LRH-1 mitigates intestinal inflammatory disease by maintaining epithelial homeostasis and cell survival

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Epithelial dysfunction and crypt destruction are defining features of inflammatory bowel disease (IBD). However, current IBD therapies targeting epithelial dysfunction are lacking. The nuclear receptor LRH-1 (NR5A2) is expressed in intestinal epithelium and thought to contribute to epithelial renewal. Here we show that LRH-1 maintains intestinal epithelial health and protects against inflammatory damage. Knocking out LRH-1 in murine intestinal organoids reduces Notch signaling, increases crypt cell death, distorts the cellular composition of the epithelium, and weakens the epithelial barrier. Human LRH-1 (hLRH-1) rescues epithelial integrity and when overexpressed, mitigates inflammatory damage in murine and human intestinal organoids, including those derived from IBD patients. Finally, hLRH-1 greatly reduces disease severity in T-cell-mediated murine colitis. Together with the failure of a ligand-incompetent hLRH-1 mutant to protect against TNFα-damage, these findings provide compelling evidence that hLRH-1 mediates epithelial homeostasis and is an attractive target for intestinal disease.
Inflammatory bowel disease (IBD) is a chronic disorder that is characterized by bouts of intense gastrointestinal inflammation, ultimately resulting in destruction of the epithelial lining of the gut. Although defects in genes expressed in the gut epithelium have been associated with IBD, the contribution of the epithelium to this disease remains understudied, particularly in comparison to the intensive interrogation of the immune component. However, the recent establishment of mouse and human intestinal organoids has provided an excellent experimental platform to explore intrinsic epithelial defects in patients and mouse models with disease.

An important regulatory factor for intestinal epithelia is Liver Receptor Homolog 1 (LRH-1, NR5A2). This nuclear receptor has been shown to be expressed in intestinal crypts, where intestinal stem cells (ISCs) reside, and where it contributes to epithelial renewal by potentiating WNT/β-catenin signaling. Recent GWAS meta-analyses of IBD patients found a significant association between LRH-1 and IBD. Animal studies using heterozygous (Lrh-1<sup>+</sup>−) or conditional knockout (Lrh-1<sup>−/−</sup>; VilCreERT2) did not note any apparent epithelial defects at baseline, but did report a defect in epithelial proliferation and susceptibility to colitis. Interestingly, the elimination of LRH-1 in mouse intestine and human colon cancer cell lines resulted in decreased glucocorticoid production, which has the potential to lead to the kind of increased intestinal inflammation observed in mouse models of colitis. This atypical nuclear receptor contains a well-ordered hormone-binding pocket, which binds signaling phospholipids including phosphoinositides. However, structural and biochemical studies have revealed major differences between the human and mouse orthologs; hLRH-1 is the relevant isoform in human disease. As illustrated in Fig. 1a, using human intestinal organoids, we uncover an essential role for LRH-1 in intestinal epithelial homeostasis and cell survival, which mitigates inflammatory injury. Our data rationalize efforts required to target this nuclear receptor for the treatment of IBD.

**Results**

LRH-1 maintains epithelial integrity and viability. In order to investigate the role of LRH-1 in gut epithelia, LRH-1 expression and the effects of its deletion were determined in mouse intestinal organoids. Similar to prior in vivo studies, LRH-1 was found in the crypt domain of intestinal organoids, but was also detected at lower levels in the villus domain (Fig. 1a). Using Lrh<sup>−/−</sup>;VilCreERT2 (Lrh<sup>1IEC-KO</sup>) mice, intestinal organoids were generated following conditional and acute deletion of LRH-1 (Fig. 1b). Consistent with the proposed role for LRH-1 in Wnt/β-catenin-regulated cell growth, deletion of Lrh−/− increased cell death and lowered organoid viability in a modified MTT reduction assay compared to control organoids from Lrh<sup>+/+</sup> mice (Fig. 1c).

Transcriptional profiling of Lrh<sup>−/−</sup> and Lrh<sup>1IEC-KO</sup> intestinal organoids revealed significant gene changes in cell survival and apoptosis pathways (Fig. 1d, e), suggesting a role for LRH-1 in intestinal epithelial homeostasis and viability. Consistent with this notion, a marked increase in activated Caspase 3 (Casp-3) was observed in the intestinal crypt domain of Lrh<sup>1IEC-KO</sup> organoids, which was further exacerbated by TNFa (Fig. 1f, g). As expected, given the documented role of LRH-1 in intestinal epithelial proliferation, Lrh<sup>1IEC-KO</sup> intestinal organoids exhibited decreased cell proliferation measured by 5-ethynyl-2-deoxyuridine (EdU) incorporation (Supplementary Figure 1).

Because epithelial damage is a major contributor to chronic inflammatory disease in IBD, we investigated whether loss of LRH-1 compromises the epithelial barrier. Indeed, significant failure of the epithelial barrier was observed in Lrh<sup>1IEC-KO</sup> intestinal organoids using a vital dye exclusion assay (Fig. 1h). Together, these data support an essential role for LRH-1 in epithelial viability and resilience.

**LRH-1 affects crypt survival and differentiation via Notch.**

Notch expression in the intestinal crypt preserves LGR5<sup>+</sup> stem cells while restricting secretory lineages and is critical for IBD survival. We then asked if the observed crypt cell death in Lrh<sup>1IEC-KO</sup> organoids might arise from impairment in Notch signaling. Indeed, both Notch1 transcripts and protein levels were diminished in Lrh<sup>1IEC-KO</sup> organoids (Fig. 2a, b). Because Notch is also a key factor in epithelial differentiation, cell numbers and markers for Paneth, goblet, and enteroendocrine cells were assessed after loss of LRH-1. As expected, lowered Notch signaling in Lrh<sup>1IEC-KO</sup> organoids resulted in downregulation of the stem cell markers Lgr5 and Olfm4, while leading to upregulation of Lyn and Muc-2; two respective markers for secretory Paneth and goblet cells (Fig. 2b). The number of goblet cells doubled in Lrh<sup>1IEC-KO</sup> intestines, and Paneth cells were visibly expanded in intestinal crypts (Fig. 2c, d). Surprisingly, rather than observing an expansion of EECs, as previously described with Notch inhibition, the number of enterochromaffin cells, a representative sub-population of EEC cells, was significantly reduced in Lrh<sup>1IEC-KO</sup> intestines (Fig. 2c, d), as were levels of EEC-specific transcripts (Fig. 1d). Collectively, these data imply that LRH-1 is necessary for maintenance of Notch signaling and cell survival and for proper allotment of intestinal epithelial cell types.

**Human LRH-1 prevents intestinal crypt death and TNFa injury.**

We next asked whether restoring or overexpressing LRH-1 might strengthen epithelial resilience to an inflammatory challenge. Human LRH-1 (hLRH-1), rather than mLrh-1, was chosen because it displays greater ligand-dependent activation and is the relevant isoform in human disease. As illustrated in Fig. 3a, hLRH-1, unlike mLrh-1, lacks the salt bridge at the mouth of the ligand-binding pocket and requires a positive charge to stabilize this domain. This role is fulfilled by the phosphate in the polar head group of its phospholipid ligand. Expression of hLRH-1 in mouse intestinal organoids was achieved by an AAV8-mediated infection protocol, which was optimized using AAV8-GFP. This method resulted in rapid and efficient gene expression (Fig. 3b) that persisted for the life of the epithelial cell (Supplementary Figure 2) and permitted dosing that could either match or exceed endogenous levels of mLrh-1 (Fig. 3c, left). Nuclear expression of hLRH-1 was detected throughout the crypt and villus zones (Fig. 3b).

Human LRH-1 expression was able to fully rescue crypt cell death and maintain viability in Lrh<sup>1IEC-KO</sup> organoids even upon challenge by TNFa (Fig. 3d–f). To ascertain if ligand binding is necessary for hLRH-1-mediated rescue, we next attempted to salvage organoid viability with the well-characterized ligand-binding-defective variant of hLRH-1 (PM; Fig. 3a). Bulky hydrophobic residues were modeled in the binding pocket to impede ligand uptake without effecting protein integrity, as previously demonstrated in cultured cell lines. Indeed, the human PM variant is stably expressed in intestinal organoids (Fig. 3c, right), and despite the fact that the hPM retains modest transcriptional activity, it failed to rescue TNFa-induced cell death in Lrh<sup>1IEC-KO</sup> intestinal organoids (Fig. 3f, light blue bar).

Expressing hLRH-1 resulted in upregulation of known downstream targets including Shp, Cyp11a1, and Cyp11b1 as well as...
robust expression of new potential LRH-1 targets (Ctbr1 and Smcp), and mediators of cell survival and anti-inflammatory responses, including the decay receptors Iilmn and Tnfrsf23 and the antiapoptotic factor Hmoxl (Fig. 3g and Supplementary Figures 3 and 4). Adding hLRH-1 to Lrh1IEC-KO organoids also restored the integrity of the epithelial barrier, as demonstrated by a reduction of vital dye-positive organoids (Fig. 3h). Moreover, increasing the dosage of hLRH-1 in the presence of wild-type mLRH-1 ameliorated TNFα-induced cell death, suggesting that elevated LRH-1 activity protects against inflammatory damage (Fig. 3i, left). This effect extends to other epithelial insults, as overexpression of hLRH-1 also protected against damage by fluorouracil (5-FU), a common chemotherapeutic with intestinal toxicity (Fig. 3i, right). Taken together, these data demonstrate that hLRH-1 fully substitutes for mLRH-1 to restore cell survival and activate anti-inflammatory programs.

To confirm the survival role of LRH-1 in vivo, we used a humanized intestinal mouse model in which mLRH-1 is deleted and hLRH-1 expressed in an inducible Cre-dependent manner (referred to as Lrh1IEC-Flex). Despite the lower protein levels of hLRH-1 as compared to endogenous mLRH-1 in Lrh1IEC-Flex organoids (Fig. 4a), expressing hLRH-1 reduced cell death by nearly 50% and restored Ctbr1 levels (Fig. 4b, c). These ex vivo results were confirmed in vivo by the near absence of cleaved Casp3 in intestinal crypts of the ileum (and to a lesser extent in villi) in hLRH1IEC-Flex mice compared to Lrh1IEC-KO (Fig. 4d). Taken together, these data reveal that human LRH-1 can promote cell survival in murine intestinal epithelia lacking mLRH-1 and following challenge by TNFa.

hLRH-1 ameliorates an immune-mediated colitis mouse model. To determine whether increased levels of LRH-1 can improve the course of disease in an immune-mediated model of colitis, VilCreRag2−/−;Rosa26-Flox-Stop-Flox hLRH-1 mice (Rag2−/−;hLrh1IEC-Tg) were generated to conditionally overexpress hLRH-1 in the intestinal epithelium in the presence of endogenous mLRH-1. As expected from our earlier data, mLRH-1 elimination greatly exacerbated T-cell transfer (TcT)-induced colitis in Rag2−/−;hLrh11KO male mice (Fig. 5a). Significantly, this result was markedly different after overexpressing hLRH-1. Disease severity was largely mitigated in Rag2−/−;hLrh1IEC-Tg mice, as evidenced by the relative preservation of body weight, prolonged disease-free survival, improved colitis histology scores, and reduced disease activity index (DAI). In fact, nearly all disease parameters were better in Rag2−/−;hLrh1IEC-Tg animals relative to animals expressing endogenous mLRH-1 (Fig. 5a). Mirroring this improvement in colitis, Rag2−/−;hLrh1IEC-Tg animals showed a decreased inflammatory cytokine profile, with lower intestinal expression of TNFa, IL-1β, and IL-6, and a corresponding increase in the anti-inflammatory cytokine IL-10 (Fig. 5b). Collectively, these in vivo data establish the critical role...
of LRH-1 as an anti-inflammatory agent in the intestinal epithelium.

**LRH-1 protects human intestinal organoids from TNFα injury.**

To determine whether the anti-inflammatory and prosurvival activity of LRH-1 translates to the human intestinal epithelium, human small intestinal organoids were derived from endoscopic biopsy samples of ileum from both Crohn disease patients and healthy individuals (Fig. 6a). Unlike murine small intestinal organoid cultures, human-derived organoids are maintained in a partially differentiated state, consisting of intestinal stem cells and partially differentiated Paneth cells, and undergo differentiation following WNT withdrawal. Human Lrh1 is expressed at similar levels in both differentiated and undifferentiated human organoids (Supplementary Figure 5), and remains broadly distributed throughout the epithelium following differentiation, as confirmed by staining for secretory goblet (MUC2) and Paneth (LYZ) cells (Fig. 6b). This pattern closely matches the broad distribution of murine Lrh1 (Fig. 1a).

Increasing hLRH-1 dosage by AAV-mediated infection caused an upregulation of the LRH-1 target Cbfb (Fig. 6c). Importantly, in human organoids from both healthy individuals and Crohn disease patients, overexpression of hLRH-1 abrogated TNFα-induced cell death (Fig. 6d). Taking all the data in this study together, we conclude that LRH-1 plays an essential role in intestinal homeostasis and in ameliorating inflammation-induced injury in human intestinal epithelia.

**Discussion**

In this study, using multiple independent mouse and human ex vivo and in vivo intestinal models, we establish that the nuclear receptor LRH-1 (Nr5a2) has a crucial role in maintaining the intestinal epithelium. Acutely knocking out mLRH-1 resulted in decreased Notch signaling and increased cell death in the intestinal epithelium. Acutely knocking out mLRH-1 resulted in decreased Notch signaling and increased cell death in the intestinal epithelium. Moreover, overexpression of hLRH-1 in both mouse and human intestinal organoids imparted epithelial resistance to both TNFα, a major inflammatory cytokine in IBD, and 5-FU, a chemotherapeutic with intestinal toxicity. In the intact animal, expression of hLRH-1 corrected these deficits. Moreover, overexpression of hLRH-1 in both mouse and human intestinal organoids imparted epithelial resistance to both TNFα, a major inflammatory cytokine in IBD, and 5-FU, a chemotherapeutic with intestinal toxicity. In the intact animal, expression of hLRH-1 corrected these deficits. Using a viral-mediated approach newly applied to intestinal organoids, we showed that efficient rescue by hLRH-1 is ligand dependent. These findings are important because they provide a compelling argument that drug targeting of LRH-1 could enhance resistance to inflammation and restore intestinal epithelial health in intestinal diseases such as IBD.
Our study extends prior studies, reporting impaired cell renewal and enhanced chemical-induced colitis in heterozygous and conditional knockout mice, by demonstrating both a fundamental role for LRH-1 in the maintenance of epithelial viability and cell types, and the therapeutic potential of LRH-1 in intestinal disease. Further, we reveal that acute loss of mLRH-1 disrupts Notch expression, triggers increased apoptosis in the crypt and results in a breach in the epithelial barrier. Our data are consistent with the findings by Samuelson and colleagues that attenuation of Notch signaling by genetic or pharmacological findings, but also appears to complement ISC apoptosis, crypt disruption, and increased intestinal inflammation in the T-cell transfer model of colitis. Importantly, increased hLRH-1 has a clear beneficial impact on disease activity and colitis scores, consistent with our organoid models.

The ability to acutely knock out mLRH-1 and replenish with hLRH-1 in intestinal organoids has provided new insights into the identity and function of species-specific LRH-1 targets in the small intestine. Based on the rapid crypt cell death and spectrum of differentially expressed genes, cell survival is the most prominent pathway affected following acute loss of mLRH-1. Interestingly, despite the known species difference in ligand binding, hLRH-1 not only functionally complements mLRH-1 but also exacerbates the immune inflammatory response. In support of this idea, we demonstrate that loss of intestinal LRH-1 expression is associated with diminished animal survival and increased intestinal inflammation in the T-cell transfer model of colitis. Importantly, increased hLRH-1 has a clear beneficial impact on disease activity and colitis scores, consistent with our organoid models.
KO increased in the intestine of conventional as opposed to germ-free upregulated threefold by hLRH-1; interestingly, this gene is also signal is also observed after expressing hLRH-1 in generated from three independent wells of enteroids (~50 organoids per well) done in triplicate.

Our data suggest that LRH-1 may play a previously unappreciated role in epithelial cell differentiation in the intestine, in addition to ISC maintenance. Indeed, a similar role for LRH-1 has been reported in the pancreas and recently in neural stem cells. Interestingly, although we observe an increase in secretory Paneth and goblet cells, consistent with reduction of Notch signaling, loss of LRH-1 also leads to a significant drop in markers defining nearly all subclasses of EECs. These data infer separate but positive roles for LRH-1 in Notch intestinal crypt signaling and EEC differentiation. Intriguingly, the latter effect may be regional, with the greatest loss of EEC cells following deletion of LRH-1 in the ileum and proximal colon (J.B. and H.I., unpublished data); both of which exhibit high LRH-1 expression. We are not yet certain how and where LRH-1 promotes lineage commitment in the gastrointestinal tract remains to be determined.

An important unanswered question is whether increased LRH-1 expression drives unchecked proliferation and promotes dysplasia in the intestinal epithelium, as suggested previously. Indeed, an earlier study showed that LRH-1 haploinsufficiency reduced tumor burden in the APCMin model, possibly through interactions with the Wnt pathway at the Cyclin D1 and Cyclin E1

**Fig. 4** In vivo rescue of Lrhfl/fl mice by hLRH-1 reverses cell death. a LRH-1 protein levels in hLrh1IEC-Flex enteroids detected by anti-LRH-1 antibody with arrowheads indicating migration of human (blue) or mouse (black) LRH-1 before or after addition of 4OHT, which eliminates mLrh1 and promotes hLRH-1 expression; protein extracts were isolated 72 h later. Relative levels of mLrh1 and a downstream LRH-1 target gene, Ctrb1 in wild-type (Lrhfl/fl), LrhIEC-KO, and hLrh1IEC-Flex enteroids, with values normalized to wild type set at 1.0. Generation of hLrh1IEC-Flex is described in Methods. For all panels, data were generated from three independent wells of untreated enteroids (−50 organoids per well) done in triplicate. Percentage of cell death in hLrh1IEC-Flex enteroids with TNFx (10 ng/ml, 40 h) after eliminating mLrh1 (gray) and expressing hLRH-1 (blue) by addition of 4OHT for 48 h. Data are also shown for treated Lrhfl/fl enteroids (black). All values are normalized to three independent wells of untreated Lrhfl/fl enteroids, which is taken to be 0%. d Immunofluorescence of wild-type (Lrhfl/fl), LrhIEC-KO, and hLrh1IEC-Flex ileum from adult male mice treated with two consecutive injections of tamoxifen. Staining for activated Casp3 (red) and CD44 (green), which marks intestinal epithelial crypt cells, is shown at lower (first column) and higher (second column) magnification. Scale bars = 50 μm. **Fig. 4** Percentage of cell death in untreated hLrh1IEC-Flex organoids and their derivatives. b Percentage of cell death in untreated enteroids is described in Methods. For all panels, data were generated from three independent wells of untreated enteroids (−50 organoids per well) done in triplicate. c Percentage of cell death in hLrh1IEC-Flex enteroids with TNFx (10 ng/ml, 40 h) after eliminating mLrh1 (gray) and expressing hLRH-1 (blue) by addition of 4OHT for 48 h. Data are also shown for treated Lrhfl/fl enteroids (black). All values are normalized to three independent wells of untreated Lrhfl/fl enteroids, which is taken to be 0%. d Immunofluorescence of wild-type (Lrhfl/fl), LrhIEC-KO, and hLrh1IEC-Flex ileum from adult male mice treated with two consecutive injections of tamoxifen. Staining for activated Casp3 (red) and CD44 (green), which marks intestinal epithelial crypt cells, is shown at lower (first column) and higher (second column) magnification. Scale bars = 50 μm. N = 2 per genotype. For panels b and c error bars are SEM with statistical analyses determined by Student’s unpaired t test, two tailed with p values of *p < 0.05, **p < 0.01, and ***p < 0.0001

**Fig. 4** In vivo rescue of Lrhfl/fl mice by hLRH-1 reverses cell death. a LRH-1 protein levels in hLrh1IEC-Flex enteroids detected by anti-LRH-1 antibody with arrowheads indicating migration of human (blue) or mouse (black) LRH-1 before or after addition of 4OHT, which eliminates mLrh1 and promotes hLRH-1 expression; protein extracts were isolated 72 h later. Relative levels of mLrh1 and a downstream LRH-1 target gene, Ctrb1 in wild-type (Lrhfl/fl), LrhIEC-KO, and hLrh1IEC-Flex enteroids, with values normalized to wild type set at 1.0. Generation of hLrh1IEC-Flex is described in Methods. For all panels, data were generated from three independent wells of untreated enteroids (−50 organoids per well) done in triplicate. c Percentage of cell death in hLrh1IEC-Flex enteroids with TNFx (10 ng/ml, 40 h) after eliminating mLrh1 (gray) and expressing hLRH-1 (blue) by addition of 4OHT for 48 h. Data are also shown for treated Lrhfl/fl enteroids (black). All values are normalized to three independent wells of untreated Lrhfl/fl enteroids, which is taken to be 0%. d Immunofluorescence of wild-type (Lrhfl/fl), LrhIEC-KO, and hLrh1IEC-Flex ileum from adult male mice treated with two consecutive injections of tamoxifen. Staining for activated Casp3 (red) and CD44 (green), which marks intestinal epithelial crypt cells, is shown at lower (first column) and higher (second column) magnification. Scale bars = 50 μm. N = 2 per genotype. For panels b and c error bars are SEM with statistical analyses determined by Student’s unpaired t test, two tailed with p values of *p < 0.05, **p < 0.01, and ***p < 0.0001
promoters, but this same study noted decreased Lrh1 in intestinal tumors. While our studies with the nonreplicating AAV vector preclude us from exploring the question of cell proliferation in infected intestinal organoids, we note that neither Cyclin D1 nor Cyclin E1 were changed after loss of mLRH-1 or Rag2–/–Lrh1IEC-KO. Nonetheless, this system allows a rapid structure-function analysis by simultaneously knocking out and adding back variants, which we put to effective use in this study to show the ligand-dependency of hLRH-1 effects. As unique molecular signatures of intestinal epithelial subtypes continue to emerge, the ability to infect large organoid fragments. On the other hand, the nonreplicating nature of AAV restricts expression to the typical ~7 days turnover for mouse intestinal organoids. Nonetheless, this system allows a rapid structure-function analysis by simultaneously knocking out and adding back variants, which we put to effective use in this study to show the ligand-dependency of hLRH-1 effects. As unique molecular signatures of intestinal epithelial subtypes continue to emerge, the ability to infect large organoid fragments.

Methods

Study approval. Animal studies were conducted in accordance with IACUC guidelines in strict accordance with the recommendations in the Guide for the Care
For panels c under differentiation conditions and then exposed to TNFα(0.8 line was generated with Lox-STOP-Lox-hLRH1 animals, gift of DeMayo (Baylor Board (IRB 15-17763) and utilized tissue from deidentified studies and procedures were approved by the Baylor College of Medicine (AN-1550) and UCSF (AN173604-01A) Institutional Animal Care and Use Committee. All animal experiments written informed consent to the study.

**Animal experiments.** Animals were housed and bred in SPF facility. Inducible IEC knockout line was created by crossing animals harboring CreERT2 under control of the villin promoter with Lrh1fl/fl animals and bred to homozygosity. Lrh1IEC-Rex line was generated with Lox-STOP-Lox-Lrh1 animals, gift of DeMayo (Baylor College of Medicine) crossed into our Lrh1IEC-Xc line. For animal knockout and activation studies, tamoxifen was dissolved in sunflower oil and delivered by two intraperitoneal injections 48 h apart (1 mg per day). Mice were sacrificed and tissue collected 5 days following the last tamoxifen dose. For the T-cell transfer model, Lrh1fl/fl;VilCre animals were bred with Rag2fl/fl Jackson Laboratory to homozygosity to generate Rag2−/−; Lrh1IEC-NOT animals. Likewise, Rosam2;Flox-Stop-Flox Lrh1fl/Lrh1;VilCre animals were crossed with Rag2−/− animals to generate Rag2−/−; Lrh1IEC-NOT animals. Chronic enterocolitis was induced by T-cell transfer of 0.5 million naive T cells per mouse, as described. Briefly, wild-type splenic CD4+ CD45RB+ cells were isolated by MACS separation and flow cytometry cell sorting (Supplementary Figure 6), and then transferred by intraperitoneal injection to Rag2−/− mice.

Clinical DAI and colitis scores: To assess the clinical DAI body weight loss, diarrhea, guaiac-positive hematochezia, and appearance were monitored daily during the experiment. The DAI was determined according to a published scoring system (Supplementary Table 1). For colon histological analysis, the colon was divided into three segments (proximal third, middle third, and distal third). Each segment was embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Histological analysis was performed in the Cellular and Molecular Morphology Core of the Digestive Disease Center at Baylor College of Medicine. The sections were blindly scored using a standard histologic colitis score. Three independent parameters were measured: severity of inflammation (0–3: none, slight, moderate, severe), depth of injury (0–3: none, mucosal, mucosal and submucosal, transmural), and crypt damage (0–4: none, basal one-third damaged, basal two-thirds damaged, only surface epithelium intact, entire crypt and epithelium lost). The score of each parameter was multiplied by a factor reflecting the percentage of tissue involvement (x1, 0–25%; x2, 26–50%; x3, 51–75%; x4, 76–100%) averaged per colon.

**Cryp culture.** Intestinal crypt cultures were derived from Lrh1fl/fl; VilCreERT2; Lrh1fl/fl, and VilCreERT2;Lrh1fl/fl;Rosa26;Lrh1−/− 6-week-old male mice. Briefly, the small intestine was isolated and flushed with ice-cold phosphate-buffered saline (PBS) and opened longitudinally. Villi were mechanically removed and the intestine cut into 1–2 mm pieces. Intestinal fragments were then incubated in an EDTA containing solution at 4 °C for 30 min. The intestinal fragment suspension was fractionated and crypt-containing fractions passed through a 70-μm cell strainer for plating in Matrigel. Crypt-Matrigel suspension was allowed to polymerize at 37 °C for 10 min. Intestinal organoids were grown in base culture media (Advanced DMEM/F12 media, HEPES, GlutMax, penicillin, and streptomycin) supplemented with growth factors (EFG, Noggin, R-spondin; Peprotech), B27 (Life Technologies), N2 (Life Technologies), and N-acytetyl cystine (NAC, Sigma). To activate genetic recombination, 2-4-hydroxytamoxifen (4OH; Sigma) was added at 300 nM for 48 h.

**Human intestinal organoid culture.** Endoscopic biopsy samples obtained from the terminal ileum were processed under a dissecting microscope to liberate intestinal crypts using EDTA chelation and mechanical disruption. Crypts were screened through a 100 μm filter, centrifuged, and suspended in ice-cold Matrigel. The suspension was plated on prewarmed cell culture plates. To induce differentiation, media was replaced with differentiation media (consisting of base culture media supplemented with 20% R-Spondin conditioned media, human EGF (Peprotech), A-83-01 (Tocris), SB202190 (Sigma), Gastrin (Sigma), Nicotinamide (Sigma), B27 (Life Technologies), N2 (Life Technologies), GlutMax (Life Technologies), and HEPES (Sigma) in F12 Advanced DMEM (Life Technologies)) was added. For the first 48 h of culture, CHIR99021 (Stemgent) and thiazovin (Stemgent) were added to support stem cell growth. To induce differentiation, media was replaced with differentiation media (consisting of base culture media supplemented with 20% R-Spondin conditioned media, human EGF (Peprotech), human Noggin (Peprotech), A-83-01, Gastrin, NAC, B27, and N2).

**Expression analysis.** Immunofluorescence (IF) and RNA in situ hybridization were performed on 5 μm cryosections using standard procedures. DIG-labeled (Roche) riboprobes were generated from pCRII-TOPO plasmid (ThermoFisher Sci) with mLRH-1 cDNA corresponding to bases 595–1683. Antibodies against Lrh1 (1:200, Sigma HPA005455), FLAG (1:300, Sigma F7425), cleaved Caspase-3 (1:1000 (WB) and 1:400 (IF), Cell Signaling 5A1E), CD-44 (1:500, Tonbo 70-0441), Lysosome (1:200, DAKO EC 3.2.1.17) and MUC-2 (1:300, Santa Cruz Bio-techology sc-15334) were used with Alexa Fluor-conjugated secondary antibodies (Millspo, Invitrogen). For Goblet staining, Rhodamine-labeled Dolichos Biflorus Agglutinin (Vector Labs) was used at 1:200 dilution.

**RNA isolation and PCR.** Intestinal organoids were washed in ice-cold PBS and suspended in Trizol solution (Ambion). RNA was isolated with Direct-zol spin and Use of Laboratory Animals of the National Institutes of Health. All animal studies and procedures were approved by the Baylor College of Medicine (AN-1550) and UCSF (AN173604-01A) Institutional Animal Care and Use Committee. All animal experiments were approved by the Baylor College of Medicine (AN-1550) and UCSF (AN173604-01A) Institutional Animal Care and Use Committee. All animal experiments were approved by the Baylor College of Medicine (AN-1550) and UCSF (AN173604-01A) Institutional Animal Care and Use Committee.

**Fig. 6** Increasing hLRH-1 in human intestinal organoids protects against TNFα. a Brightfield view of human small intestinal organoid. Scale bar = 100 μm. b Immunofluorescence for LRH-1 (green, top panels) in human intestinal organoid sections shows expression throughout the organoid with strongest expression occurring in the crypt domain (yellow dashed box and zoomed image, right). Differentiation markers for Paneth (Lyz, left) and goblet (Muc2, right) cells are shown below. Scale bar = 100 μm. c Expression of LRH-1 target gene Cbtl in human intestinal organoids is upregulated 72 h after infection with AAV-hLRH1 (blue) but not with control AAV (black). d Overexpression of hLRH-1 by AAV confers resistance to TNFα-mediated cell death. Human organoids from healthy donor and a Crohn disease patient were infected with AAV-hLRH1 (blue) or AAV-Control (black) (3.3×10^10 genome copies) for 72 h under differentiation conditions and then exposed to TNFα (20 ng/ml, 40 h). Data represent an N of at least three replicates with -50 organoids per well. For panels c and d error bars are SEM with statistical analyses determined by Student’s unpaired t test, two tailed with p values of *p < 0.05.
performed as per the manufacturer’s recommendations on 5 μM prewarmed organoid growth media. For proliferation assays, intestinal organoid cultures were incubated with EdU (10 μM) for 2 h and then fixed in 4% paraformaldehyde. Click-It chemistry was performed as per the manufacturer’s recommendations on 5 μM cryosections (Life Technologies). For 5-FU experiment, organoids were incubated with 5-FU (5 μg/ml in DMEM, Millipore) for 24 h and viability determined as above.

Immunoblotting. Protein was isolated from intestinal organoids grown in 24-well culture plates in RIPA buffer containing protease inhibitors (Roche). Samples were processed in a Bioruptor prior to gel loading. Antibodies include LRH-1 (R&D Systems PP-H2325-00), P-Actin (Ambion AM4302), FLAG (Sigma F1804), cleaved Caspase-3 (Cell Signaling 9662), and cleaved Notch1 (Cell Signaling D3B8). Full blots are shown in Supplementary Materials (Supplementary Figure 7).

Dextran exclusion assay. Intestinal organoids were exposed to mTNFα for 24 h and then incubated in 1 mg/ml Texas Red labeled dextran (average weight 10 kDa; Life Technologies) for 30 min. Following incubation, excess dye was removed by serial washes with PBS and the plate imaged immediately. Wells were scored for fraction of dye-retaining intestinal organoids. 30–50 intestinal organoids were seeded per well. Eight wells per experiment were scored for each condition. Opened intestinal organoids were excluded from analysis. Results were validated by a blinded, independent observer.

AAV-directed gene expression. AAV viral particles expressing LRH1 or GFP under direction of the thyroxine-binding globulin promoter were obtained from the University of Pennsylvania Viral Core. Intestinal organoids were isolated in cold PBS, pelleted at 1000 × g, and resuspended in ice-cold Matrigel. The mixture was added to chilled Eppendorf tubes containing virus on wet ice and then aliquoted immediately onto prewarmed cell culture plates. After Matrigel was set, organoid growth media was added.

Imaging. Live cell and intestinal organoid immunofluorescence imaging was performed on an Olympus IX51 microscope equipped with a DP71 imager. Mouse intestinal imaging was obtained on a Nikon Eclipse Ti equipped with a DS-Q2i imager or an Olympus BX40 microscope with Magnafire imaging.

Data availability. Deep sequencing data are archived under GEO accession number GSE116563. Reagents including mouse and organoid lines will be made available by request.

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J.R.B., H.W., R.J.F., O.D.K., D.D.M., and H.A.I. conceived and designed the research. J.R.B., H.W., D.D.M. and H.A.I. performed the data analysis and wrote the manuscript. All authors reviewed the final manuscript.

Additional information

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