ImP production or signalling may represent therapeutic avenues in PSC.

The gut and liver are closely connected: bile from the liver flows through the bile ducts to the intestine and contributes to shaping its environment, while nutrient-rich portal blood from the gut exposes the liver to absorbed dietary, endogenous and microbial compounds. Intestinal diseases are commonly associated with liver co-morbidities, but there is still limited understanding of the molecular interactions of the gut–liver axis. PSC can be considered a model disease for a disturbed gut–liver axis. PSC is an autoimmune disease affecting young adults, characterized by chronic inflammation of the large intrahepatic and extrahepatic bile ducts, leading to progressive fibrotic strictures causing cholestasis and eventually end-stage liver disease^{1,2}. Up to 80% of individuals with PSC have concomitant inflammatory bowel disease (IBD), most commonly ulcerative colitis, representing strong evidence of a link between the gut and the liver. Furthermore, PSC is one of the most difficult remaining challenges in hepatology, with no disease-modifying medical therapy available and liver transplantation (LT) as the only treatment option¹. The median time from diagnosis to LT or death is 13–21 years¹. A subgroup of people with both PSC and IBD eventually need a colectomy owing to active colitis or colorectal cancer. Interestingly, a proctocolectomy with ileostomy improves the prognosis of

the liver disease³, which may suggest potential disease-contributing mechanisms from the colonic microbiota.

Chronic inflammatory disorders like IBD or autoimmune liver diseases are associated with altered gut microbiota structure or function⁴. The gut microbiota in PSC is characterized by lower diversity than in healthy individuals^{5–7}, and transfer of gut microbiota from individuals with PSC worsens biliary disease in mice⁸. The mechanisms are probably multiple and complex, including specific bacteria or functional alterations in bacterial metabolism⁹. This includes reduced capacity to synthesize vitamin B6 and branched-chain amino acids, with corresponding reduced circulating levels of these essential nutrients, which also associate with a more severe disease course in PSC¹⁰. Diet and gut microbiota are the strongest determinants of circulating metabolites in humans¹¹, and gut microbial diversity predicts levels of circulating metabolites¹².

In the present study, we aimed to identify microbial metabolites driving PSC by performing comprehensive untargeted metabolomic profiling of peripheral blood, representing biochemical footprints of microbial activity. Metabolomic profiling is challenging in liver diseases because the liver is a key regulator of metabolism, and extensive

 e-mail: j.e.r.hov@medisin.uio.no

secondary changes are expected. However, we previously showed that the PSC-associated microbiota alterations persist after LT⁷, and by focusing specifically on metabolites persistently elevated after LT, we found the microbially produced amino acid metabolite ImP to be associated with PSC and its progression. In cholangiocyte organoids derived from people with PSC, we found that ImP promotes a secretory, pro-fibrogenic phenotype. ImP activates the mTORC1 signalling pathway in cholangiocytes and mediates portal inflammation, biliary fibrosis and ductular reaction in the mouse liver in a p38-dependent manner, which is upstream of mTORC1. Taken together, our findings suggest that the dysbiotic gut in PSC favours production of ImP with the potential to impact disease progression.

Results

Identification of ImP as a persistent disease modifier in PSC

To identify metabolites that can modify PSC, we performed untargeted metabolomic profiling of plasma from individuals with ($n = 132$) and without ($n = 32$) PSC (Fig. 1a and Supplementary Table 1). We found a large shift in the circulating metabolome in individuals with PSC (PSC pre-LT), with 261 out of 1,108 (24%) of the quantified metabolites showing associations with the disease after adjusting for confounding factors and at a false discovery rate (FDR) threshold of 0.01 (Supplementary Table 2). Furthermore, with increasing PSC disease severity as measured by risk scores based on clinical features and liver biochemistry, the metabolomic profiles diverged even further from that of healthy controls (HCs) (Extended Data Fig. 1a,b). Notably, we observed a clear correlation between metabolite associations with PSC and disease score severity (Extended Data Fig. 1c,d), suggesting that a substantial proportion of the variation in the PSC circulating metabolome could be attributed to liver disease itself.

Out of the 261 metabolites associated with PSC, 38 demonstrated a significant association with LT-free survival after adjusting for sex and disease severity (Fig. 1b and Supplementary Table 3). With the rationale that metabolites contributing to PSC should be produced independently of liver function, we subsequently retained only metabolites with similar concentrations before and 5 years after LT (Supplementary Table 4). This filtering criterion yielded nine candidate metabolites (Supplementary Table 5). To test the internal validity of these metabolites, we bootstrapped the complete filtering pipeline. ImP, a microbially derived amino acid derivative previously found to associate with type 2 diabetes¹³, heart failure¹⁴ and wound healing¹⁵, was the most frequently selected metabolite (71%) in the bootstrap samples and was chosen for further validation (Fig. 1c). To confirm elevated levels of ImP in PSC, we used targeted liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis in serum samples from an expanded Norwegian cohort, denoted NO-1, consisting of 164 PSC pre-LT and 100 HC cross-sectional samples, 117 and 29 of which had also been part of the untargeted metabolomics experiment. Additionally, we examined serum from an independent Norwegian PSC pre-LT cohort, designated NO-2 ($n = 151$; Supplementary Tables 6 and 7). The relative ImP levels were strongly correlated with targeted measurements (adjusted $R^2 = 0.91$; Extended Data Fig. 2a).

We confirmed elevated ImP levels in PSC (Fig. 2a and Extended Data Fig. 2b), and we replicated this observation in external validation cohorts from Sweden (SE; $n = 139$ PSC and $n = 51$ HCs) and the USA (US; $n = 609$ PSC; Fig. 2b). In individuals with paired serum–plasma samples, ImP levels were strongly correlated (291 pairs, adjusted $R^2 = 0.80$; Extended Data Fig. 2c), showing that both matrices can be used. In cross-sectional cohorts with available control populations, ImP demonstrated an association with PSC after accounting for age and sex (Extended Data Fig. 2d).

Elevated levels of ImP were consistently linked to a decrease in LT-free survival across all four cross-sectional PSC cohorts (Fig. 2c–f; baseline characteristics in Supplementary Table 8). Furthermore, in a longitudinal cohort in which individuals with PSC were sampled before

and serially after LT ($n = 567$ samples from 158 individuals)¹⁶, plasma ImP levels were highest immediately before LT (Fig. 2d), falling slightly after LT to the levels seen in the cross-sectional NO-1 and NO-2 PSC cohorts. These analyses suggest that ImP levels are largely unaffected by LT, thus confirming our discovery in the untargeted metabolomics (discovery) cohort. Notably, recurrent PSC (rPSC) after LT is a significant clinical problem¹. Higher circulating ImP levels were associated with an increased risk of rPSC after LT (hazard ratio per doubling = 1.18, 95% CI = 1.01–1.39, $P = 0.041$; hazard ratio for ImP > median = 1.98, 95% CI = 1.24–3.17, $P = 0.0041$; Fig. 2h) (Supplementary Table 9).

ImP levels are elevated in PSC compared to other liver and inflammatory conditions

ImP is known to be produced by commensal microbiota, but the key factors influencing its aberrant levels in PSC remain unknown^{13,17}. To address this knowledge gap, we first examined serum ImP levels in individuals with PSC stratified by prior colectomy type. Individuals who had undergone a proctocolectomy with ileostomy had reduced, but not abolished, serum ImP levels compared to those with other types of colectomies and reconstructions, including ileal pouch–anal anastomoses and those who had not undergone colectomy (Fig. 3a), suggesting a contribution of colon-resident microbiota to circulating ImP.

ImP levels were consistent among people with PSC regardless of the presence of concurrent IBD and the IBD disease activity (Fig. 3b,c). However, in individuals with longstanding IBD but without PSC (Supplementary Table 10), the median concentration of ImP in serum was 10.5 nM. This was higher than in HCs (8.5 nM) but substantially lower than in the Norwegian PSC pre-LT cohorts (19.1 nM in the pooled Norwegian cohort; Fig. 3d).

In other liver diseases, serum ImP was also lower than in PSC (Fig. 3d and Extended Data Fig. 3a). This included primary biliary cholangitis (PBC), an autoimmune liver disease that affects the small bile ducts of the liver (median ImP = 11.9 nM and 11.0 nM in a Danish and an Italian cohort, respectively; Supplementary Tables 11 and 12) and metabolic dysfunction-associated liver disease (MASLD; 12.0 nM; Supplementary Table 13). Positive correlations between increasing disease stage and circulating ImP levels were found both in PSC (Spearman's ρ with the clinical Mayo PSC risk score = 0.45, $P = 2.3 \times 10^{-17}$; $\rho = 0.36$, $P = 1.4 \times 10^{-5}$; and $\rho = 0.27$, $P = 3.0 \times 10^{-8}$, in the NO, SE and US cohorts, respectively) (Fig. 3f), and PBC, with a positive correlation with liver stiffness measured by elastography (Spearman's ρ with elastography = 0.24, $P = 0.0002$). Given that these cross-disease comparisons could be confounded by differences in disease severity and the measures of disease severity, we stratified by platelet counts, which were available for the majority of participants. A platelet count below $150 \times 10^9 \text{ l}^{-1}$, commonly seen in advanced liver diseases with portal hypertension, was used as a surrogate marker and was associated with generally higher serum ImP concentrations (Fig. 3e). Among individuals with normal platelet counts, ImP levels were significantly higher in PSC than in PBC and MASLD, but not in those with low platelet counts (Fig. 3e), a finding supported by age-adjusted linear models (Extended Data Fig. 3b). The observation of higher ImP levels in advanced liver disease was supported by samples measured at time of listing for LT, in which ImP levels were similarly elevated in a cohort of transplant candidates with PSC, PBC or alcohol-related liver disease (Extended Data Fig. 3c). Overall, this suggests that ImP was clearly higher in PSC than other diseases in earlier stages of disease, and there was a convergence towards high ImP concentrations in very advanced liver disease, irrespective of aetiology.

Liver conditions such as PBC, MASLD and alcohol-related liver disease are, like PSC, known to involve gut microbial alterations^{4,18}, and cirrhosis itself—in particular in a decompensated state—is associated with shared gut environmental and microbial alterations across the spectrum of liver diseases¹⁹. The particularly elevated ImP in PSC may therefore reflect PSC-specific microbial changes that are later masked

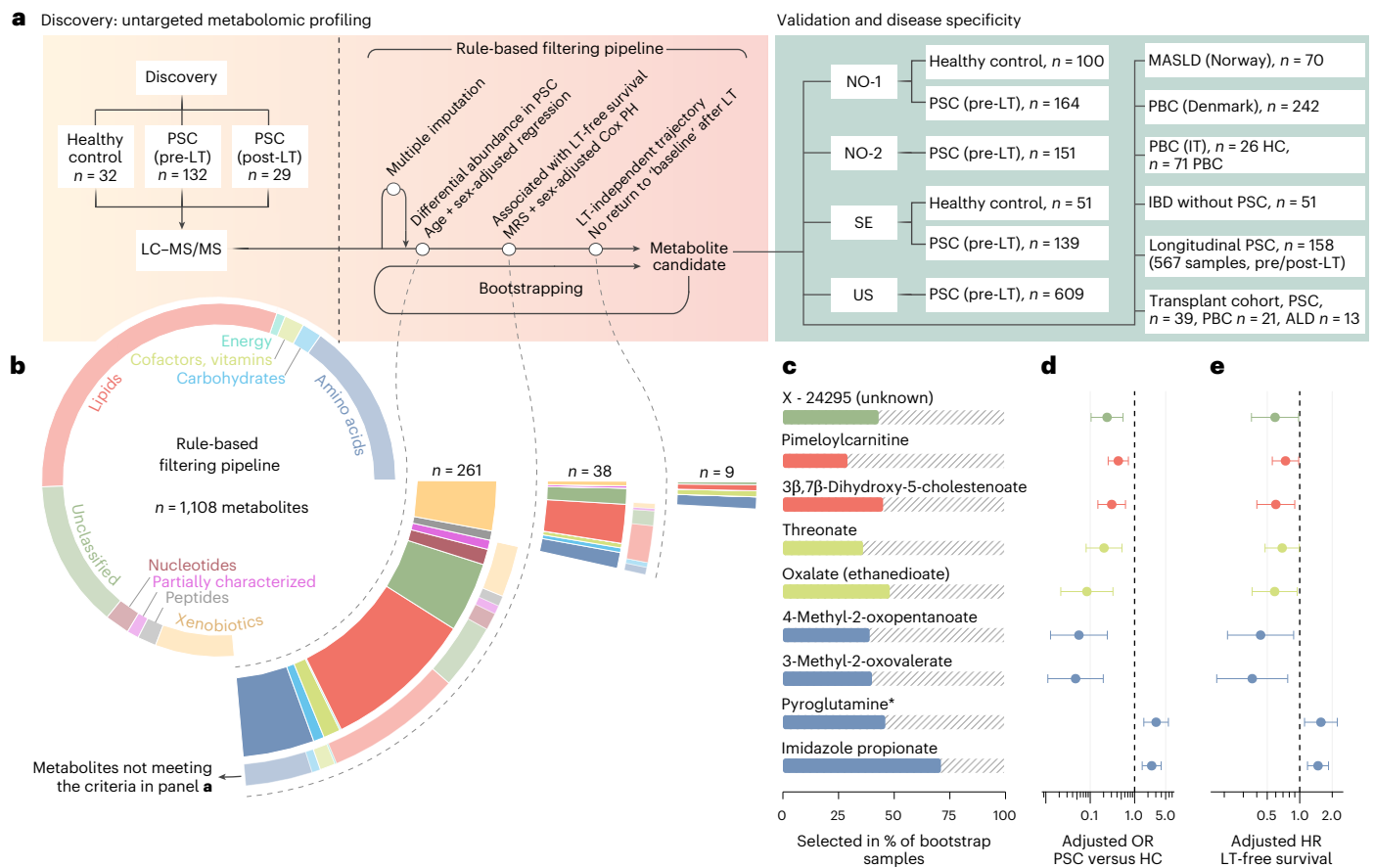


Fig. 1 | Discovery of ImP as a potential disease modifier in PSC. a, Flowchart of the discovery pipeline and cohorts used for targeted validation and evaluation of disease specificity. MRS, Mayo PSC risk score; PH, proportional hazard. **b**, Visualization of the number and classes of metabolites sequentially meeting the criteria at the different steps of the rule-based filtering pipeline shown in **a** (Methods). Faded, thinner bars indicate metabolites excluded at each step. **c**, Frequency with which the candidate metabolites from **b** fulfilled

the three criteria in the resampled datasets. **d**, Pooled odds ratios (ORs) and 95% confidence intervals (CIs) from multivariable binary logistic regression analyses on multiply imputed data to predict disease status (PSC versus HCs, HCs as reference) for the selected metabolites. **e**, Pooled hazard ratios (HRs) and 95% CIs from multivariable Cox proportional hazards analyses (adjusted for sex and MRS) on multiply imputed data to predict LT-free survival for the selected metabolites.

by cirrhosis and portal hypertension-driven microbial alterations. To investigate the microbial contribution to ImP, we analysed temporally matched blood and stool samples (taken up to 14 days apart) from 138 individuals with PSC. Here, high ImP was associated with reduced gut microbial diversity, including a lower number of observed genera and species, and reduced Shannon diversity (Fig. 3e). At an FDR threshold of 0.05, we identified 19 species that were positively correlated with circulating ImP levels (Supplementary Table 14), including *Enterocloster clostridioformis* and *Enterocloster boltae*, both previously shown to be increased in PSC¹⁰, and *Ruminococcus gnavus*, previously linked to ImP production¹⁷.

There were no substantial differences in ImP levels between users and non-users of ursodeoxycholic acid, a commonly prescribed drug in PSC (Extended Data Fig. 3d). Levels of urocanate, which can be produced both by the host and bacteria and is reduced exclusively by bacterial urocanate reductase (urcA) into ImP¹³, were comparable between individuals with PSC and HCs (Extended Data Fig. 3e,f), and individuals with PBC and HCs (Extended Data Fig. 3g), supporting that microbial activity rather than substrate availability affect the circulating levels of ImP in humans.

ImP activates human cholangiocytes and induces a pro-inflammatory and pro-fibrotic phenotype

Activated cholangiocytes secrete pro-inflammatory and pro-fibrotic substances, a feature believed to occur early in the cascading

development of cholangiopathies like PSC²⁰. To test whether ImP could function as a cholangiocyte-activating substance, we cultured endoscopic retrograde cholangiopancreatography-derived cholangiocytes from three people with PSC as organoids ex vivo and treated them with ImP or urocanate for 6 days (Fig. 4a). The concentrations of pro-inflammatory and pro-fibrotic cytokines were elevated in conditioned media from ImP-treated organoids compared to urocanate and vehicle (Fig. 4b and Supplementary Fig. 1). In addition, ImP treatment impaired the ability of the organoids to maintain a spherical morphology (Fig. 4c), suggesting that ImP directly interacts with cholangiocytes.

Chronic administration of ImP induces biliary inflammation and liver fibrosis in mice

Next, we investigated whether chronic ImP exposure induces cholangiocyte injury in vivo by administering ImP to 6-week-old wild-type C57BL/6J mice in the drinking water for 8 weeks (Fig. 5a). There were no significant differences in water intake, body weight or organ weights of the mice by treatment group (Extended Data Fig. 4a–c). Plasma concentrations of ImP, but not urocanate, increased in ImP-treated mice compared to vehicle-treated controls (Ctrl) (Fig. 5b). ImP-treated mice also demonstrated elevated ImP levels in liver tissue, gallbladder tissue and bile, as measured in a limited number of mice (Extended Data Fig. 4d), indicating biliary excretion and ImP exposure of cholangiocytes. Quantifications of villi and crypt length and Ki67 staining (a marker of epithelial cell proliferation) indicated no differences in small and large

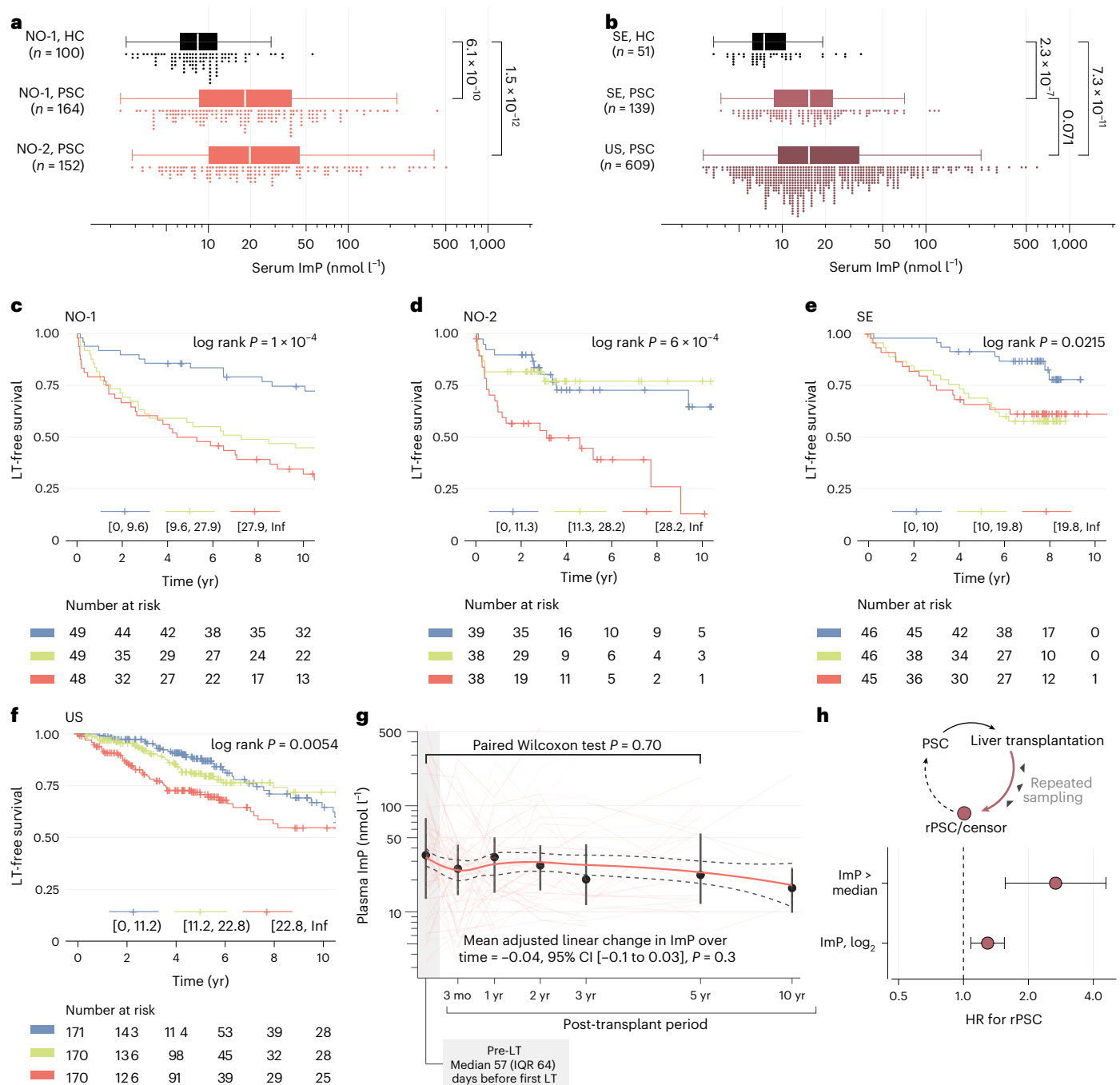


Fig. 2 | Targeted validation of the rule-based filtering pipeline and ImP as a disease modifier in PSC. a, ImP levels in serum samples from HCs and people with PSC in the expanded Norwegian cohort NO-1 and independent targeted cohort NO-2, measured using targeted LC-MS/MS. **b**, Serum ImP levels in HCs and people with PSC in external validation cohorts from Sweden (SE; measured using a different targeted LC-MS/MS protocol) and the USA (US). Differences between groups were tested using two-sided Mann-Whitney *U*-tests. **In a and b**, points represent individual ImP measurements. Box plot centre lines indicate the median, the bounds of the box indicate the 25th and 75th percentiles and the whiskers indicate the minimum and maximum values within 1.5 × the interquartile range (IQR). **c–f**, LT-free survival curves of individuals with PSC in NO-1, NO-2, SE and US cohorts, stratified by ImP tertiles. Differences in overall survival distributions were tested using log-rank tests. **g**, Individual longitudinal trajectories of ImP in the longitudinal PSC cohort, including only samples taken

before an rPSC diagnosis (*n* = 403 samples from 143 individuals). A LOESS curve (red line with dotted lines indicating 95% CIs) was fit to the data, and median and IQRs are shown for each timepoint in black. The average adjusted change in log(ImP) over time was estimated using a mixed-effects model, with patient ID as a random effect and age and sex as fixed covariates (model estimates are shown in the plot). The model allowed for correlation in both the intercept and the effect of time for each subject by specifying a first-order autoregressive structure. A paired Wilcoxon signed-rank test was performed to test for differences in ImP levels in paired samples from pre-LT and 5-year samples. **h**, Schematic illustration of rPSC as a time-to-event outcome in post-transplant PSC; triangles indicate serial sampling. The forest plot shows univariable HR point estimates and 95% CIs for ImP (dichotomized by the median and log₂-transformed) modelled as time-varying predictors of rPSC (*n* = 300 samples from 119 individuals; 75 rPSC diagnoses).

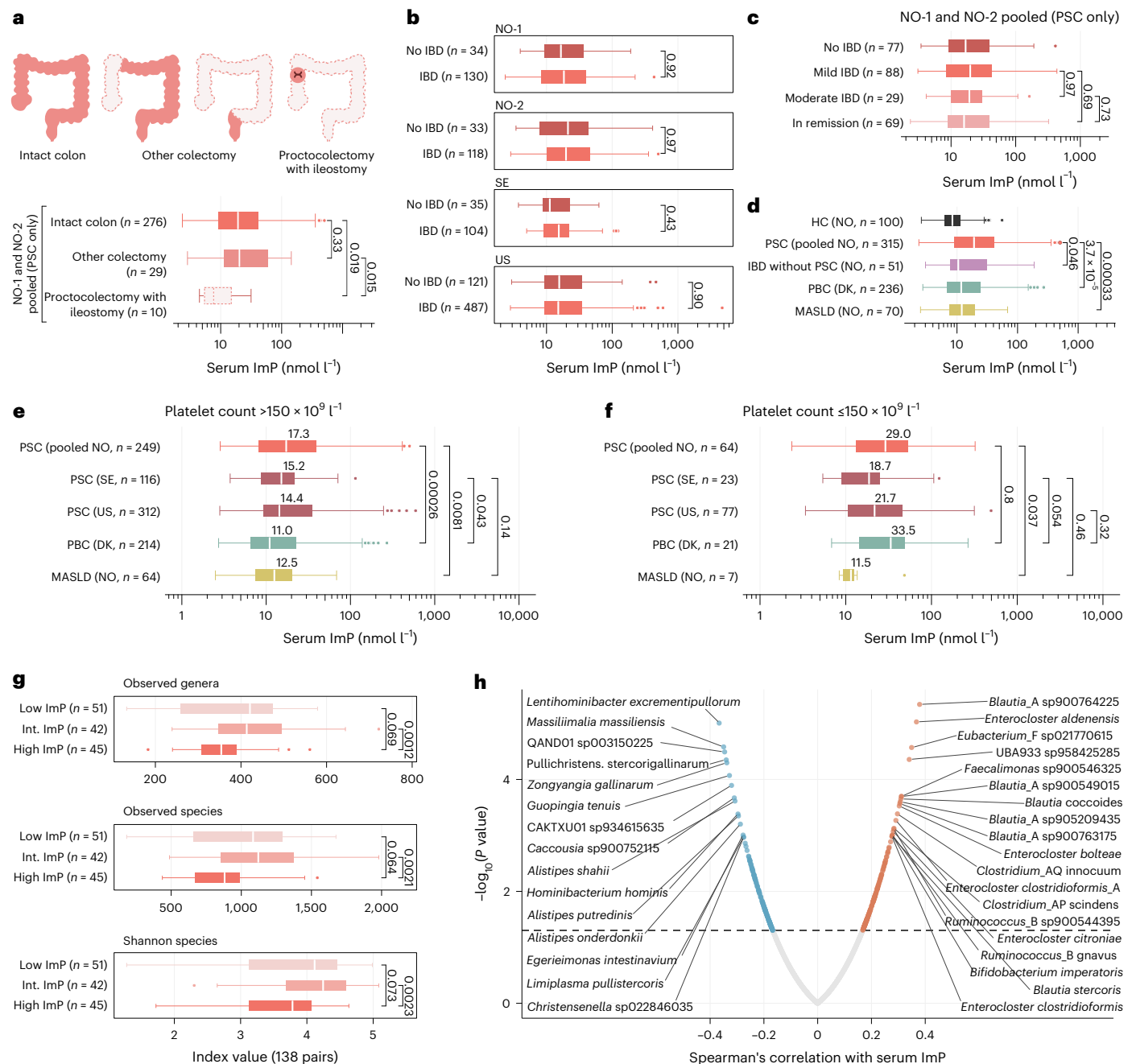


Fig. 3 | Disease specificity of ImP. **a**, Schematic illustration of different types of colectomy, and distributions of serum ImP in people with PSC by colectomy status at the time of sampling (NO-1 and NO-2 cohorts pooled). ‘Other colectomy’ encompasses other types of colectomies, including colectomy with ileorectal anastomosis or ileal pouch–anal anastomosis. **b**, Serum ImP in the PSC pre-LT cohorts by IBD concurrency. **c**, Serum ImP in the NO-1 and NO-2 cohorts pooled, by IBD activity at time of sampling. **d**, Serum ImP in individuals with IBD without PSC, PBC and metabolic dysfunction-associated steatotic liver disease (MASLD), alongside HCs and individuals with PSC from the NO-1 and NO-2 cohorts (same data used to produce Fig. 2a). **e, f**, Serum ImP in various liver diseases stratified by platelet counts (above versus below 150 × 10⁹ L⁻¹). The numbers above the box plots indicate medians. **g**, Observed gut microbial genera, species and Shannon

species in stool samples from people with PSC who had a blood sample taken within ±14 days of stool collection, stratified by ImP categories (same thresholds as in Fig. 2f). **h**, Correlations between individual gut bacterial species and serum ImP levels were calculated using Spearman’s rank correlation, with the x axis showing Spearman’s rank correlation and the y axis showing the corresponding unadjusted P value. Associations with raw P values of <0.05 are coloured blue or red, while species with text annotation indicate a Benjamini–Hochberg-adjusted P < 0.05. For **a–g**, differences in distributions were tested using two-sided Mann–Whitney U-tests. In **a–g**, box plot centre lines indicate the median, the bounds of the box indicate the 25th and 75th percentiles and the whiskers indicate the minimum and maximum values within 1.5 × the IQR. Data points beyond the whiskers are plotted individually as outliers.

intestine morphology (Supplementary Fig. 2). By contrast, ImP-treated compared with Ctrl mice exhibited increased liver portal inflammation (Fig. 5c). This finding was supported by increased F4/80-positive macrophages in the livers of ImP-treated mice, consistent with macrophage accumulation in portal areas that was also evident in human

PSC liver sections (Extended Data Fig. 5a–d). No differences were observed in CD3⁺ T cell infiltration between the treatment groups (Extended Data Fig. 5a,b), which contrasts with human PSC, where there was evidence of increased infiltration of T cells (CD3) and B cells (CD20) (Extended Data Fig. 5c,d). ImP-treated mice also showed increased

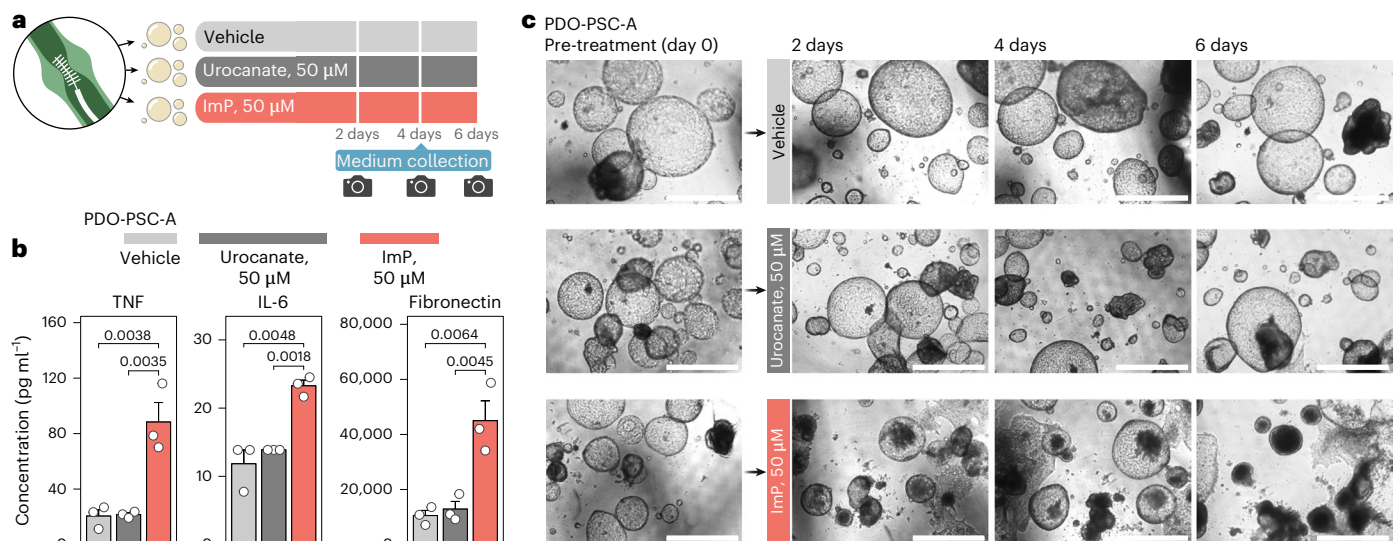


Fig. 4 | Chronic administration of ImP induces duct inflammation and fibrosis in human-derived cholangiocyte organoids. **a**, Schematic representation of the experimental set-up. **b**, Concentrations of tumour necrosis factor (TNF), interleukin-6 (IL-6) and fibronectin in conditioned medium collected at day 4 from cholangiocyte organoids derived from a donor with PSC (PDO-PSC-A), after the organoids were treated for 4 days with ImP (50 μ M), urocanate (Uro, 50 μ M) or vehicle. **c**, Light microscopy time-series images of PDO-PSC-A organoids at

baseline (day 0, pre-treatment) and after 2, 4 and 6 days of treatment with ImP (50 μ M), Uro (50 μ M) or vehicle. The organoid experiment was performed in technical triplicate; bars represent mean values; error bars, s.e.m. Statistical significance was calculated using ANOVA with Tukey's honestly significant difference post hoc test. Data on additional markers and experimental models from two additional donors (yielding similar results) are shown in Supplementary Fig. 1. Scale bars, 100 μ m. PDO, patient-derived organoid.

histological features of liver fibrosis, especially in the portal areas (Fig. 5c,d), a finding we confirmed using an artificial intelligence-based, quantitative analysis of the same liver sections (Supplementary Fig. 3). Moreover, the proportion of proliferating cytokeratin 19-positive (CK19⁺) cholangiocytes was increased in ImP-treated compared with Ctrl mice (Fig. 5c). Despite these histological changes, serum alkaline phosphatase (ALP) as well as aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels were comparable between groups. The expression levels of pro-inflammatory and pro-fibrotic genes tended to be elevated in whole livers from ImP-treated mice (Extended Data Fig. 4d–f).

To test whether physiologically relevant, gut-microbiota-derived ImP could induce cholangiocyte injury in vivo, we colonized germ-free Swiss Webster mice, an outbred strain well suited for bacterial colonization²¹, with *Shewanella oneidensis* and administered urocanate to the mice through their drinking water (Extended Data Fig. 6a). *S. oneidensis* is known to express the rate-limiting bacterial UrdA²², which is required for ImP synthesis from urocanate. No differences were observed in body weight or organ weights between germ-free and *S. oneidensis*-colonized mice, except for a significant reduction in caecum size, a well-documented consequence of microbial colonization (Extended Data Fig. 6b–d). Colonization with *S. oneidensis* increased ImP plasma concentrations into the micromolar range (Extended Data Fig. 6e,f). Histological analysis revealed that *S. oneidensis*-colonized mice displayed statistically significant increases in portal inflammation and liver fibrosis as well as increased ductular reaction compared with germ-free controls, recapitulating the major pathological features observed following chronic ImP administration in drinking water (Extended Data Fig. 6g,h).

Next, we investigated the effect of ImP administration in a diet-induced model of PSC²³ by supplementing mice on chow diet with 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) for 2 weeks (Fig. 5e). Plasma concentrations of both ImP and urocanate increased in ImP-treated mice compared with Ctrl mice (Fig. 5f). There were no significant differences in water intake, body weight or organ weights of the mice by treatment group (Extended Data Fig. 7a–c). As expected,

administration of DDC led to an overall increase in portal inflammation, liver fibrosis and ductular reaction compared to Ctrl mice. However, despite the potency of DDC, liver fibrosis, portal inflammation and ductular reaction were more prominent in ImP-treated compared to Ctrl mice (Fig. 5g,h). Serum ALP as well as ASAT levels were comparable between groups, with a small significant reduction of ASAT levels in ImP-treated mice compared to Ctrl. There were no differences in the expression of hepatic pro-inflammatory and pro-fibrotic genes between the ImP and Ctrl groups (Extended Data Fig. 7e,f), again possibly a result of ImP primarily affecting portal areas (Supplementary Fig. 3).

ImP activates mTORC1 in cholangiocytes

To identify molecular targets of ImP in cholangiocytes, we performed RNA sequencing of ImP-treated human cholangiocyte H69 cells. We observed a significant dose-dependent impact on the transcriptome, illustrated by enrichment of gene sets annotated to hypoxia, tumour necrosis factor signalling by nuclear factor kappa-light-chain-enhancer of activated B cells and mTORC1 signalling, while no such effects were observed in urocanate-treated cells (Fig. 6a,b and Extended Data Fig. 8). These findings were consistent with our previous observation in hepatocytes that short-term ImP induces mTORC1 pathway activity by promoting the upstream p38 γ / δ activity¹³. To assess whether this mechanism was also active in vivo, we stained livers of mice treated with ImP with an antibody against phosphorylated (active) mTOR (p-mTOR). ImP treatment increased the number of p-mTOR⁺ cholangiocytes in mice fed either standard chow or chow supplemented with DDC (Fig. 6c–f). A similar trend was observed in *S. oneidensis*-colonized mice (Fig. 6c,e). To determine whether mTOR signalling is similarly induced in human disease, we performed p-mTOR staining on liver sections from individuals with PSC and HCs. We observed increased p-mTOR staining in PSC samples compared to HCs (Fig. 6g,h), consistent with the possibility that disease-associated factors like ImP contribute to mTOR activation in PSC. In line with a role of mTOR activation in PSC, our data suggest that mTOR inhibition may be protective: in a cohort of $n = 182$ people who received LT for PSC (Supplementary Table 15), exposure to the mTOR inhibitor sirolimus at or before the 1 year follow-up visit was

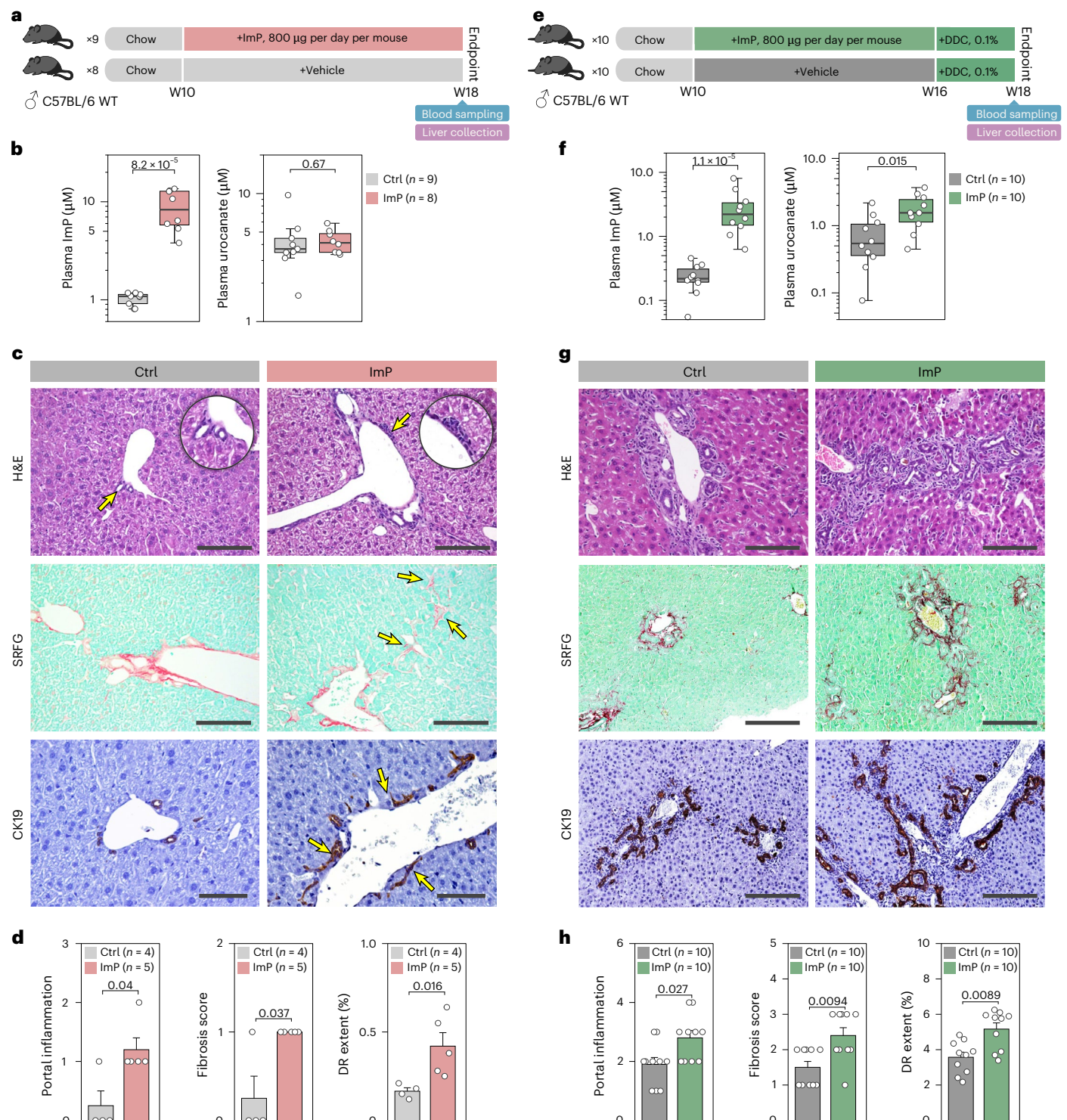


Fig. 5 | Chronic administration of ImP induces duct inflammation and fibrosis in vivo. **a**, Schematic representation of the experimental design using C57BL/6J wild-type (WT) mice on a chow diet (n = 8 or 9 mice per group). **b**, Plasma levels of ImP and urocanate in WT mice treated with vehicle (Ctrl) or ImP for 8 weeks on a chow diet. **c,d**, Representative images (c) and relative quantification (d) for haematoxylin and eosin (H&E), Sirius red fast green (SRFG) and immunohistochemistry for CK19 in livers of mice treated without or with ImP on a chow diet. Scale bars, 100 µm (H&E, SRFG) and 50 µm (CK19). Arrows indicate areas of portal inflammation (magnified in the circles; H&E), collagen fibres (SRFG) or ductular reaction (DR; CK19). Among the eight and nine mice per group, four and five were randomly selected for liver histology analyses.

e, Schematic representation of the experimental design using WT mice on a 0.1% DDC diet (n = 10 per group). **f**, Plasma levels of ImP and urocanate in WT mice treated with vehicle (Ctrl) or ImP for 8 weeks on a 6-week chow diet followed by a 2-week DDC diet. **g,h**, Representative images and relative quantification for H&E, SRFG and immunohistochemistry for CK19 in livers of mice treated with or without ImP on a chow diet. Scale bars, 100 µm (H&E, SRFG) and 50 µm (CK19). For **b** and **f**, box plot centre lines indicate the median, the bounds of the box indicate the 25th and 75th percentiles and the whiskers indicate the minimum and maximum values within 1.5× the IQR. For **d** and **h**, bars represent the mean values; error bars, s.e.m. *P* values were calculated using two-sided Mann–Whitney *U*-tests.

less common among those who subsequently developed rPSC ($n = 7$, 6%) than among those who did not ($n = 11$, 16%; $P = 0.04$).

Genetic ablation of p38 γ / δ abrogates ImP-induced biliary inflammation and fibrosis

We have previously demonstrated that ImP can induce mTORC1 signalling through p38 γ and, to a lesser extent, p38 δ (refs. 13,24). To investigate whether this pathway mediates ImP-induced biliary inflammation and liver fibrosis in vivo, we generated mice with a whole-body deletion of p38 γ / δ (double knockout, DKO) (Supplementary Fig. 4) and administered ImP in the drinking water to both 6-week-old wild-type and DKO mice for 8 weeks (Fig. 7a). As expected, ImP treatment increased circulating ImP but not urocanate to similar levels in both the DKO mice and wild-type littermate controls (Fig. 7b). Neither ImP treatment nor genotype affected food intake, body weight or organ weights (Extended Data Fig. 9a–d). As expected, ImP administration to wild-type mice increased liver p-mTOR staining, portal inflammation, liver fibrosis and ductular reaction compared to wild-type control mice (Fig. 7c,d). By contrast, p-mTOR staining was abolished and ImP did not induce portal inflammation, liver fibrosis or ductular reaction in DKO mice (Fig. 7c,d). Serum ALP, ASAT and ALAT were increased in DKO mice treated with ImP compared to Ctrl mice (Extended Data Fig. 9e). This finding may suggest that ImP had additional effects on the liver not mediated by mTORC1. There were no differences in the expression of hepatic inflammatory and pro-fibrotic genes in ImP-treated or Ctrl groups (Extended Data Fig. 9f, g).

When the DKO mice were challenged with a 2-week DDC diet (Fig. 7e,f and Extended Data Fig. 10), we did not observe any effect of ImP treatment on mTORC1 activation or worsening of fibrosis, inflammation or ductular reaction (Fig. 7g,h), showing that ImP-driven biliary injury in mice was dependent on p38 γ / δ and thus functional mTORC1 signalling. There were no differences in the expression of hepatic pro-inflammatory and pro-fibrotic genes in ImP-treated or Ctrl groups (Extended Data Fig. 10f, g).

Discussion

We identified the bacterial histidine metabolite ImP as a driver of PSC by alternative p38 activation. Our findings represent a molecular mechanism linking the gut alterations and liver manifestations in PSC, highlighting pathogenetic principles that are relevant for other diseases involving the gut–liver axis.

We demonstrated broad metabolic alterations in PSC, but a strict filtering pipeline to detect potential disease drivers enabled the identification of circulating ImP as being consistently elevated in PSC cohorts compared with healthy individuals, as well as when considering non-advanced disease and other metabolic or cholestatic liver diseases. Our findings are consistent with prior untargeted metabolomics data in which ImP was reported among metabolites elevated in PSC compared with HCs²⁵, as well as earlier observations of increased urinary ImP excretion in individuals with gastrointestinal diseases²⁵. However, ImP has not been specifically investigated in PSC.

Mechanistically, we identified the alternative p38 signalling through mTORC as a molecular target for cholangiocyte pro-fibrotic and inflammatory phenotypes. This finding is consistent with a previous study that demonstrated that the mTORC1 pathway was upregulated in bile ducts in individuals with PSC²⁶. mTORC has been shown to drive cholangiocyte inflammation, proliferation and fibrosis, both in chronic liver diseases and in cholangiocarcinoma^{26,27}, which can develop in PSC. Our data showed that deletion of p38 γ / δ prevents ImP-driven inflammation and fibrosis, indicating that targeting p38 MAPK or mTORC directly could be efficacious in PSC. Although DKO models provide mechanistic support, they may also introduce off-target effects and should therefore clearly be interpreted with caution. However, pirfenidone (a p38 inhibitor with antifibrotic effect) has been shown to reduce cholestatic liver injury in a chemically induced

mouse model of cholestasis²⁸. Furthermore, in a small pilot study of pirfenidone in PSC, a trend towards reduced histological fibrosis was observed, although the drug was poorly tolerated²⁹. The associations observed between ImP and later rPSC, and less exposure to sirolimus in individuals with later recurrence, also provide a rationale for further testing of pharmacological inhibition of these pathways; for example, using mTOR inhibitors.

ImP is a marker of microbial ecosystems with low diversity¹², such as in IBD^{30,31}. Individuals with IBD without PSC exhibited elevated ImP levels compared with controls, although these were lower than in PSC, and ImP levels were similar in PSC irrespective of concurrent IBD. ImP was only modestly elevated in PBC and MASLD, collectively suggesting a general increase in microbial ImP production across disease-associated gut environments, with the highest levels observed in PSC. Although ImP levels converge across liver diseases of different aetiologies at the time of LT, they are disproportionately elevated in PSC earlier in the disease course, supporting a PSC-specific component driving ImP production. This interpretation is further supported by the persistent elevation of ImP after LT for PSC, suggesting that increased ImP is not simply a consequence of impaired liver function but may instead reflect ongoing dysbiosis⁷. Despite these signs of specificity, high ImP has been observed in, for example, type 2 diabetes and cardiovascular disease^{13,14,17,32–34}, which indicates that ImP may be relevant in several conditions in which the mTORC pathway is involved. There is limited overlap between risk of PSC and, for example, cardiovascular disease³⁵, suggesting that there are other determinants of ImP-driven disease specificity, and the role and effect of ImP in any individual is a consequence of multiple factors like genetic susceptibility, age, sex and other exposures or risk factors.

ImP is produced by gut bacterial enzymes encoded by the *urda* gene; however, accurate quantification at the DNA level is challenging owing to substantial sequence heterogeneity among *urda*-encoding genes¹³. Circulating ImP levels have been repeatedly associated with an overlapping set of specific bacteria in other metabolic conditions^{14,17,36}. Here, we showed that bacteria that have been previously reported to be increased in PSC, such as *E. clostridioformis* and *E. boltae*¹⁰, or that are known to be associated with ImP-circulating levels, such as *R. gnavus*¹⁷, are associated with high circulating levels of ImP and with low bacterial diversity. Together with the lack of normalization of gut microbiota composition after LT for PSC⁷ and the known impact of advanced liver disease on promoting dysbiosis and expansion of pathobionts¹⁹, these observations suggest that the intestinal environment in PSC predisposes to increased ImP production. As disease progresses towards cirrhosis, non-PSC-specific factors related to advanced liver disease may then become the dominant determinants of circulating ImP.

Experimental data in mice have shown that broad-spectrum antibiotics reduce microbial ImP production³⁷, and we show here that colonization with an *urda*-expressing strain, combined with dietary supplementation with urocanate, increased systemic ImP levels and signs of biliary injury. Together, these findings form a mechanistic link between gut microbial ImP biosynthesis and a biliary phenotype, and principally suggest that targeting the gut or gut microbes may be a therapeutic avenue. In humans, however, broad-spectrum antibiotics are unlikely to constitute a viable strategy and may paradoxically increase ImP levels by favouring a low-diversity, ImP-producing microbial ecosystem³⁷. In addition, such interventions may further impair microbiome integrity and promote antimicrobial resistance. Nonetheless, our finding that ImP was particularly low in proctocolectomized individuals with ileostomy could point towards a clinical benefit of intervention-based restriction of gut ImP production, and is consistent with prior data demonstrating better clinical outcomes in PSC after proctocolectomy with ileostomy³. Potential translational strategies may include more targeted approaches, such as limiting access to the dietary ImP substrates histidine and urocanate and reducing the

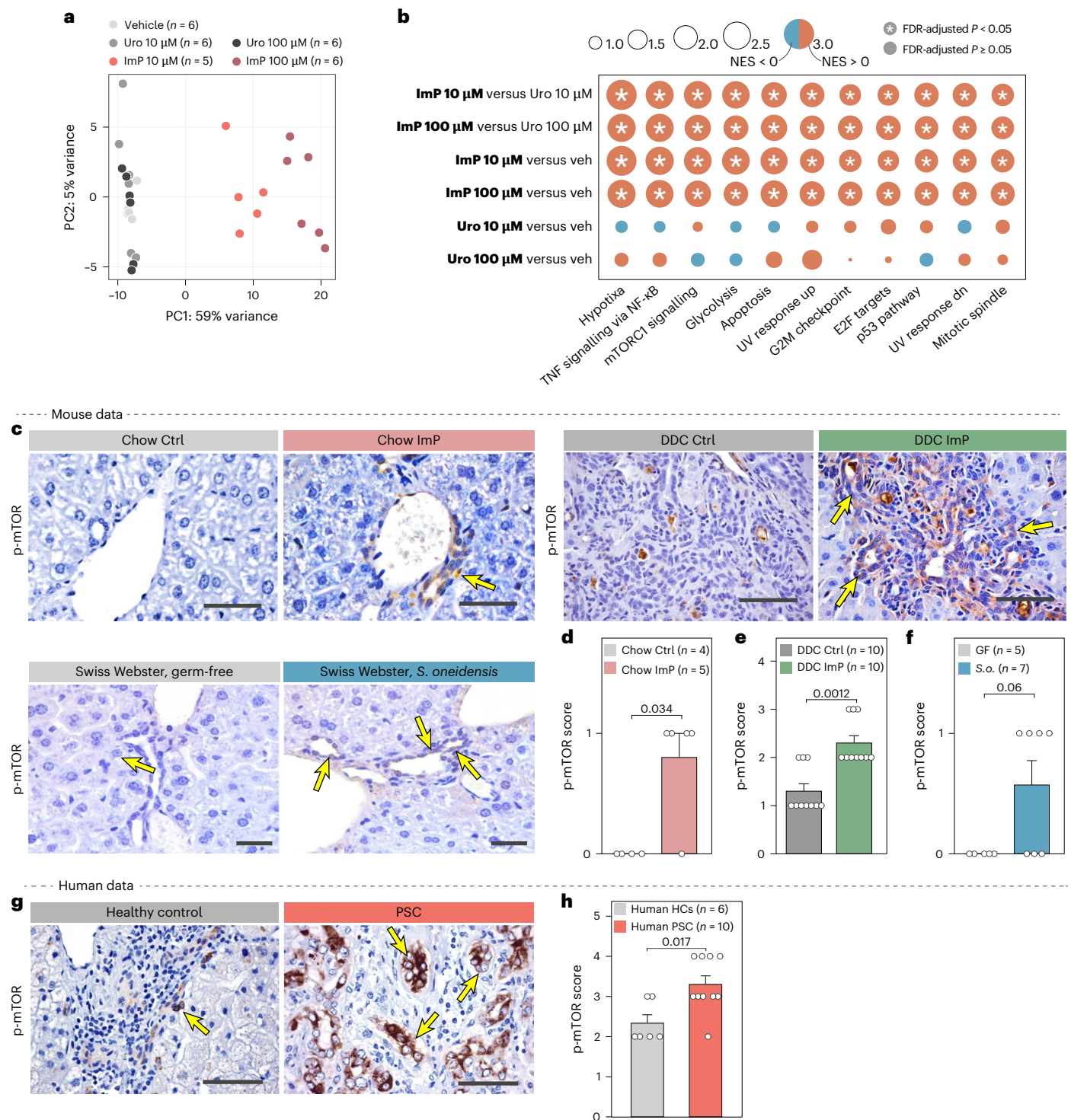


Fig. 6 | ImP activates the mTORC1 pathway in cholangiocytes. **a**, Principal component analysis of normalized RNA sequencing data from H69 cholangiocytes stimulated with ImP or urocanate at 10 μ M and 100 μ M. The first two principal components (PCs) are shown. One sample from the 10 μ M ImP group was excluded as a clear outlier. The experiment was performed in three independent biological replicates, each with two technical replicates. **b**, Gene set enrichment analysis (GSEA) of Hallmark gene sets across DESeq2 comparisons (non-bold text indicates reference categories). Enrichment P values were calculated using two-sided tests (using `fgsea::fgsea()`) and adjusted for multiple comparisons using the FDR. Gene sets with an FDR-adjusted $P < 1 \times 10^{-6}$ from the GSEA in the ImP 100 μ M versus Uro 100 μ M comparison are shown.

NES, normalized enrichment score. **c–f**, Representative liver sections stained for p-mTOR (**c**) and quantification of p-mTOR⁺ cells in mice fed chow ($n = 4$ and 5 per group) (**d**) or DDC diet ($n = 10$ per group) (**e**), vehicle-treated controls (Ctrl) or ImP-treated, germ-free (GF) and *S. oneidensis* (*S.o.*)-colonized mice ($n = 5$ and 7 per group) (**f**). **g, h**, Representative liver sections stained for p-mTOR (**g**) and quantification of p-mTOR⁺ cells in liver samples from HCs ($n = 6$) and individuals with PSC ($n = 10$) (**h**). Arrows indicate p-mTOR⁺ biliary epithelial cells ($n = 6$ and 10 per group). For **d, e, f** and **h**, bars represent the mean values; error bars, s.e.m. Statistical significance for individual comparisons was tested using two-sided Mann–Whitney U -tests. Scale bars, 50 μ m.

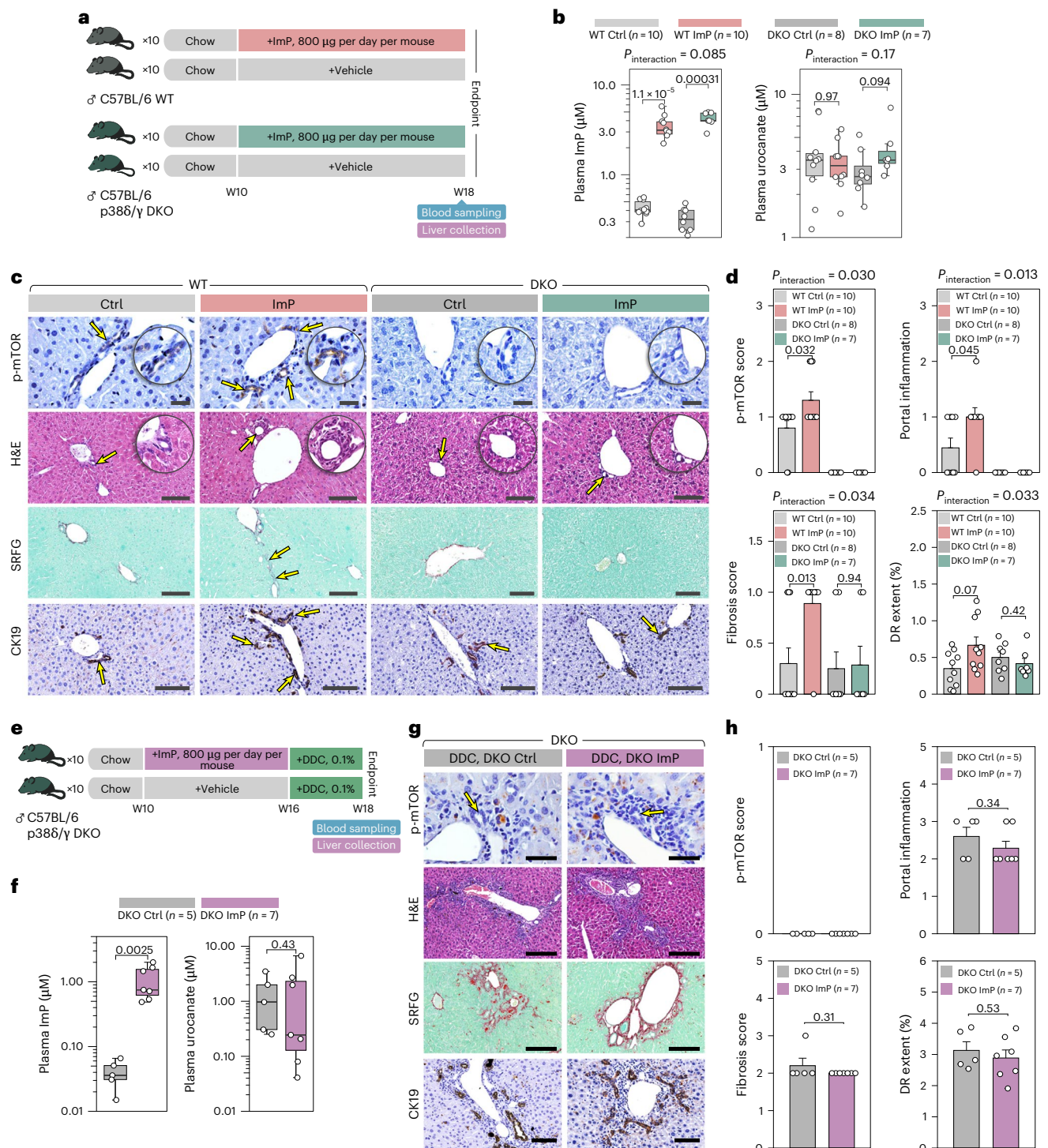


Fig. 7 | p38 γ/δ is necessary for ImP-induced biliary inflammation and fibrosis. **a**, Schematic representation of the experimental design using C57BL/6 WT mice and p38 γ/δ whole-body DKO mice on a chow diet ($n = 7$ and 10 mice per group). **b**, Plasma levels of ImP and urocanate in WT and DKO mice treated with vehicle (Ctrl) or ImP for 8 weeks on a chow diet. **c**, **d**, Representative images (**c**) and relative quantification (**d**) of H&E, SRFG and immunohistochemistry for CK19 in livers of WT and DKO mice treated with or without ImP on a chow diet. Scale bars, 100 μm (H&E, SRFG) and 50 μm (CK19). Arrows indicate areas of portal inflammation (magnified in the circles; H&E), collagen fibres (SRFG) or ductular reaction (CK19). **e**, Schematic representation of the experimental design using DKO mice on a 0.1% DDC diet ($n = 5$ and 7 per group). **f**, Plasma levels of ImP and urocanate in DKO mice treated with vehicle (Ctrl) or ImP for 8 weeks, on a 6-week

chow diet followed by 2 weeks on a DDC diet. **g**, **h**, Representative images (**g**) and relative quantification (**h**) for H&E, SRFG and immunohistochemistry for CK19 in livers of mice treated with or without ImP on a chow diet. Scale bars, 100 μm (H&E, SRFG) and 50 μm (CK19). Arrows indicate areas of portal inflammation (H&E), collagen fibres (SRFG) or ductular reaction (CK19). For **b** and **f**, box plot centre lines indicate the median, the bounds of the box indicate the 25th and 75th percentiles and the whiskers indicate the minimum and maximum values within $1.5 \times$ the IQR. For **d** and **h**, bars represent the mean values; error bars, s.e.m. P values for two-group comparisons were calculated using two-sided Mann–Whitney U -tests. For comparisons involving genotype and treatment, P values were derived from factorial analyses including genotype, treatment and their interaction.

capacity of ImP-producing strains to produce ImP through chronic UrdA inhibition^{37,38}.

PSC typically, over decades of progressive bile duct injury, causes liver failure and death³⁹. The exact cause of PSC is unclear but involves a complex interaction of genetic, environmental and immune factors. The impact of ImP in experimental models *in vivo* was mainly seen on liver histology and must be considered modest. In line with the natural history, we speculate that continuous exposure to elevated circulating levels of ImP over time, together with other factors, contributes to sclerosis of the bile ducts and disease progression. The physiological relevance of compound concentrations used in experimental science is also an important discussion point. We speculate that the long exposure time in humans could, in part, also explain the modest effect of short-term high ImP concentration in the *in vivo* models compared with the potent disease associations observed with lower serum ImP concentrations in humans. Notably, in the colonization model with an urdA-expressing strain, circulating ImP concentrations were within the upper range of what we find in human PSC, suggesting that these concentrations may be sufficient to induce biliary injury *in vivo*. The single-timepoint cross-sectional serum ImP concentrations in PSC varied from 2 nM to 500 nM, suggesting that the gut microbial potential to produce ImP—and hence its clinical impact—varies considerably between individuals. This is illustrated by the strongest effect on PSC survival seen in the upper tertile of ImP levels across all studied populations, suggesting that this is a subgroup in which ImP-directed therapies could be especially relevant.

Importantly, ImP may reach the cholangiocyte in portal blood directly perfusing large bile ducts⁴⁰, by systemic (arterial) circulation or even from the bile duct, as ImP is present in human bile⁴¹, in line with its presence in mouse liver tissue, gallbladder tissue and bile shown here. Indeed, exposing cholangiocytes to ImP led to a major transcriptomic rewiring and increased secretion of pro-inflammatory and pro-fibrogenic factors, thus resembling activated cholangiocytes responding to inflammation. Although the exact concentrations of ImP used in *in vitro* systems are high and not directly translatable to *in vivo* conditions, the experiments provide proof of principle of mechanisms underlying the effects observed *in vivo*. The recent discovery of the imidazoline 1 receptor as a probable target of ImP could lead to further molecular characterization of the ImP–mTORC pathway and potentially new therapeutic opportunities. As ImP may also influence macrophages and other cells expressing its receptor³⁴, and given the autoimmune features of PSC, studies of the impact of ImP on, for example, different immune cell subsets should also be a priority.

At present, there is no cure for PSC except LT for advanced cases, after which the disease often recurs. Detailed knowledge of disease mechanisms is a prerequisite for the development of an effective treatment. We have identified ImP as a circulating metabolite associated with PSC transplant-free survival. By demonstrating that ImP can activate cholangiocytes *ex vivo* and *in vivo* and elucidating the molecular pathways involved, we highlight that the development of treatments that can reduce production of ImP or inhibit ImP-induced cellular signalling may represent an important step forward in this orphan disease.

Methods

Participants

Inclusion, ethics and consent. Unless stated otherwise, there were no explicit clinical exclusion criteria, and sample availability determined sample sizes. All study subjects gave informed, written consent. The participants were not given any compensation. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the relevant institutional review boards (IRBs) and ethics committees in each participating country: Norway (2010/1540, 2011/2572, 2015/2140, 2012/286, 2016/1690 and 568878); Sweden (Dnr 571-18 and 2018/1111-32); Italy (PBC322 numbers 2969

and 5129/541-1); the USA (Mayo Clinic IRB for PSC studies, IRB numbers 670-02 and 16-005892); and Denmark (Central Denmark Region Ethics Committee, 1-10-72-146-16).

Untargeted discovery cohort. The discovery cohort consisted of cross-sectionally collected EDTA plasma samples from 132 liver pre-LT and 29 post-LT patients with PSC (5 year blood sample, no rPSC diagnosis made at time of sampling) who were prospectively recruited between 2008 and 2015 at the tertiary care hospital Oslo University Hospital, Rikshospitalet (Oslo, Norway). Thirty-three age-matched healthy donors from the Norwegian Bone Marrow Donor Registry were also included. Ethical approval was obtained from the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway (2011/2572, 2015/2140 and 2016/1690), which covers all analyses of Norwegian patients with PSC before or after transplant as well as HCs (that is, NO-1, NO-2 and the longitudinal PSC cohort; additional cohorts listed below).

Expanded targeted cohort (NO-1). An expanded, cross-sectional, technical validation cohort consisted of 164 individuals with pre-LT PSC and 100 age-matched HCs attending Rikshospitalet. Of these, 117 individuals with PSC and 29 HCs temporally overlapped with the discovery cohort (that is, samples were taken from the same blood draw). Both plasma and serum were analysed in parallel.

Independent targeted cohort (NO-2). An independent validation cohort from Rikshospitalet consisted of serum from 151 individuals with pre-LT PSC who had blood sampled cross-sectionally.

External validation cohort (SE). An external validation cohort consisted of 139 individuals with pre-LT PSC who were recruited at Karolinska University Hospital (Stockholm, Sweden) between 2008 and 2012, as well as 51 healthy donors recruited at Sahlgrenska University Hospital (Gothenburg, Sweden). All participants had donated one serum sample. Ethical approval: Dnr: 571-18 and 2018/1111-32.

External validation cohort (US). An external validation cohort from the USA ($n = 609$) was obtained from the PSC Scientific Community Resource⁴². Ethical approval was obtained from the Mayo Clinic (IRB numbers 670-02 and 16-005892).

Longitudinal PSC cohort. A longitudinal Norwegian cohort consisted of 158 individuals with PSC who underwent LT at Rikshospitalet between 1990 and 2016 (ref. 16) and for whom EDTA plasma samples were available. Individuals in this cohort were sampled both directly before and serially after (3 months, 1, 2, 3, 5 and 10 years) their first LT (totalling 567 plasma samples; median four samples donated; range, 1–7). In total, 51 participants overlapped with the expanded technical validation cohort, albeit with temporally non-matched plasma samples. Individuals included in the final analyses (see outcomes and definitions below) are described in Supplementary Table 9. Details regarding clinical definitions in the post-transplant setting are described in the Supplementary Note.

For the study of the effect of sirolimus on later rPSC, adults who underwent LT for PSC between 1988 and 2017 and who were evaluated at a 1 year follow-up were included (Supplementary Table 15).

PBC cohorts. A cross-sectional cohort comprised 71 consecutive individuals with PBC and 26 HCs enrolled at Bicocca University Hospital, Milan, and Policlinico Umberto I, Rome, for whom serum samples were available for ImP measurement. Ethical approval was obtained in Milan, Italy (PBC322; $n = 2969$), and Rome, Italy ($n = 5129/541/1$).

A cross-sectional cohort comprised 236 individuals with either incident ($n = 79$) or prevalent ($n = 157$) PBC, from whom serum samples were obtained at Aarhus University Hospital and Aalborg University

Hospital, Denmark⁴³. The study was approved by the ethical committee of the Central Denmark Region (1-10-72-146-16).

IBD cohort. A total of 51 serum samples were obtained from the prospective, population-based IBD cohort (IBSEN^{16,44}). The samples were drawn from a set of samples collected 20 years after IBD diagnosis from individuals who had no evidence of PSC as evaluated by MR cholangiopancreatography at the time of blood collection. Ethical approval was obtained from the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway (2010/1540).

MASLD cohort. The cross-sectional cohort comprised 71 adults with MASLD (42 males (59.2%); median age, 52 years (IQR, 23)) who were referred for liver elastography from gastroenterology, diabetes and morbid obesity outpatient clinics and consecutively enrolled at Haraldsplass Deaconess Hospital, Bergen, Norway, between 2023 and 2025. Ethical approval was obtained from the Regional Committee for Medical and Health Research Ethics in Western Norway (number 568878).

All participants met criteria for a MASLD-compatible diagnosis, defined by hepatic steatosis, absence of clinically significant alcohol intake (>12 units per week for males or >6 units per week for women) and exclusion of alternative or concomitant liver disease. Participants had a mean BMI of 35.6 ± 6.5 (s.d.), and 70 (98.6%) had at least one metabolic trait. Overall, 69 participants (97.2%) had overweight or obesity (BMI ≥ 25 kg m²), including 62 (87.3%) with obesity (BMI ≥ 30 kg m²); 30 (42.3%) had hypertension, 23 (32.4%) had hypercholesterolaemia and 21 (29.6%) had type 2 diabetes. Individuals receiving systemic corticosteroids or other immunosuppressive agents within 12 weeks before study inclusion were not eligible. All participants underwent standardized B-mode ultrasound examination on the day of serum or plasma sampling to confirm hepatic steatosis, defined as diffusely hyperechogenic liver parenchyma compared with right kidney parenchyma, followed by liver stiffness assessment in the same session⁴⁵.

Transplant cohort. For analysis of ImP levels in advanced-stage liver disease, serum samples obtained at LT evaluation were selected from 39 individuals with PSC, 21 with PBC and 13 with alcohol-related liver disease who subsequently underwent LT. Individuals with low-grade or high-grade dysplasia as the indication for transplantation were excluded. Samples were obtained within 1 year before transplantation.

Human PSC liver samples. For immunohistochemical analysis of human PSC liver tissues, formalin-fixed liver explant tissue was obtained from ten individuals who underwent LT for PSC in Oslo, Norway, between 2004 and 2012. All patients provided written informed consent. Median age at transplant was 37 years (range, 27–61 years). At the time of transplantation, median (range) biochemical parameters were 20 (4–219) $\mu\text{mol l}^{-1}$ for bilirubin, 41 (22–49) g l⁻¹ for albumin, 86 (20–258) U l⁻¹ for AST, 129 (44–290) U l⁻¹ for ALT, 250 (73–859) U l⁻¹ for ALP and 1.0 (0.9–2.4) for INR. No tissue is available for additional analyses. Ethical approval was obtained from the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway (2012/286).

Human control liver samples. As histological controls, human liver samples with normal histology ($n = 6$) were obtained from organ donors at the 'Paride Stefanini' Department of General Surgery and Organ Transplantation, Sapienza University of Rome, Italy. No donor organs were obtained from executed prisoners or other institutionalized individuals. Ethical approval was obtained in Rome, Italy (541/1).

Sex and gender reporting. Sex was incorporated into the study design and was determined from population records. Gender was not assessed. Adjustment for sex in the initial analyses of ImP levels did

not materially influence the observed associations, and therefore no further or post hoc analyses specifically examining sex-dependent effects were performed, given that there was no a priori hypothesis related to sex-specific effects.

Sex was also considered during the design of the animal experiments. In accordance with the principles of the 3Rs (replacement, reduction and refinement), we chose to perform the study using a single sex to minimize animal use. Only male mice were included because PSC shows a clear male predominance in humans, making male animals the most clinically relevant initial choice.

Outcomes, definitions and diagnostic criteria. PSC was defined according to accepted criteria¹. For the cross-sectional cohorts, the composite endpoint event was LT or death. LT was restricted to transplantations performed for PSC, irrespective of clinical or symptomatic burden. LT-free survival was calculated as the time from sampling to endpoint. For these analyses, subjects who donated blood, presumably in conjunction with a referral for LT ($n = 25$), or who had hepatobiliary cancer at the time of sampling ($n = 13$) were excluded.

Untargeted metabolomics using ultra-high performance LC–MS/MS

A total of 193 plasma samples were analysed by ultra-high performance liquid chromatography with tandem mass spectrometry (UPLC–MS/MS) at Metabolon Inc. Metabolites were identified by comparison with library entries for purified standards or recurrent unknowns, by matching retention time/index, mass-to-charge ratio and chromatographic data. Of 1,265 detected metabolites, 243 were of unknown identity (termed 'unclassified metabolites'). Further details are described in the Supplementary Note.

Targeted ImP and urocanate measurements. ImP and urocanate in serum samples from the external validation (SE) and PBC (Italy) cohorts were measured using targeted UPLC–MS/MS analysis as reported previously¹³, with minor modifications outlined in the Supplementary Note. For the NO-1, NO-2, US, longitudinal PSC, IBSEN IBD, PBC (DK) and MASLD (NO) cohorts, plasma and/or serum ImP was analysed using a panel for B-vitamins, kynurenines and a set of microbial metabolites at BEVITAL (Panel D, bevital.no) by LC–MS/MS as described in a previous publication⁴⁶.

Stool shotgun metagenomics (USA). Sampling protocol. Individuals with PSC from the USA were recruited through the PSC Scientific Community Resource⁴². Stool specimens were self-collected using additive-free mail-in kits, shipped overnight to the Mayo Clinic on ice packs and stored at -80°C before downstream processing. Genomic DNA was subsequently isolated at the University of Minnesota Genomics Center using the Qiagen DNeasy 96 PowerSoil Pro QIAcube HT kit.

Sequencing and shotgun metagenomic read processing. Shotgun metagenomic sequencing was performed using an Illumina NovaSeq 6000 at the University of Minnesota Genomics Center with the Nextera library preparation kit. Sequencing generated 150 bp paired-end reads, with a mean sequencing depth of 9.16 million read pairs per sample. Raw reads underwent quality control and host DNA decontamination, followed by taxonomic classification using Kraken 2 (ref. 47) against the GTDB reference database (v.220)⁴⁸, as previously described⁴⁹.

Data analysis. To account for compositionality, species-level count data were transformed using the centred log-ratio transformation with the `compositions::clr` function and a pseudocount of 1. Before centred log-ratio transformation, we removed rare species by excluding taxa with zero counts in more than 80% of the samples.

In vivo experiments. Male wild-type C57BL/6J mice were purchased from Charles River Laboratories (Germany). Mice were maintained under a 12 h light cycle with access to food and water ad libitum. A chow diet (TD.130066, LabDiet) was used, enriched with 0.1% DDC (Merck; 137030-25G).

Mice harbouring loxP sequences flanking exon 3 p38 γ (*Mapk12*) and exon 3 p38 δ (*Mapk13*) were generated by Cyagen Biosciences (Supplementary Fig. 4). The strain was generated by homologous recombination in C57BL/6N embryonic stem cells, followed by microinjection into C57BL/6N blastocysts. The resulting offspring were crossed with C57BL/6NTac-*Gt(ROSA)26Sor^{tm16Ar}* Cre deleter mice to excise the neomycin selection cassette (Taconic Biosciences) and subsequently crossed with p38 γ / $\delta^{fl/fl}$. Deletion of 95 bp and 127 bp from exon 3 of *Mapk12* and *Mapk13*, respectively, led to a frameshift resulting in a premature stop codon. No functional domain would be present on these putative proteins. All animal procedures were approved by the Gothenburg Animal Ethics Committee (Dnr 5.8.18-16056/2019).

Male mice (6-week-old C57BL/6J or p38 γ / δ knockout) were randomized to receive either ImP (Santa Cruz, sc294276; 800 μ g per day per mouse) or no ImP (Ctrl) in drinking water. Water and food consumption was determined twice weekly. After 8 weeks of treatment, all mice were killed, which was the defined endpoint for all mouse experiments, and body and organ weights were determined. Peripheral serum from the vena cava and liver tissue were collected and stored at -80°C for further analysis. Serum ASAT, ALAT and ALP were measured using Alinity c (Abbott Laboratories). Total RNA was isolated using the RNeasy kit with DNase treatment (Qiagen), and cDNA templates were generated using the high-capacity cDNA reverse transcription kit using random hexamers (Applied Biosystems). Reverse transcription qPCR assays were performed in 10 μ l reactions with SYBR Green Master Mix buffer (Thermo Scientific). In a subgroup of C57BL/6J mice, blood was collected from the tail vein following vehicle or ImP treatment. Mice were then perfused with PBS through the inferior vena cava to remove blood before collecting organs. Gene expression data were normalized to the ribosomal protein L32. Primers were purchased from Sigma-Aldrich; sequences are listed in Supplementary Table 16.

Germ-free male Swiss Webster mice, 10–12 weeks old, were maintained in flexible film isolators under a strict 12 h light cycle. Germ-free status was verified regularly by anaerobic culturing in addition to PCR for the bacterial 16S rRNA gene. Experiments were performed on autoclaved chow diet (LabDiet) ad libitum. *S. oneidensis* MR-1 (ATCC 700550) was grown in Trypticase Soy Yeast Extract Medium (TSBYE) (Becton Dickinson) supplemented with 0.25% yeast under aerobic conditions at 29°C . Bacteria were collected at a density of $\sim 10^9$ cells per ml and administered to mice by oral gavage (200 μ l per mouse) following a 4 h fasting period on day 0. Colonized mice were monitored closely in experimental isolators to ensure controlled conditions. Urocanate at a concentration of 500 μM in the drinking water was provided to colonized mice from week 3 onwards. The colonization experiment was performed on one batch of mice.

Liver histology and immunohistochemistry

Details regarding liver histology and immunohistochemistry are described in the Supplementary Note.

In vitro and ex vivo experiments

Details regarding in vitro experiments in H69 cells and ex vivo experiments in cholangiocyte organoids are described in the Supplementary Note.

Statistical analyses

Handling of missing data. In our discovery (untargeted metabolomics) dataset, we detected a total of 1,265 metabolites, with 42,540 (17.3%) values missing. ImP was detected in all samples. There were no discernible differences in the distribution of missing values between the

sample groups (Supplementary Fig. 5). We excluded metabolites missing in $>50\%$ of samples (157), of which 89% were annotated as drugs or unclassified. For the remaining 1,108 metabolites, missing values were imputed using multivariate imputation by chained equations predicted mean matching (MICE-pmm) method⁵⁰ in the UnMetImp script in R, which specifically takes into account that xenobiotics are unlikely to be missing at random and should therefore have missing values imputed to zero. Age at sampling, leucocyte counts, platelet counts, bilirubin, albumin, ASAT, ALAT and ALP were included in the imputation. Missing data in the NO-1, NO-2 and SE cohorts were imputed for each cohort separately using the mice::mice function. Age and blood biochemical parameters were included in the imputation. ImP was quantified and detected in all samples. Missing data in the longitudinal cohort were not imputed given that only around half of the theoretical maximum of samples were collected and the mechanisms underlying the missing data could not be determined.

Rule-based feature selection pipeline (untargeted metabolomics cohort). In the discovery cohort, we defined three rules to pinpoint putative disease-driven, gut microbiota-derived candidate metabolites in plasma samples. The candidate metabolite had to be (1) differentially abundant in PSC versus HCs in a multivariable binary logistic regression model that included age and sex as fixed covariates ($\text{FDR} < 0.01$); (2) associated with LT-free survival in a multivariable Cox proportional hazards model with sex and Mayo PSC risk score as fixed covariates ($P < 0.05$); and (3) the metabolite must show no evidence (P value threshold of 0.05) of returning to its corresponding level in HCs in PSC post-LT samples. To identify apparent metabolite candidates, we generated 40 imputed datasets and pooled model estimates from each of the three rule-based feature selection pipeline criteria using Rubin's rules (mice::pool). Metabolites were considered candidates if they fulfilled the three specified criteria.

We next evaluated internal validity of the metabolites by bootstrapping (100 iterations) the complete rule-based feature selection pipeline whereby each resampled dataset maintained the distribution of classes (HCs, PSC pre-LT and PSC post-LT) from the original sample. Within each bootstrap sample, we generated 40 imputed datasets and pooled model estimates within each resampled dataset using Rubin's rules. A metabolite that fulfilled all three criteria in a bootstrap sample was considered a winner within that sample. This enabled us to rank the metabolites by how frequently they appeared in the 100 bootstrap samples.

Time-to-event modelling (targeted, cross-sectional cohorts). We used Kaplan–Meier survival curves to visualize the survival distributions of groups categorized by tertiles of circulating ImP levels. For analyses of rPSC, longitudinal ImP measurements from patients who had undergone LT once for PSC but with no recorded diagnosis of rPSC at the time of sampling were modelled as time-dependent covariates using Cox proportional hazards regression with start–stop intervals.

Longitudinal analyses. To test whether ImP overall changed longitudinally in the longitudinal cohort, we fitted a mixed-effects model using nmle::glms with ImP ($\log_2$) as outcome and time (continuous), age at LT and sex as fixed covariates. We defined an autoregressive correlation structure that allowed for subject-specific intercepts and slopes while allowing correlation between two observations within the same subject to decrease as the time differences between them increased.

Experimental data analyses. All cell culture experiments were performed in ≥ 3 biological replicates. Unless specified otherwise, $P < 0.05$ was considered statistically significant. To assess whether the effects of treatment on various outcomes differed depending on genetic background, we included interaction terms between treatment type and genetic background in our analysis. For ordinal outcomes, we applied

the aligned rank transform for nonparametric factorial ANOVAs, while for continuous outcomes, we used ANOVA. For ANOVA, we visually inspected the model's residuals to detect any clear departures from normality. We used two-sided Mann–Whitney *U*-tests to test for differences in distributions of ordinal and continuous variables.

Other statistical analyses. We tested the strengths of bivariate linear relationships using Spearman's rank correlation. Where indicated, the Benjamini–Hochberg FDR procedure was performed to account for multiple testing.

R was used for all data processing, visualization and statistical analyses.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Individual participant data have not been deposited in public repositories owing to Norwegian regulations and lack of consent, but will be made available to other researchers upon reasonable request. A request should be e-mailed to the corresponding author, with a very brief description of the aim and methods of the planned analyses. If this is covered by participant consent, an amendment will be filed for ethical approval of the analyses, and a material and data transfer agreement must be established between the institutions. Source data underlying all figures are published alongside this article. Source data underlying supplementary figures are provided in the Supplementary Data. RNA sequencing data have been deposited to Gene Expression Omnibus (accession number [GSE341409](https://doi.org/10.5555/GSE341409)). Correspondence and requests for materials or data should be addressed to the corresponding author. Source data are provided with this paper.

Code availability

The analysis RMarkdown files and associated code are available via Zenodo at <https://doi.org/10.5281/zenodo.21531744> (ref. 51).

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Acknowledgements

We thank all study participants for their contributions and the IBSEN study group for sharing samples and data from the first IBSEN study. C. Morelli (Wallenberg laboratory in Gothenburg) provided technical support with mouse liver gene expression analysis. H. Aggarwal and S. Ivarsson (Wallenberg laboratory in Gothenburg) are acknowledged for assistance with animal models. I. Sohail (Oslo University Hospital) is acknowledged for help with the transplantation cohort. S. Åkra, L. Wenche Thorbjørnsen and H. Guldsten (Oslo University Hospital) provided laboratory assistance with study cohorts and samples.

Author contributions

P.R.B., M.N., A.H.A., L.B., B.D.J., H.M.R., I.B., K.G., A.A., T.F., M. Cornillet, H.H., B.M.M., M. Carbone, L.C., V.C., M.L.H., L.K.E., S.T., M.V., T.H.K., H.G., K.N.L., A.B., E.M. and J.R.H. contributed to the collection of samples and clinical follow-up data of human subjects. K.R.B., A.M.C. and P.M.U. performed the targeted metabolomics and pre-processing of the targeted metabolomics data. A.M., A.C.P., L.G. and A.L. performed the experimental in vivo and in vitro work. G.C., D.O. and E.G. performed the stains and scoring of mouse and human liver tissue. A.M., P.R.B., P.H., A.F., E.A.I., T.F. and E.M. contributed to the design and analysis of ex vivo models. A.M., P.R.B., M.N., E.M., J.R.H. and F.B. performed the bioinformatics and statistical analyses. P.R.B., A.M., J.R.H. and F.B. were responsible for the conceptualization and design of the study, as well as the first drafting of the manuscript. All authors commented on the paper and approved the final version.

Funding

J.R.H. was funded by grants from the European Research Council (StopAutoimmunity, number 802544 and FatVersusBile, number 101170353) and grants from the Regional Health Authority South-Eastern Norway (numbers 2017022, 2022074 and 2023018). While funded by the European Union, views and opinions expressed are those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Council Executive Agency. Neither the European Union nor the granting authority can be held responsible for them. The study was funded by Knut and Alice Wallenberg Foundation (2017.0026). A.M. was funded by the United European Gastroenterology (UEG) Fellowship, Mag tarm fonden, WCMTM starting grant and the ALF-agreement (ALFGBG-1006719). E.G. was funded by the Italian Ministry of University and Research (“Progetti di rilevante interesse nazionale”: PRIN-2022HT2MAT) and by Sapienza University of Rome (Bandi di Ricerca di Ateneo). D.O. was funded by the Italian Ministry of University and Research (“Progetti di rilevante interesse nazionale”: PRIN PNRR P2022H7JYZ). K.N.L. was supported by the National Institutes of Health (RC2 DK118619) and the Halloran Family Foundation. E.M. was funded by the Research Council of Norway (grant numbers 325435 and 315399).

Competing interests

F.B. is a co-founder and shareholder in Implexion Pharma and Roxbiosens, and receives research funds from BioGaia. A.M., A.C. and K.R.B. are shareholders in Implexion Pharma. The other authors declare no competing interests.

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s42255-026-01600-1>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s42255-026-01600-1>.

Correspondence and requests for materials should be addressed to Johannes Roksund Hov.

Peer review information *Nature Metabolism* thanks Patrice Cani and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Yanina-Yasmin Pesch, in collaboration with the *Nature Metabolism* team.

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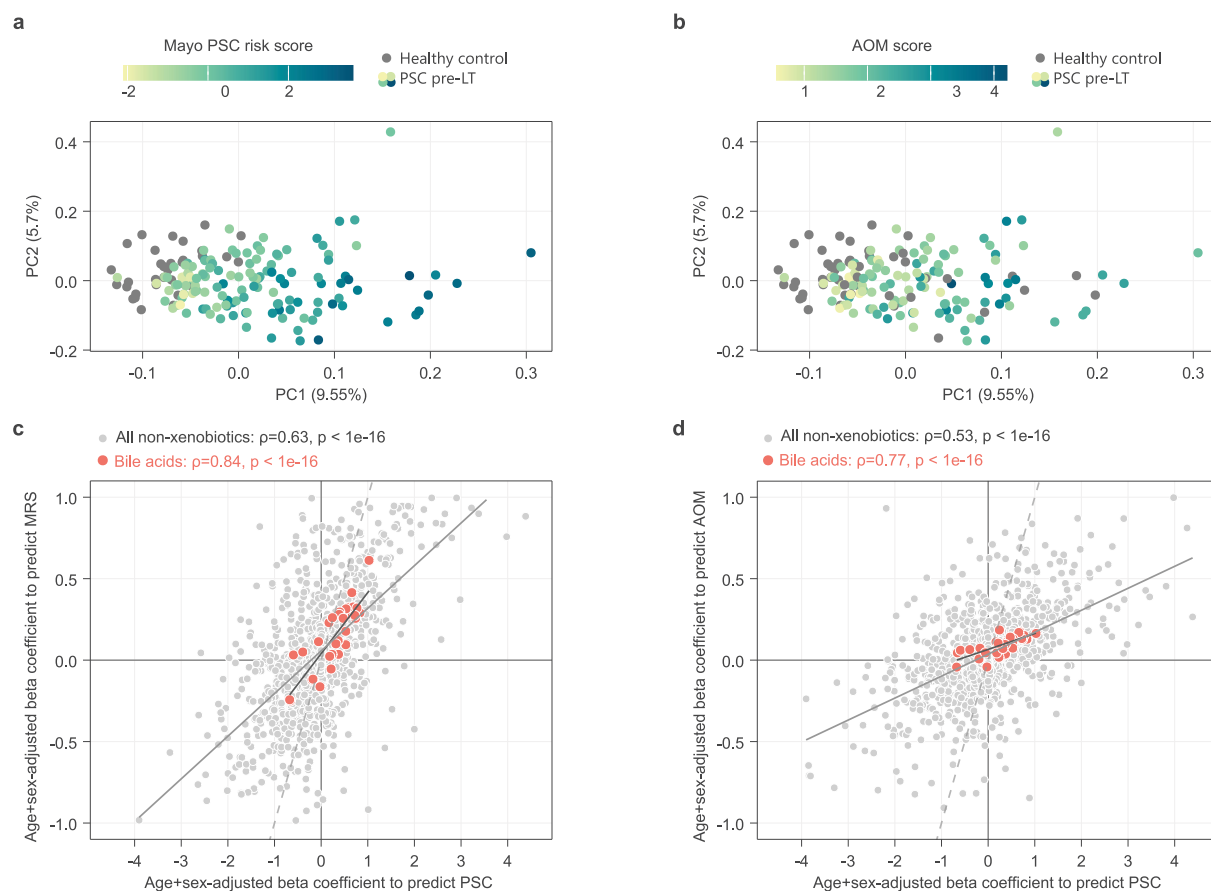
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Antonio Molinaro^{1,2,3,27}, **Peder Rustøen Braadland**^{1,4,5,27}, **Guido Carpino**⁶, **Alba Carreras**², **Marios Nikolaidis**⁷, **Petra Hanzely**^{1,4,5}, **Katharina R. Beck**², **Ahmad H. Ali**⁸, **Lars Bossen**^{9,10}, **Anna Frank**^{1,4,5,11}, **Annika Lundqvist**¹², **Brian D. Juran**⁷, **Diletta Overi**¹², **Lei Geng**², **Enya Amundsen-Isaksen**^{1,4,5,14}, **Henrik Mikael Reims**¹², **Ida Björk**¹³, **Krzysztof Grzyb**¹², **Andreas Abildgaard**¹³, **Trine Folseraas**^{1,4,5,14}, **Martin Cornillet**¹⁵, **Hólmfríður Helgadóttir**^{1,16}, **Bryan M. McCauley**⁷, **Laura Cristofori**¹⁷, **Marco Carbone**^{18,19}, **Vincenzo Cardinale**²⁰, **Marte L. Høivik**^{5,21}, **Lise Katrine Engesæter**^{1,4,5,14}, **Sara K. V. Tjønnfjord**^{1,4,5,14}, **Adrian McCann**²², **Per M. Ueland**²², **Mette Vesterhus**^{1,16,23}, **Tom H. Karlsen**^{1,4,5,11,14}, **Henning Grønbaek**^{9,10}, **Eugenio Gaudio**⁶, **Konstantinos N. Lazaridis**⁷, **Annika Bergquist**²⁴, **Espen Melum**^{1,4,5,11,14}, **Fredrik Bäckhed**^{12,25,26,28} & **Johannes Roksund Hov**^{1,4,5,14,28} ✉

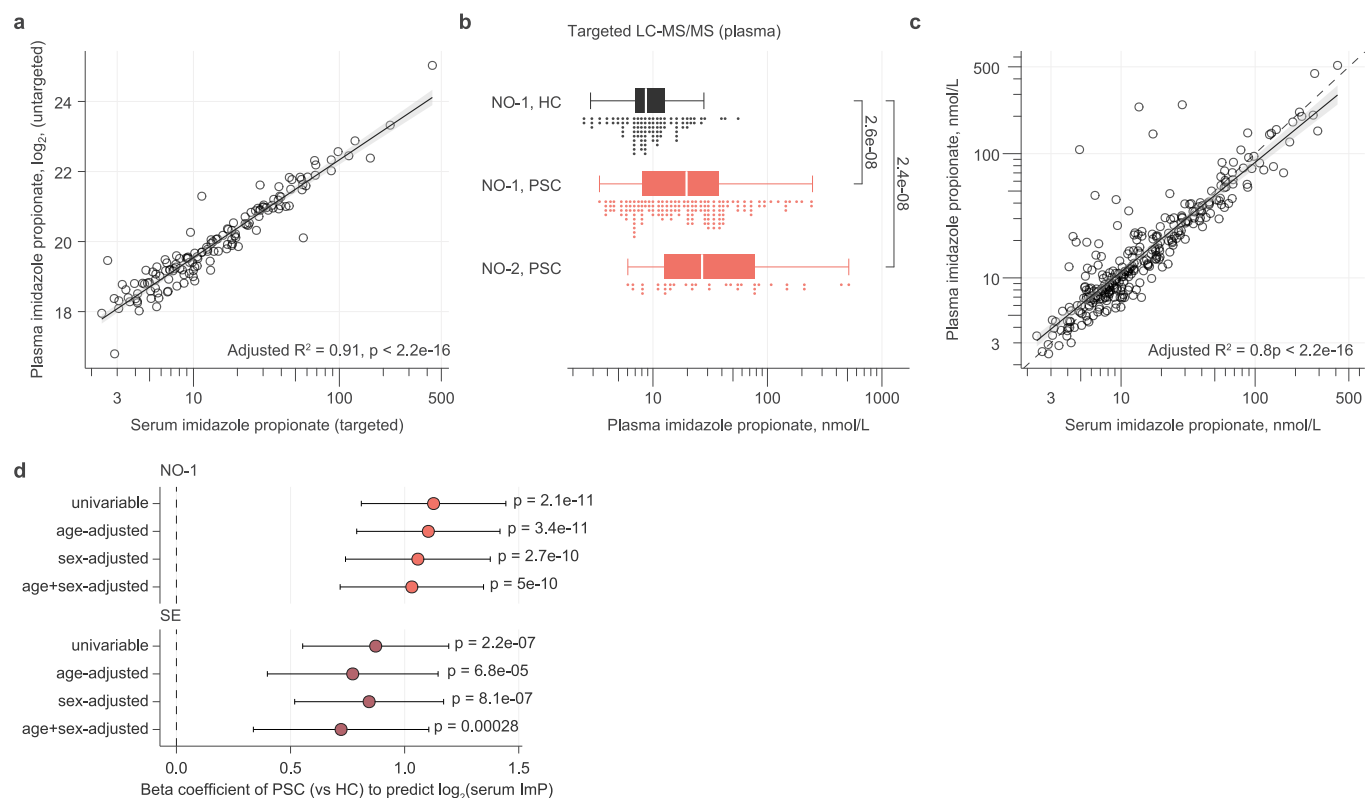
¹Norwegian PSC Research Center, Department of Transplantation Medicine, Division of Surgery and Specialized Medicine, Oslo University Hospital, Oslo, Norway. ²The Wallenberg Laboratory, Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. ³Department of Medicine, Sahlgrenska University Hospital, European Reference Network on Hepatological Diseases (ERN Rare-Liver), Region Västra Götaland, Gothenburg, Sweden. ⁴Research Institute of Internal Medicine, Division of Surgery and Specialized Medicine, Oslo University Hospital, Oslo, Norway. ⁵Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway. ⁶Department of Anatomical, Histological, Forensic Medicine and Orthopedics Sciences, Sapienza University of Rome, Rome, Italy. ⁷Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA. ⁸Division of Gastroenterology and Hepatology, School of Medicine, University of Missouri, Columbia, MO, USA. ⁹Department of Hepatology & Gastroenterology, Aarhus University Hospital, Aarhus, Denmark. ¹⁰Clinical Institute, Aarhus University, Aarhus, Denmark. ¹¹Hybrid Technology Hub – Centre of Excellence, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, Norway. ¹²Department of Pathology, Oslo University Hospital, Oslo, Norway. ¹³Division of Radiology and Nuclear Medicine, Oslo University Hospital, Oslo, Norway. ¹⁴Section of Gastroenterology, Department of Transplantation Medicine, Division of Surgery and Specialized Medicine, Oslo University Hospital, Oslo, Norway. ¹⁵Department of Medicine Huddinge, Center for Infectious Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden. ¹⁶Department of Medicine, Haraldsplass Deaconess Hospital, Bergen, Norway. ¹⁷Division of Gastroenterology, Center for Autoimmune Liver Diseases, European Reference Network on Hepatological Diseases (ERN RARE-LIVER), IRCCS Fondazione San Gerardo dei Tintori, Monza, Italy. ¹⁸Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy. ¹⁹Liver Unit, ASST Grande Ospedale Metropolitano (GOM) Niguarda, Milan, Italy. ²⁰Department of Translational and Precision Medicine, Sapienza University of Rome, Rome, Italy. ²¹Department of Gastroenterology, Division of Medicine, Oslo University Hospital, Oslo, Norway. ²²Bevital AS, Bergen, Norway. ²³Department of Clinical Science, University of Bergen, Bergen, Norway. ²⁴Unit of Gastroenterology and Nutrition, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden. ²⁵Department of Clinical Physiology, Sahlgrenska University Hospital, Region Västra Götaland, Gothenburg, Sweden. ²⁶Novo Nordisk Foundation Microbiome Health Initiative and the National Food Institute, Technical University of Denmark, Kongens Lyngby, Denmark. ²⁷These authors contributed equally: Antonio Molinaro, Peder Rustøen Braadland. ²⁸These authors jointly supervised this work: Fredrik Bäckhed and Johannes Roksund Hov.

✉ e-mail: j.e.r.hov@medisin.uio.no



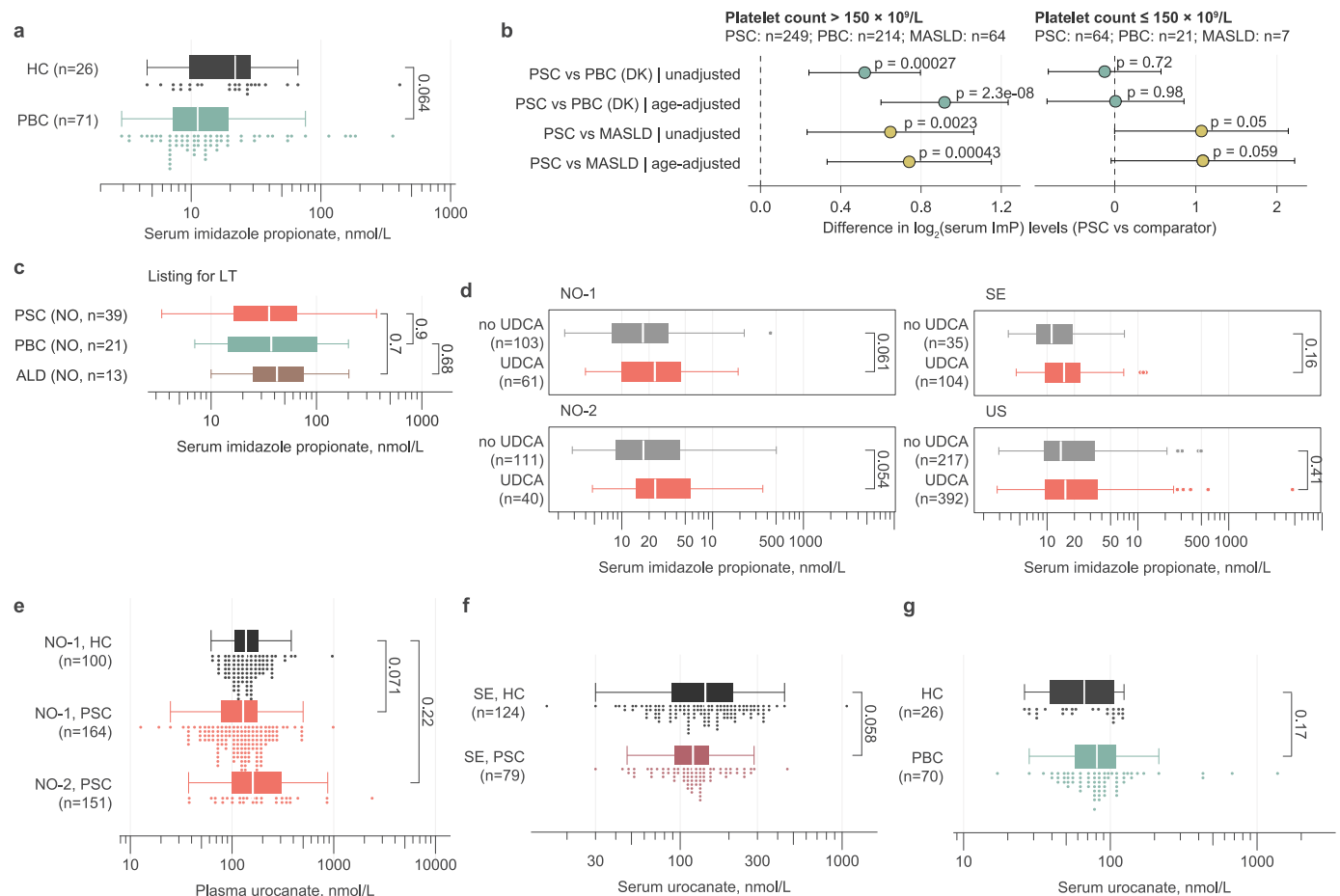
Extended Data Fig. 1 | Metabolomic changes in PSC plasma correlate with disease severity. a–b. Plots of the first two principal components of metabolomic profiles of samples from healthy controls and PSC pre-liver transplantation (LT) in the untargeted metabolomic profiling (discovery) cohort, derived using principal component analysis. The PSC samples are colored based on their donor's Mayo PSC risk score (MRS) (**a**) or Amsterdam-Oxford model (AOM) (**b**) scores at time of sampling. **c–d.** Bivariate plots of (i) each metabolite's age- and

sex-adjusted beta coefficient from linear regressions to predict MRS (**c**) or AOM (**d**) and (ii) each metabolite's age- and sex-adjusted beta coefficient from binary logistic regressions to predict PSC (versus HC). Spearman's rank correlation (p) was calculated for all metabolites and for primary bile acids (PBAs) selectively. HC: healthy controls, LT: liver transplantation, PC: principal component, PSC: primary sclerosing cholangitis, MRS: Mayo risk score, AOM: Amsterdam-Oxford PSC model.



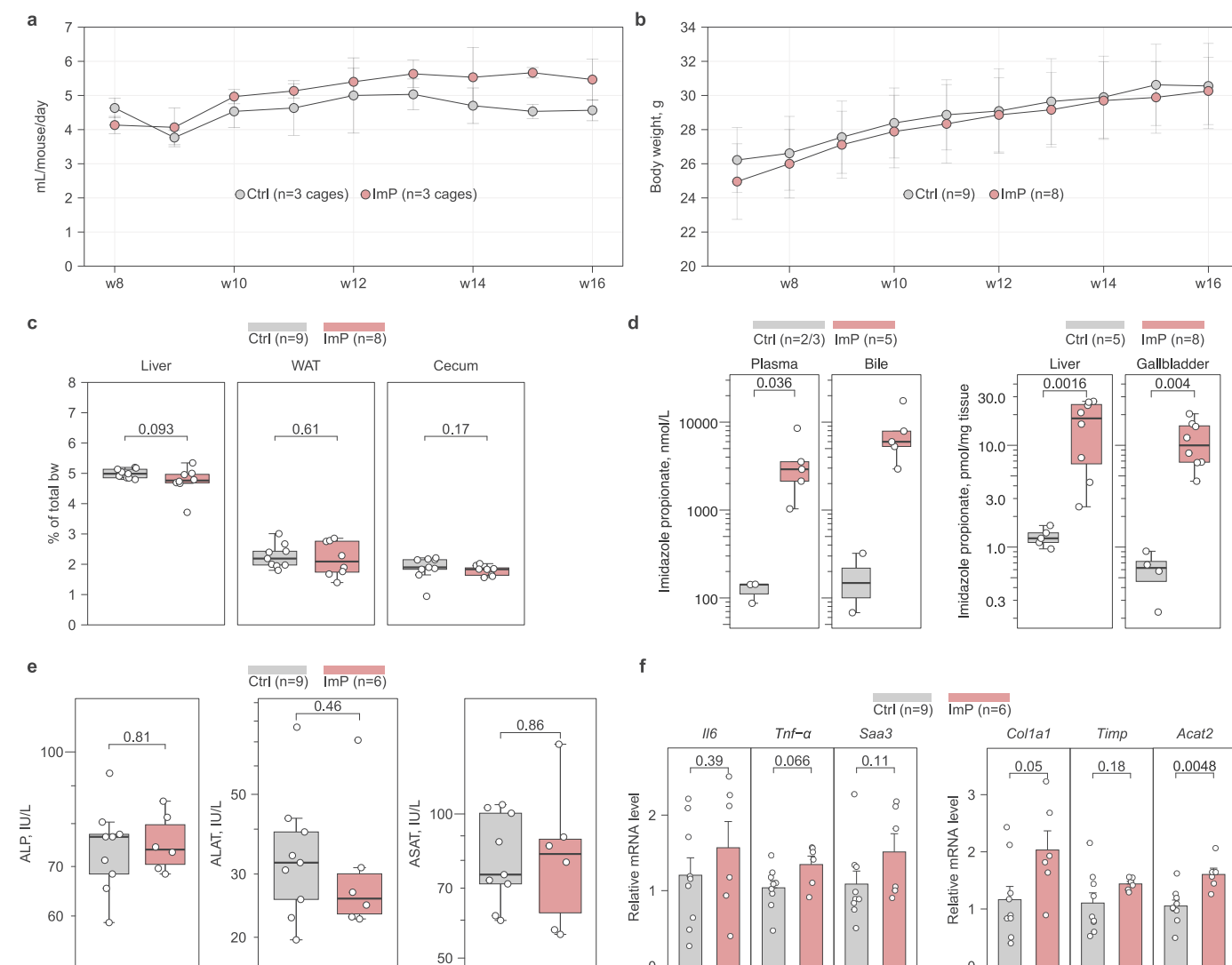
Extended Data Fig. 2 | Targeted validation of imidazole propionate, supplementary analyses to Fig. 2. **a.** Shows the relation between scaled, $\log_2$ -transformed imidazole propionate (ImP) levels in the untargeted metabolomic profiling (discovery) cohort and the absolute concentrations of ImP measured in the same blood samples ($n = 146$ plasma pairs) using a targeted method. Linear regression was used to quantify the univariable strength of the association (adjusted R^2 is shown). The black line indicates the best-fitting linear regression line, and the shaded area indicates the 95% confidence interval around the regression line. **b.** Plasma ImP levels in healthy controls ($n = 100$), NO-1 ($n = 155$) and NO-2 ($n = 36$) cohorts measured using a targeted method (see methods). Points indicate individual ImP measurements. Box plot center lines indicate the median, the bounds of the box indicate the 25th and 75th percentiles,

and the whiskers indicate the minimum and maximum values within $1.5 \times$ the interquartile range. Statistical significance was tested using two-sided Mann-Whitney U -tests. **c.** Relationship between absolute concentrations of ImP measured in paired serum and plasma from pooled PSC and healthy control samples ($n = 291$ pairs) using the same, targeted method. Linear regression was used to quantify the strength of the univariable association (adjusted R^2 is shown). The black line indicates the best-fitting linear regression line, and the shaded area indicates the 95% confidence interval around the regression line. **d.** Forest plot showing the univariable and multivariable (adjusted for age and sex) associations between $\log_2$ -transformed ImP and disease status (PSC versus HC, HC as reference). Beta coefficient point estimates are shown as filled circles with error bars corresponding to 95% confidence intervals.



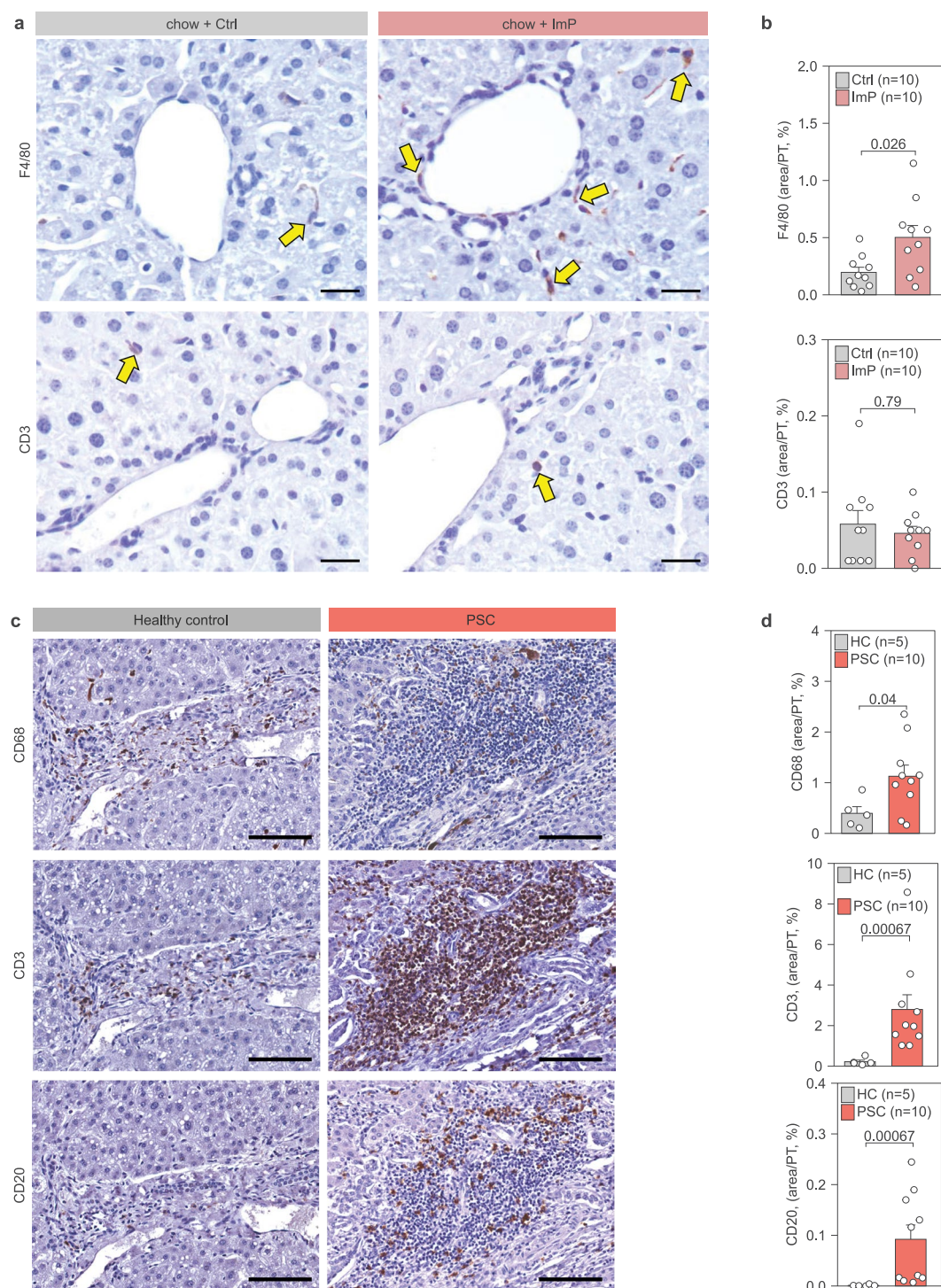
Extended Data Fig. 3 | Disease specificity of imidazole propionate, supplementary analyses to Fig. 3. **a.** Serum levels of imidazole propionate (ImP) in people with primary biliary cholangitis (PBC) and healthy controls from the Italian PBC cohort. **b.** Univariable and age-adjusted linear regression estimates (points indicate beta coefficient point estimates and error bars indicate 95% confidence intervals) of the association between disease group (PSC versus PBC or MASLD) and $\log_2(\text{ImP})$, stratified by platelet count (above versus below $150 \times 10^9/\text{L}$). Regression coefficients for PSC indicate the adjusted difference in $\log_2(\text{ImP})$ compared with PBC or MASLD. **c.** Distributions of serum ImP in people with PSC, PBC and alcohol-related liver disease (ALD) listed for liver transplantation (LT). **d.** Distributions of ImP in people with PSC in the PSC

pre-LT cohorts by ursodeoxycholic acid (UDCA) treatment. **e.** Distributions of plasma urocanate, measured using a targeted method, in serum samples from the Norwegian PSC pre-LT cohorts. **f.** Serum levels of urocanate in people with PSC and healthy controls in the Swedish PSC pre-LT cohort. **g.** Serum levels of urocanate in people with primary biliary cholangitis (PBC) and healthy controls from the Italian PBC cohort. For panels **a**, **c**, **d**, **e**, **f** and **g**, box plot center lines indicate the median, the bounds of the box indicate the 25th and 75th percentiles, and the whiskers indicate the minimum and maximum values within $1.5 \times$ the interquartile range. For panels **c** and **d**, data points beyond the whiskers are plotted individually as outliers. Aside from in panel **b**, statistical significance was tested using two-sided Mann-Whitney *U*-tests.



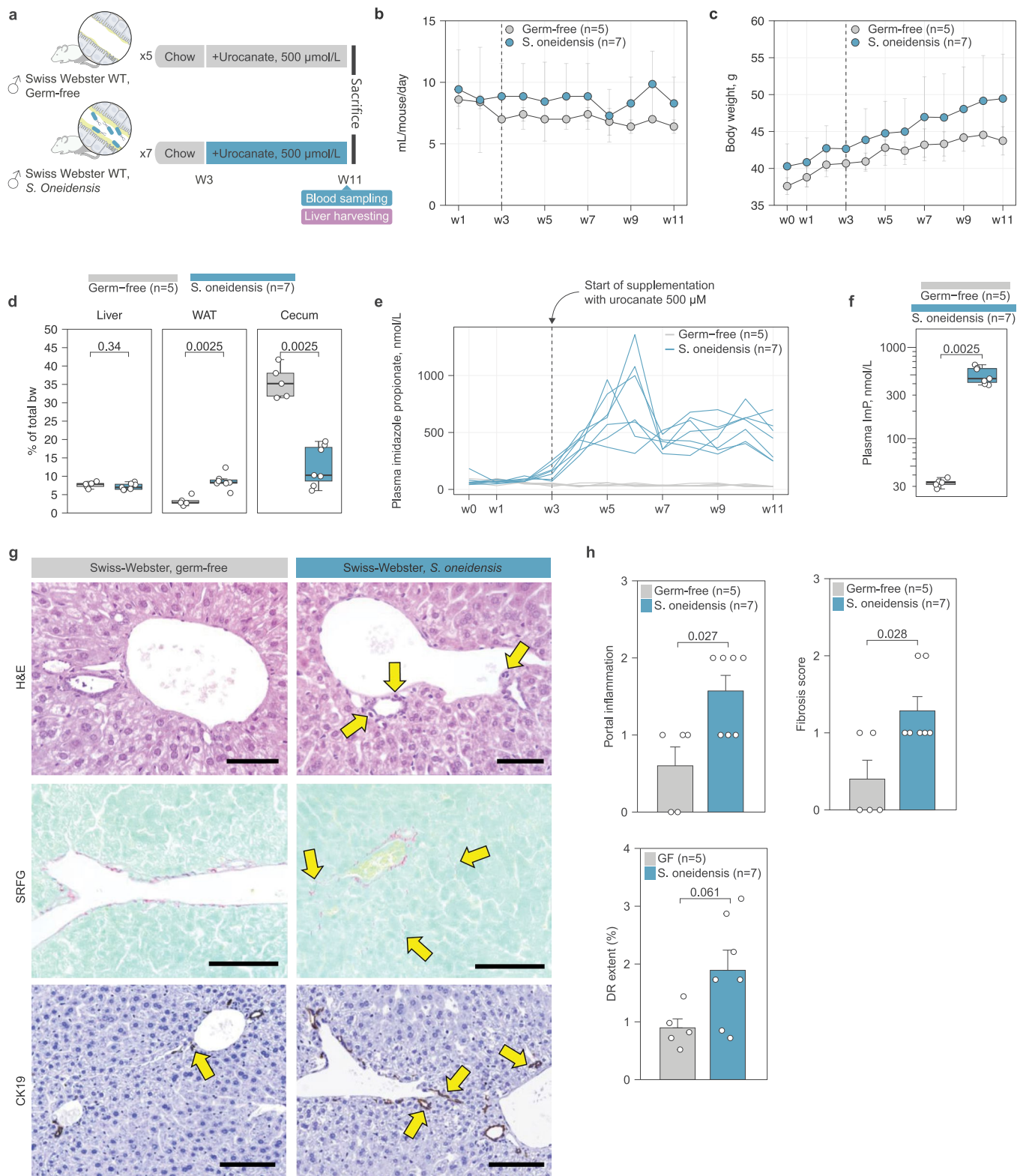
Extended Data Fig. 4 | Anthropometric parameters, serum liver enzyme and gene expression in the livers of wild type mice treated with and without imidazole propionate on a chow diet. a–b. Water intake and body weight in 8-week-old mice treated with vehicle (Ctrl) or imidazole propionate (ImP) on a chow diet ($n = 8$ and 9 per group with data shown per cage). **c.** Organ weights (as percentage of body weight) at sacrifice in mice treated with or with ImP. **d.** Levels of ImP in plasma, bile, gallbladder and liver in mice treated with ImP for 8 weeks (independent cohort from the other panels in this figure, $n = 3$ and 8 per group). **e.** Serum levels of ALP, ASAT and ALAT at sacrifice. For panels **c–e**, box plot center lines indicate the median, the bounds of the box indicate the 25th and

75th percentiles, and the whiskers indicate the minimum and maximum values within $1.5 \times$ the interquartile range. **f.** Relative mRNA levels of inflammation- and fibrosis-related genes in mouse liver extracts. Bars represent the mean, and error bars represent the standard error of the mean (SEM). For panels **c–f**, p-values were calculated using two-sided Mann-Whitney *U*-tests. ALP: alkaline phosphatase, ASAT: aspartate aminotransferase, ALAT: Alanine aminotransferase, *Il6*: interleukin 6, *Saa3*: serum amyloid 3, *Tnfα*: tumor necrosis factor alpha, *Acat2*: Acetyl-CoA acetyltransferase, *Col1a1*: alpha-1 type I collagen, *Timp*: TIMP metalloproteinase inhibitor 1, WAT: white adipose tissue.



Extended Data Fig. 5 | Immune cell staining in mice treated with imidazole propionate and in individuals with and without PSC. a–b. Representative pictures and relative quantification of F4/80 and CD3 in wild type mice treated with vehicle (Ctrl) or imidazole propionate (ImP, $n = 10$ per group; same mice used for Fig. 7). Scale bars = 20 μm . **c–d.** Representative pictures and relative

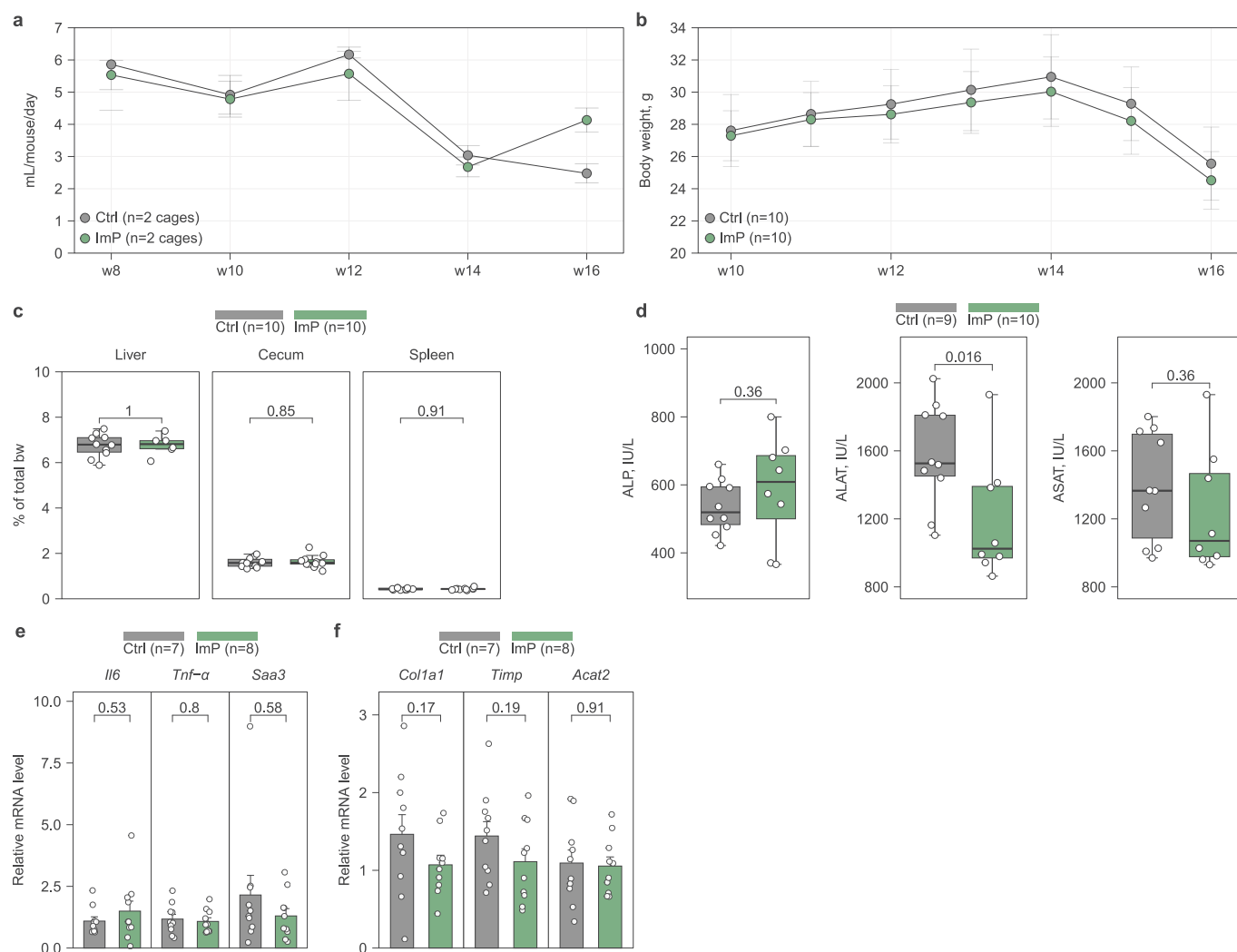
quantification of CD68, CD3 and CD20 in human healthy controls and individuals with PSC ($n = 6$ and 10 per group, same individuals as in Fig. 6 panels g–h). Scale bars = 100 μm . Bars represent the mean, and error bars represent the standard error of the mean (SEM). P-values were calculated using two-sided Mann-Whitney U -tests.



Extended Data Fig. 6 | Imidazole propionate produced by *Shewanella oneidensis* impacts bile ducts inflammation and fibrosis in colonized mice.

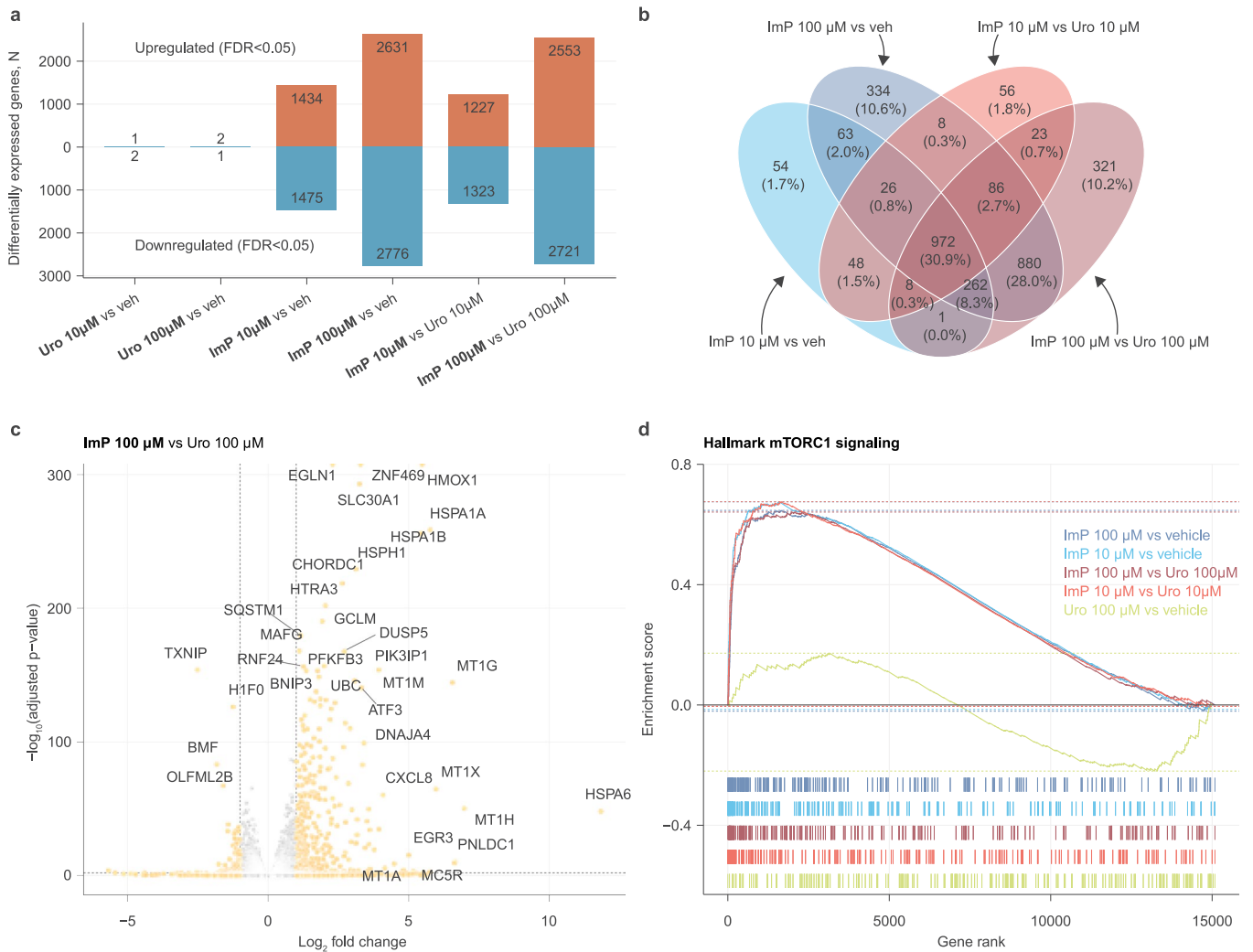
a. Schematic representation of the colonization experiment with *Shewanella oneidensis*. **b–c.** Water intake and body weight in 11-week-old gnotobiotic mice colonized with *S. oneidensis* and germ-free mice ($n = 4$ and 7 per group). **d.** Organ weights (as percentage of body weight) at sacrifice in *S. oneidensis*-colonized and germ-free mice. **e–f.** Imidazole propionate (ImP) levels during treatment and at sacrifice in *S. oneidensis*-colonized and germ-free mice. The dotted line in (**e**) refers to the start of supplementation with urocanate (500 µM) in the drinking water. Values in (**f**) are median concentrations per mouse for weeks

4–11. **g–h.** Representative images and relative quantification for hematoxylin and eosin (H&E), Sirius red fast green (SRFG) and immunohistochemistry for cytokeratin 19 (CK19) in livers of *S. oneidensis*-colonized and germ-free mice. Scale bars = 100 µm (H&E, SRFG) and 50 µm (CK19). Arrows indicate areas of portal inflammation (H&E), collagen fibers (SRFG) or ductular reaction (CK19). Bars represent the mean, and error bars represent the standard error of the mean (SEM). For panels **d** and **f**, box plot center lines indicate the median, the bounds of the box indicate the 25th and 75th percentiles, and the whiskers indicate the minimum and maximum values within $1.5 \times$ the interquartile range. P-values were calculated using two-sided Mann-Whitney *U*-tests.



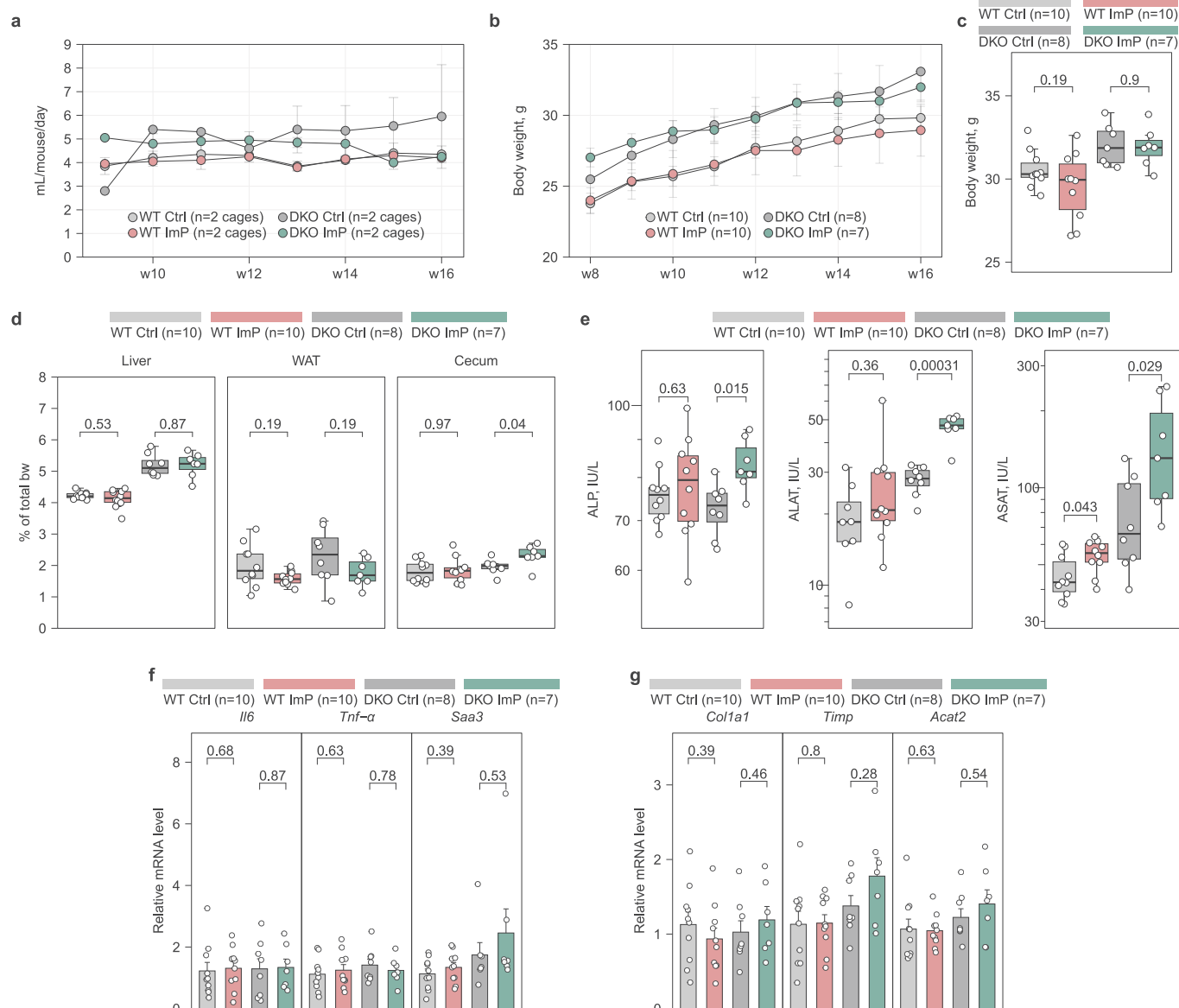
Extended Data Fig. 7 | Anthropometric parameters, serum liver enzymes and gene expression levels in liver of wild type mice treated with and without imidazole propionate on a 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet. a-b. Water intake and body weight in 8-week-old mice treated with vehicle (Ctrl) or imidazole propionate (ImP) (data are shown per cage) on a 6-week chow diet followed by 2 weeks on a DDC diet ($n=10$ per group). **c.** Organ weights (as percentage of body weight) at sacrifice in mice treated with and without ImP. **d.** Serum levels of ALP, ASAT and ALAT at sacrifice. For panels **c-d**, box plot center lines indicates the median, the bounds of the box

indicate the 25th and 75th percentiles, and the whiskers indicate the minimum and maximum values within $1.5 \times$ the interquartile range. **e-f.** Relative mRNA levels of inflammation- and fibrosis-related genes in mouse liver extracts. Bars represent the mean, and error bars represent the standard error of the mean (SEM). P-values were calculated using two-sided Mann-Whitney U -tests. ALP: alkaline phosphatase, ASAT: aspartate aminotransferase, ALAT: Alanine aminotransferase, *Il6*: interleukin 6, *Saa3*: serum amyloid 3, *Tnfα*: tumor necrosis factor alpha, *Acat2*: Acetyl-CoA acetyltransferase, *Col1a1*: alpha-1 type I collagen, *Timp*: TIMP metalloproteinase inhibitor 1, WAT: white adipose tissue.



Extended Data Fig. 8 | Imidazole propionate-driven activation of mTORC signaling-related genes in human cholangiocyte H69 cells. a. Shows the number of upregulated and downregulated genes from differential expression analyses using different comparisons, based on DESeq2 two-sided Wald test Benjamini-Hochberg false discovery rate (FDR) adjustment (FDR-adjusted p -value < 0.05). **b.** Venn diagram of overlapping and unique differentially expressed genes (DESeq2 two-sided Wald tests with FDR-adjusted p -value < 0.05). **c.** Volcano plot illustrating the relationship between individual gene

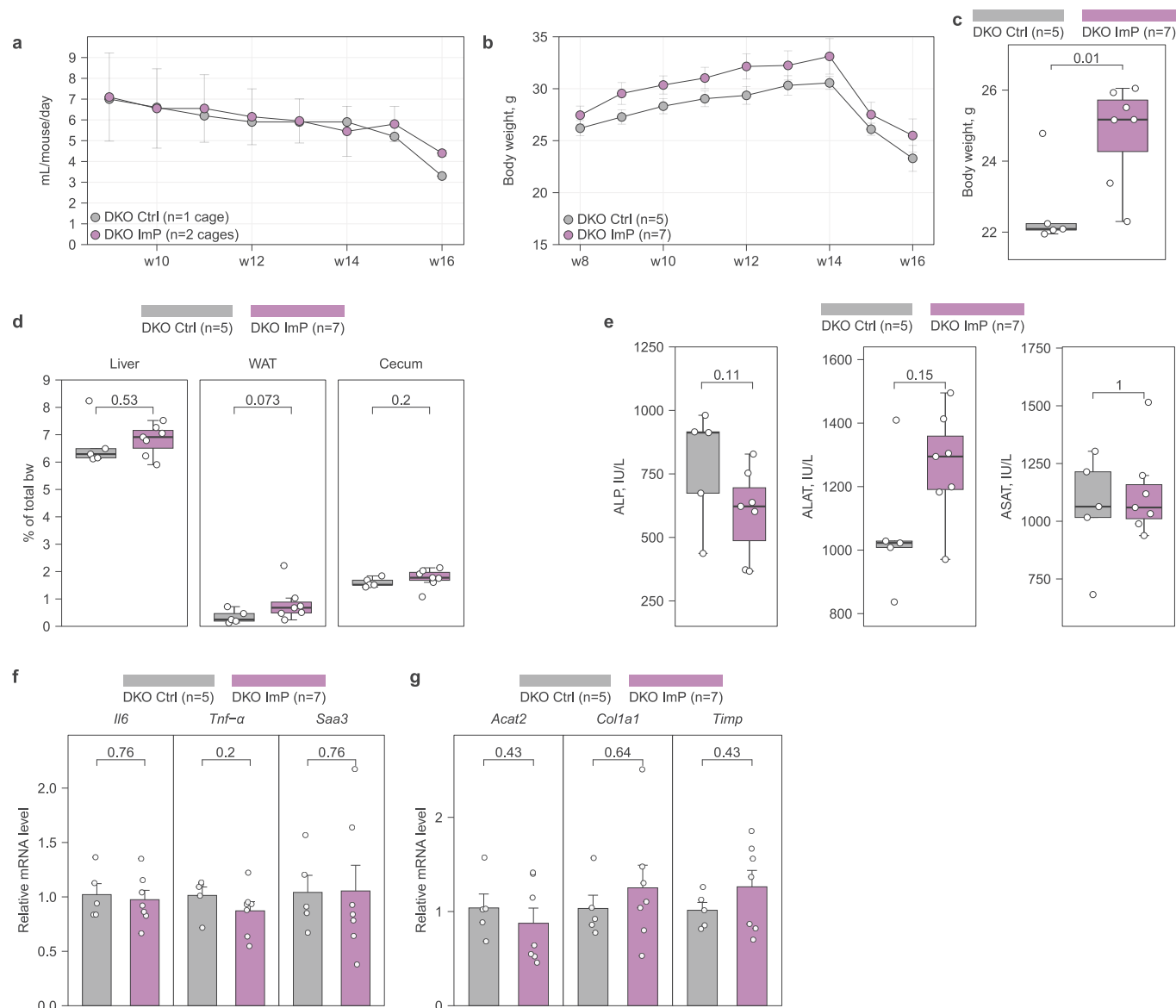
$\log_2$ (fold changes) and $-\log_{10}$ (FDR)-adjusted p -values, estimated using DESeq with log fold change shrinkage (lfcShrink), in H69 cells treated with 100 μ M imidazole propionate (ImP) compared to 100 μ M urocanate (uro). Genes with an adjusted p -value < $1e-06$ and an absolute $\log_2$ (fold change) > 3 are labeled. **d.** Enrichment plot of the Hallmark mTORC1 signaling pathway genes for various comparisons, with rug plots showing each gene's individual rank (based on t -statistics from the DESeq analyses). veh = vehicle.



Extended Data Fig. 9 | Anthropometric parameters, serum liver enzymes and gene expression levels in the liver of wild type (WT) and p38 γ - δ knock out mice (DKO) treated with and without imidazole propionate on a chow diet.

a-b. Water intake and body weight in 8-week-old WT and DKO mice treated with vehicle (Ctrl) or imidazole propionate (ImP) on a chow diet ($n=7$ and 10 per group with data shown per cage). **c.** Body weights at time of sacrifice. **d.** Organ weights (as percentage of body weight) at sacrifice in mice treated with and without ImP. **e.** Serum levels of ALP, ASAT and ALAT at sacrifice. For panels **c-e**, box plot center lines indicate the median, the bounds of the box indicate the 25th and 75th

percentiles, and the whiskers indicate the minimum and maximum values within $1.5 \times$ the interquartile range. **f-g.** Relative mRNA levels of inflammation- and fibrosis-related genes in mouse liver extracts. Bars represent the mean, and error bars represent the standard error of the mean (SEM). P-values were calculated using two-sided Mann-Whitney U -tests. ALP: alkaline phosphatase, ASAT: aspartate aminotransferase, ALAT: Alanine aminotransferase, *Il6*: interleukin 6, *Saa3*: serum amyloid 3, *Tnf α* : tumor necrosis factor alpha, *Acat2*: Acetyl-CoA acetyltransferase, *Col1a1*: alpha-1 type I collagen, *Timp*: TIMP metalloproteinase inhibitor 1, WAT: white adipose tissue.



Extended Data Fig. 10 | Anthropometric parameters, serum liver enzymes and liver gene expression levels in p38 γ - δ knock out (DKO) mice treated with and without imidazole propionate on a 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) diet. a–b. Water intake and body weight in DKO 8-week-old mice treated with vehicle (Ctrl) or imidazole propionate (ImP) (data are shown per cage) on a 6-week chow diet followed by two weeks on a DDC diet ($n=10$ per group). **c.** Body weights at time of sacrifice. **d.** Organ weights (as percentage of body weight) at sacrifice in mice treated with or without ImP. **e.** Serum levels of ALP, ASAT and ALAT at sacrifice. For panels **c–e**, box plot center lines indicate the median, the bounds of the box indicate the 25th and 75th percentiles,

and the whiskers indicate the minimum and maximum values within $1.5 \times$ the interquartile range. **f–g.** Relative mRNA levels of inflammation and fibrosis related genes in mouse liver extracts. Bars represent the mean, and error bars represent the standard error of the mean (SEM). P-values were calculated using two-sided Mann-Whitney U -tests. ALP: alkaline phosphatase, ASAT: aspartate aminotransferase, ALAT: Alanine aminotransferase, *Il6*: interleukin 6, *Saa3*: serum amyloid 3, *Tnf α* : tumor necrosis factor alpha, *Acat2*: Acetyl-CoA acetyltransferase, *Col1a1*: alpha-1 type I collagen, *Timp*: TIMP metalloproteinase inhibitor 1, WAT: white adipose tissue.

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

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| ☐ | ☒ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
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<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
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| ☐ | ☒ | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☐ | ☒ | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ | Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Internal database system at Oslo University Hospital (Medinsight)
Data analysis	R was used for all data processing, visualization, and statistical analyses, HALO® and HALO AI®, LAS X Core, Bio-Plex Manager™ for in vivo and ex vivo data analysis

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

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All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Individual participant data have not been deposited in public repositories due to Norwegian regulations and lack of consent. Individual participant data will be made available to other researchers upon reasonable request, following amendments to the ethical approvals and a material and data transfer agreement between the institutions. Likely time-frame: 3 months. However, fully anonymized source data for all figures will be provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Sex is reported in all the human data presented. Participant sex is based on the population registry and hence not self-reported. The initial comparisons of key features (ImP level) between patients and controls showed no effect of sex (Extended data figure 2D), hence sex was not studied specifically later. Additional sex-focused data must be considered clearly post-hoc and would have very limited high scientific value. We also lack permission and consent to deposit individual level data. Taken together, data disaggregated for sex is therefore not provided for source data nor for summary tables or individual comparisons/statistical tests. The underlying individual data of the study may be shared according to the data availability statement.

Reporting on race, ethnicity, or other socially relevant groupings

Ethnicity is not considered relevant or reported in our data analysis

Population characteristics

Patients with PSC, PBC, MASLD, ALD and IBD under follow-up in specialized center for the treatment and follow-up for chronic liver diseases.

Recruitment

Performed at local centers or at a tertiary care hospital

Ethics oversight

No specific risk for the patients as this is a cross-sectional observational study performed in already collected blood samples.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

5-10 mice per group in each experiment, 5 cells replicate per condition, 3 organoids separate experiments generated from 3 independent donator

Data exclusions

None of mice have been excluded from the analysis

Replication

No applicable

Randomization

Mice were randomly assigned to IMP or Vehicle (1:1 per cage)

Blinding

All histological quantification were performed blindly by two expert pathologists and MRIs re-reviewed blinded by two radiologists

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

- | | |
|-------------------------------------|---|
| n/a | Involved in the study |
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☐ | ☒ Animals and other organisms |
| ☐ | ☒ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

Methods

- | | |
|-------------------------------------|---|
| n/a | Involved in the study |
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

Antibodies

Antibodies used	CK19: abcam, code #ab133496, dilution 1:100; p-mTOR: Cell Signaling Technologies, code #2976S, dilution 1:100; CD3: Dako, Agilent, M725401, dilution 1:100; CD20: Dako, Agilent, M0755, dilution 1:100; CD68: Dako, Agilent. Code: M0876, dilution 1:100
Validation	Not performed due to commercially available antibodies used in all the experiments

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	H69 were kindly gifted from Prof. Domenico Alvaro, organoids were derived from patient bile duct brushes
Authentication	Not performed
Mycoplasma contamination	Tested negative (several times)
Commonly misidentified lines (See ICLAC register)	Not used

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	C57b6/J mice and Swiss Webster germ free mice, Gt(ROSA)26Sortm16(cre)Arte – Cre Deleter were purchased from Charles River, MAPK12-13 flox mice were generated by Charles River upon investigator request
Wild animals	not used
Reporting on sex	only male mice were used. The main reason for this is the 3Rs (reduce, refine replace) where sex differences would necessitate a doubling of the number of animals. Male mice were chosen as PSC is a male dominated disease (2:1 male:female ratio)
Field-collected samples	not used
Ethics oversight	Dnr 5.8.18-16056/2019 approved by the Gothenburg animal ethics committee

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	(No clinical interventions performed), but the research projects are covered by the following approvals: Norway (2010/1540, 2011/2572, 2015/2140, no.568878, 2012/286, 2016/1690, 568878), Sweden Dnr: 571-18 and 2018/1111-32, Italy PBC322 (n.2969), n.5129 (541/1). USA PSC Mayo Clinic Institutional Review Board (IRB) #s 670-02 and 16-005892. Denmark PBC ethical committee of the Central Denmark Region (1-10-72-146-16)
Study protocol	Blood/stool sampling of patients with PBC, PSC, MASLD, ALD, IBD and healthy controls at baseline and/or during follow-up and after transplantation. No specific interventions have been performed in all the cohorts analyzed
Data collection	Clinical data collected at time of blood/stool sampling.
Outcomes	Investigated clinical (observational outcomes): 1. Transplant-free survival and 2. recurrence of PSC post-transplantation

Plants

Seed stocks	not used
Novel plant genotypes	not used
Authentication	not applicable