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FXR mediates ILC-intrinsic responses to intestinal inflammation

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The pleiotropic actions of the Farnesoid X Receptor (FXR) are required for gut health, and reciprocally, reduced intestinal FXR signaling is seen in inflammatory bowel diseases (IBDs). Here, we show that activation of FXR selectively in the intestine is protective in inflammation-driven models of IBD. Prophylactic activation of FXR restored homeostatic levels of pro-inflammatory cytokines, most notably IL17. Importantly, these changes were attributed to FXR regulation of innate lymphoid cells (ILCs), with both the inflammation-driven increases in ILCs, and ILC3s in particular, and the induction of *Il17a* and *Il17f* in ILC3s blocked by FXR activation. Moreover, a population of ILC precursor-like cells increased with treatment, implicating FXR in the maturation/differentiation of ILC precursors. These findings identify FXR as an intrinsic regulator of intestinal ILCs and a potential therapeutic target in inflammatory intestinal diseases.

inflammatory bowel disease | bile acids | FXR | innate lymphoid cells | IL17

Significance

In addition to promoting lipid and fat-soluble vitamin absorption, bile acids (BAs) function as signaling molecules that coordinate persistent changes in intestinal cells. As such, BA homeostasis is tightly regulated by the Farnesoid X Receptor (FXR). This study elucidates a previously unknown role for FXR in regulating innate lymphoid cells (ILCs), wherein activation of FXR abrogates ILC3-dependent intestinal inflammation. Establishing FXR as an intrinsic regulator of ILCs provides a functional connection between diet and innate immunity. Moreover, with synergistic effects in both intestinal epithelial and immune cells, these findings implicate FXR agonism as an integrative therapeutic strategy for chronic intestinal inflammation.

Inflammatory bowel disease (IBD) is a chronic intestinal disorder that affects 4 million individuals worldwide. While the underlying cause(s) is not fully understood, defective epithelial barrier function and aberrant intestinal immune responses contribute to the inflammatory responses that drive the chronic disease. Loss of epithelial tight junctions exposes innate immune cells to commensal microbiota to induce cytokines that subsequently initiate an inflammatory response in adaptive immune cells (1–3). Thus, a complex interplay between epithelial cells, the innate and adaptive immune systems, and the microbiome contributes to the pathogenesis of IBD (2–5).

Innate lymphoid cells (ILCs) characteristically express the IL-7 receptor and are found in mucosal tissues including that in the intestine (6, 7). ILCs are subtyped into 3 major groups based on signature transcription factor expression and cytokine secretion, analogous to helper T cell grouping (8). Unique tissue-specific ILC repertoires have been attributed to the differentiation of circulating precursors in response to local environmental cues (9). Within the intestine, ILCs are important for intestinal barrier function and innate immunity, where integration of microbial signals generates phenotypic and functional plasticity (8–11). Consistent with the notion of cellular plasticity, recent single-cell studies identified multiple discrete ILC subsets in human ileal lamina propria (LP), including cells coexpressing markers of ILC3s and ILC1s (12). Notably, an increase in the number of actively proliferating ILCs was seen in the LP of Crohn's disease patients (2, 10, 11, 13–16).

Consistent with a role in maintaining intestinal homeostasis, the development of precursor ILCs can be influenced by nutritional cues that tailor the frequencies of ILC subtypes to specific intestinal challenges, thereby directly coupling the intestinal innate immune response with diet (11). Similarly, the adaptive immune system has been shown to be responsive to dietary-related signals, whereby intestinal T cell homeostasis is dependent on the ability of T_{eff} cells to appropriately respond to bile acids (BAs) (17). BAs are cholesterol-based metabolites that regulate mucosal homeostasis and inflammation. Secreted into the duodenum to facilitate the absorption of dietary lipids and fat-soluble vitamins, they are subsequently reabsorbed in the ileum where they accumulate in the LP prior to transiting to the portal circulation. In addition to solubilizing fats, BAs function as key signaling molecules involved in the regulation of metabolic processes. Indeed, BA homeostasis is tightly controlled by the transcriptional activities of the farnesoid x receptor (FXR) (18), and loss of BA homeostasis has been implicated in the pathogenesis of IBD. We have evolved gut-selective small-molecule FXR agonists (fexaramine (Fex) and FexD) (19) and shown that Fex treatment results in dramatic metabolic improvements in obesity and related metabolic diseases (20).

Cellular crosstalk between the epithelium and the intestinal immune system is critical in maintaining gut integrity and protecting against pathogen-initiated inflammation (1, 3). Increased intestinal levels of the cytokines IL17 and IL23 have been causally associated with IBD (21–23). While IL17A is implicated in the maintenance of intestinal tight junctions, IL17F is associated with intestinal damage, and antibodies to IL17F are

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protective in the mouse model of colitis (24). Th17 and ILC3 cells secrete IL17; however, the relative contributions of specific immune cell types to IBD have not been clearly established (13, 23). Here, we elucidate a previously unknown role for FXR in the regulation of IL17 production in intestinal ILC3s. Moreover, the increase in an ILC precursor-like population seen with FexD treatment implicates FXR in the maturation/differentiation of intestinal ILCs.

Results

Inflammation Reshapes the Villi Structure. The crypt-villus architecture is integral for normal intestinal regeneration and bile acid homeostasis (20, 25–28). Here, we used a dextran sulfate sodium (DSS)-induced model of colitis to explore the consequences of inflammatory damage to the villi structure (29). Acute DSS (ADSS) treatment (5% DSS in drinking water for 5 to 7 d, ADSS) caused profound morphological changes including epithelial erosion, irregular hyperplastic crypt formation, a reduction in villi length, and aberrantly shaped Goblet and Paneth cells in wild-type (WT) mice (*SI Appendix,* Fig. S1 *A–D*). These changes were associated with reduced expression of intestinal *Fxr* and its target genes (*Fgf15, Ibabp, Ostα,* and *Ostβ*) and a ~2-fold increase in intestinal permeability (*SI Appendix,* Fig. S1 *E* and *F*) (27, 28). Consistent with reduced ileal FXR signaling, total BA levels were increased ~2.9 fold, with a disproportionate increase in primary BAs (*SI Appendix,* Fig. S1*G*). These changes were due, in large part, to pronounced increases in the levels of the primary BAs β-muricholic acid (βMCA), taurocholic acid (T-CA), and cholic acid (CA) and the secondary BAs deoxycholic acid and ω-muricholic acid upon ADSS treatment (*SI Appendix,* Fig. S1*H*). In addition, the serum levels of the proinflammatory cytokines IL17 and IL6 approximately doubled, in agreement with the marked increases in ileal *Il17a*, *Il6*, *Il22,* and *Il23* expression in response to the ADSS challenge (*SI Appendix,* Fig. S1 *I* and *J*) (5). As expected with epithelial damage, expression of intestinal stem cell (ISC) (30) signature genes *Lgr5* and *Olfm4* was upregulated (*SI Appendix,* Fig. S1*J*) (25). The concurrent increase in the stem cell IL17 receptor *Il17rd* raised the possibility that ISC proliferation may be driven by IL17. In support of this notion, IL17 but not IL6, stimulated the growth of intestinal organoids as measured by ATP release and stem cell marker gene expression (*SI Appendix,* Fig. S1 *K* and *L*).

FXR Protects against ADSS-Induced Inflammation. To explore a causal association between reduced ileal FXR signaling and epithelial dysregulation, we utilized the intestinally restricted FXR agonist, FexD (19, 20, 25). Prophylactic treatment of WT mice with FexD (50 mg/kg/day p.o. for 4 wk prior to the ADSS challenge) was protective against DSS-induced damage, with improvements in intestinal morphology and body weight (Fig. 1 *A* and *B* and *SI Appendix,* Fig. S2*A*). Notably, ileal FXR signaling was largely maintained in FexD-treated mice (Fig. 1*C*). The DSS-induced increase in total serum BAs and the associated compositional changes were partially prevented by FexD treatment; a possible consequence of the severity of the ADSS model (Fig. 1*D* and *SI Appendix,* Fig. S2*B*). In addition, FexD treatment prevented DSS-induced increases in intestinal permeability and reduced the systemic inflammatory response as indicated by spleen size and serum IL17 and IL6 levels (Fig. 1*E* and *SI Appendix,* Fig. S2 *C–F*). The reduction in proinflammatory cytokines including *Il17* and IL17-induced genes in immune cells enriched from intestinal LP led to speculation that in addition to its established role in epithelial cells, FXR signaling directly affected immune cell populations (*SI Appendix,* Fig. S2*G*) (17, 26). Notably, in an adoptive T cell transfer model of colitis, FexD-treated mice displayed a less inflammatory phenotype including reduced weight loss and reduced expression of key inflammatory cytokines (*SI Appendix,* Fig. S2 *H–N*).

To support a role for FXR in immune cells, the FXR-induced changes in immune cell populations were determined. Prophylactic FexD treatment largely blocked the ADSS-induced increases in $IL17A^+$ and $IFN\gamma^+$ $CD4^+$ T cells in ileal LP, with minimal effects on splenic and mesenteric lymph node (MLN) cells (Fig. 1*F* and *SI Appendix,* Fig. S3 *A–E*). In addition, the disproportionate increase in ILCs (~10 to 18% of total immune cells in DSS-treated mice) was abrogated by FexD treatment (Fig. 1*G* and *SI Appendix,* Fig. S3*F*). In particular, DSS-induced increases in $CCR6^+$ and $CD4^+$ ILC3s and the ~2-fold increase in $IL17A^+$ and $IL17F^+$ ILC3s were prevented by FexD treatment (Fig. 1*G* and *SI Appendix,* Fig. S4 *A–E*). These findings indicate that activation of FXR was sufficient to restore T cell and ILC homeostatic levels in this intestinal inflammation model.

To support this notion, mice deficient in T cells and ILCs (NSG mice) were prophylactically treated with FexD prior to ADSS (Fig. 1*H*). The activation of FXR target genes in the intestinal epithelium led to reduced morphological changes; an effect attributed in part to a reduction in fibrotic gene expression (Figs. 1 *I–J* and *SI Appendix,* Fig. S5 *A–C*). In contrast, while the inflammatory response to ADSS was markedly reduced in NSG mice, FexD treatment failed to restore intestinal BA homeostasis and gut integrity and did not attenuate inflammatory damage (Fig. 1 *K–N* and *SI Appendix,* Fig. S5 *D–F*). These findings reveal that the beneficial histological effects of FXR activation in the epithelium are separable from the antiinflammatory response in this ADSS model.

FXR Affects Innate and Adaptive Immunity via ILCs. To explore the mechanism underlying how FXR modulates the function of immune cells in the ADSS model, we initially determined the functional consequences of FXR signaling in T cells. Naive splenic T cells were differentiated in vitro into Th1, Th2, Th17, and iTreg cells prior to exposure to the synthetic FXR agonist FexD or the natural antagonistic BA tauro-β-muricholic acid (T-βMCA) (*SI Appendix,* Fig. S6*A*). Somewhat unexpectedly, neither activation nor inhibition of FXR signaling affected signature functional markers in Th17, Th1, Th2, or iTregs (*SI Appendix,* Fig. S6 *B–D*). Treatment with additional synthetic FXR agonists and antagonists, as well as endogenous BA ligands, largely replicated these findings and was consistent with the lack of *Fxr* expression in these cell populations. As in vitro differentiated T cells may not fully recapitulate the activities of in vivo activated T cells, similar experiments were performed with total immune cell populations isolated from both the spleen and MLNs of ADSS-treated mice (*SI Appendix,* Fig. S6*E*). Immune cells were cultured ex vivo prior to treatment with either the FXR agonist FexD or the antagonist T-βMCA. Subsequent gene expression profiling established that these ex vivo activated cell populations were also largely refractory to FXR ligands (*SI Appendix,* Fig. S6*F*). These findings, in combination with essentially undetectable *Fxr* levels, excluded a direct effect of FexD in T cells in attenuating the ADSS-induced phenotype.

Next, the ex vivo responses of immune cells isolated from the small intestinal LP of ADSS-challenged mice were determined. $IL17A^+$ T cell numbers were not altered by ex vivo FexD treatment (*SI Appendix,* Fig. S6*G*), in agreement with the findings with splenic and MLN T cells. In contrast, a marked reduction in the proportion of ILC3s including the percentage of $IL17A^+$ ILC3s and decreased IL22 secretion were seen with ex vivo FexD treatment (*SI Appendix,* Fig. S6 *H–K*). This sensitivity of ILCs to FXR

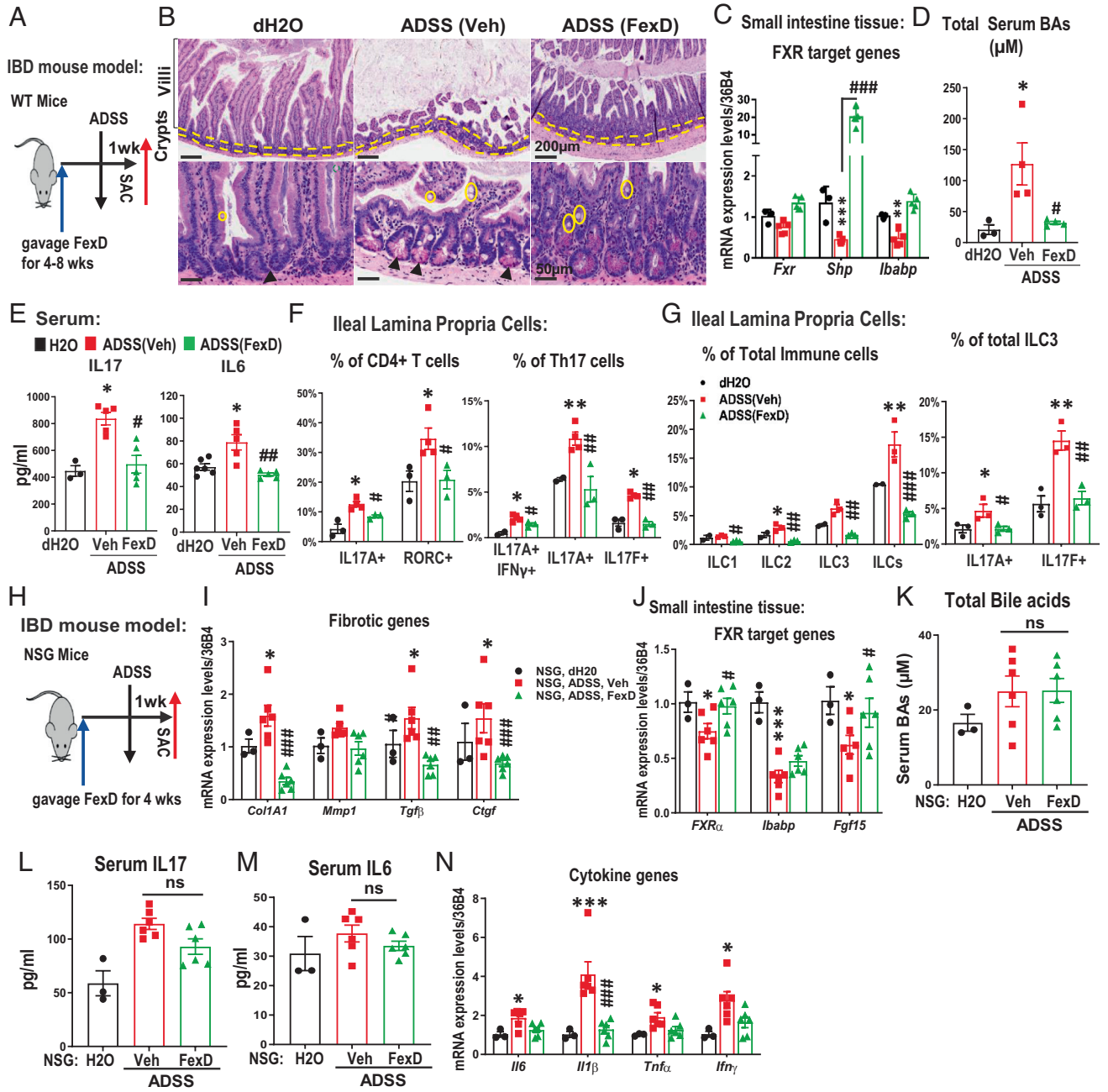

Fig. 1. FXR protects against ADSS-induced inflammation. (*A*) Schematic of ADSS (ADSS, 5% DSS in drinking water) regimen in WT mice treated with vehicle or FexD (50 mg/kg/day p.o.). H_2O, n = 3; DSS, n = 5; DSS + FexD, n = 5. (*B*) Representative H&E staining of small intestine. Yellow lines delineate villi and crypt structures, and goblet (yellow circles) and crypt Paneth (arrowheads) cells are indicated. (Scale bars, 200 μm (*Upper*) and 50 μm (*Lower*).) (*C*) Ileal expression of *Fxr* and FXR target genes (n = 5). (*D* and *E*) Total serum bile acid (BA) levels (n = 3 to 4) (*D*) and serum IL17 and IL6 levels (n = 5) levels in 18-wk-old ADSS mouse models (*E*). (*F*) Total (*Left*) and cytokine positive Th17 cells (*Right*) in small intestinal tissue (n = 3 to 5). Representative FACS analyses in *SI Appendix*, Fig. S3*E*. (*G*) Total ILC subtype (*Left*) and cytokine positive ILC3s (*Right*) in small intestinal tissue (n = 3 to 5). Representative FACS analyses in *SI Appendix*, Fig. S3*F*. (*H*) Experimental scheme (NSG mice treated with H_2O, n = 3; DSS, n = 6; and DSS + FexD, n = 6). (*I*) Expression of fibrotic genes in small intestine, measured by qRT-PCR. (*J*) Expression of FXR target genes in small intestine, measured by qRT-PCR. (*K–M*) Total bile acid (*K*) and serum IL17 (*L*) and IL6 (*M*) levels. (*N*) Expression of cytokine genes in small intestine, measured by qRT-PCR. WT mice under ADSS were independently replicated ten times and NSG experiments were independently replicated two times, and representative data in one experiment are shown as the mean ± SEM. * ADSS versus vehicle; # FexD versus ADSS; * and # $P < 0.05$; ** and ## $P < 0.01$; *** and ### $P < 0.005$; and ns—not significant. One-way ANOVA test followed by multiple comparisons were used for three group comparisons.

ligands was supported by gene expression profiling, where both FXR target genes and signature cytokine genes including *Il17a*, *Il22*, and *Ifnγ* were responsive to agonist and antagonist treatments (*SI Appendix*, Fig. S6 *L–M*).

FXR Modulates ILC Response to Inflammatory Insult. The disparity between in vivo and ex vivo effects of FexD on IL17A$^+$ T cells raised the possibility of ILC/T cell crosstalk (Fig. 1*F* compared to *SI Appendix*, Fig. S6*G*) (31–33). Indeed, proinflammatory cytokines such as IL6 are known to affect immune cells locally and systemically. To explore possible crosstalk, T and ILC populations from the LP of ADSS-treated mice were sorted prior to treatment with FXR ligands (Fig. 2*A*). As seen with in vitro differentiated T cells, *Fxr* expression and regulation of FXR target genes were not seen in the isolated T cell population (*SI Appendix*, Fig. S6*N*). In contrast, the sorted ILC population had measurable levels of *Fxr*

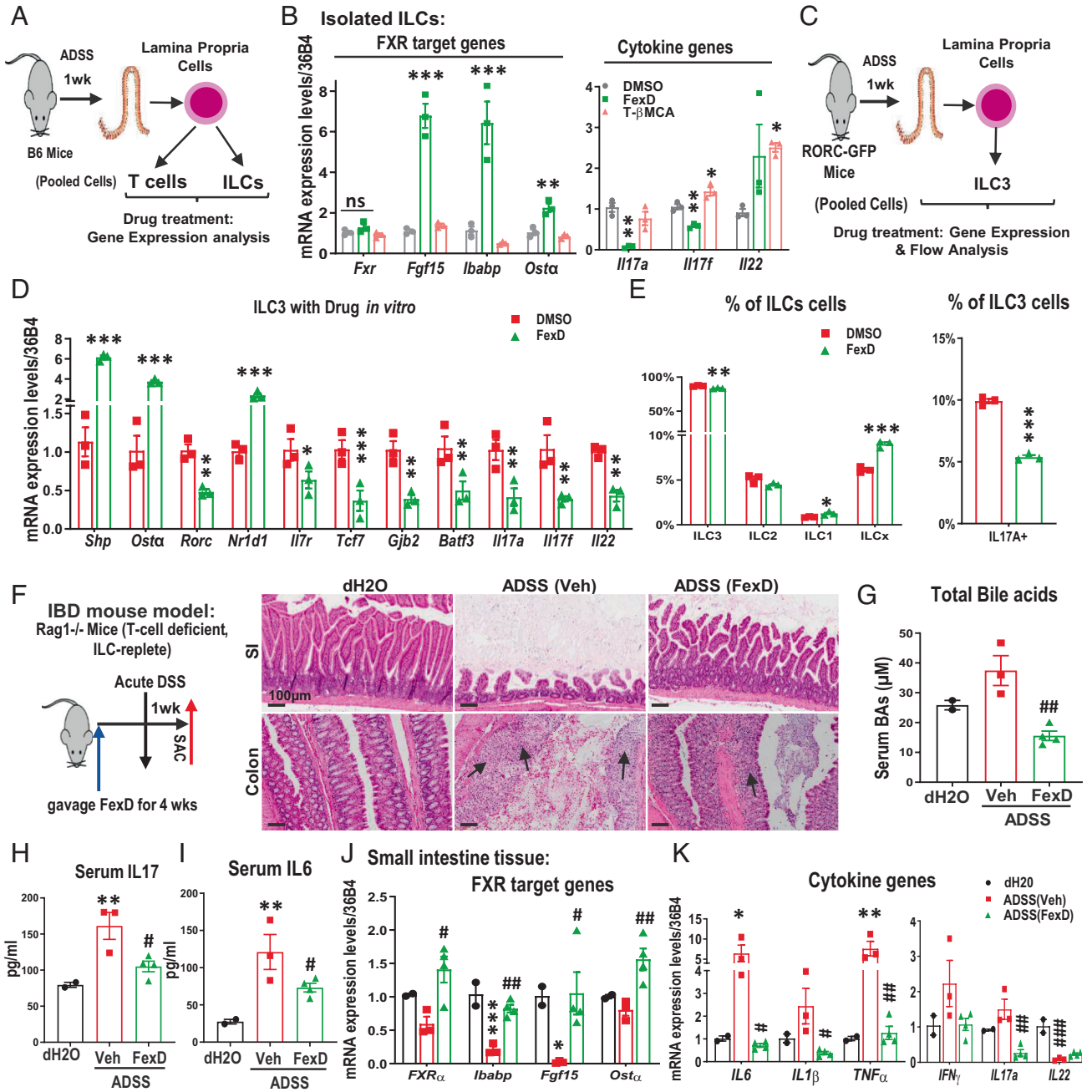

Fig. 2. FXR modulates ILC responses to inflammatory insults. (*A* and *B*) Experimental scheme (n = 12 pooled) (*A*), and FXR target and selected cytokine gene expression in isolated ILCs (*B*) (n = 3). (*C* and *D*) Experimental scheme (n = 12 pooled) (*C*), and gene expression in isolated ILC3s (*D*). (*E*) Quantification of ILCs and ILC3 percentages. (*F*) Experimental scheme and representative H&E staining of small intestine (SI) and colon. Arrows indicate neutrophil infiltration (Scale bar, 100 μm.) (*G–I*) Total serum bile acid (*G*), IL17 (*H*), and IL6 levels (*I*) (n = 3 and 4). (*J* and *K*) Expression of FXR target (*J*) and marker cytokine (*K*) genes in small intestinal tissue, measured by qRT-PCR. Experiments were independently replicated two times, with representative data shown as the mean ± SEM. * ADSS versus vehicle; # FexD versus ADSS; *,$^{\#}P$ < 0.05; **, $^{\#\#}P$ < 0.01; ***, $^{\#\#\#}P$ < 0.005; and ns—not significant. Student's unpaired *t* test was used for two group comparisons; one-way ANOVA test followed by multiple comparisons was used for three-way comparisons.

expression, and FXR target genes including *Fgf15* and *Ibabp* were induced upon FexD treatment (Fig. 2*B*). Notably, FexD treatment reduced *Il17a* expression only in the ILC population, suggesting that the beneficial effects of FXR activation are mediated by ILCs (Fig. 2*B*). These findings were recapitulated in ILC3s sorted from Rorc-EGFP mice, where ex vivo FexD treatment decreased the expression of identity markers (*Rorc*, *Tcf7*, *Gjb2*, and *Batf3*) and cytokine genes (*IL17a* and *IL17f*) (Fig. 2 *C* and *D* and *SI Appendix*, Fig. S7*A*), implicating FXR in the functional maturation of ILC3s. Moreover, changes in the ILC subtype composition were evident with FexD treatment, including reductions in 1L17A^{+} and CCR6^{+} ILC3 subpopulations, suggestive of a developmental role for FXR (Fig. 2*E* and *SI Appendix*, Fig. S7 *A–C*).

The above ex vivo findings implicated FXR signaling in ILCs in the protective effects of FexD treatment. To further support this notion, T cell-deficient mice (Rag1−/− mice) were prophylactically treated with FexD prior to ADSS. In the absence of T cells, FexD treatment was able to reduce intestinal damage, body weight loss, and serum BA and largely normalize both the intestinal expression and serum levels of key inflammatory cytokines (Fig. 2 *F–K* and *SI Appendix*, Fig. S7 *D* and *E*). Moreover, ILC compositional changes similar to those seen in WT mice were

found in the small intestine LP, most notably reduced CCR6$^+$ and CD4$^+$ ILC3s (*SI Appendix,* Fig. S7 *F–H*).

In agreement with a protective role of FXR, a more severe inflammatory phenotype was seen in whole-body FXR knockout (FXRKO) mice challenged with DSS, including increased serum IL17 levels (Fig. 3 *A–D* and *SI Appendix,* Fig. S8 *A–D*). The percentages of ILCs, and ILC3s in particular, were ~5–7 fold higher in FXRKO mice (Fig. 3*E* and *SI Appendix,* Fig. S8*E*). This increase in ILC3s was compounded by a ~4-fold increase in functionality, as determined by the proportion of IL17A$^+$ cells and the increase in expression of signature cytokines (Fig. 3 *E* and *F* and *SI Appendix,* Fig. S8*E*). In addition, the frequency of pathogenic IL17$^+$IFNγ$^+$ Th17 cells was doubled in FXRKO mice (*SI Appendix,* Fig. S8*D*). Similarly, an exaggerated ADSS phenotype was evident in WT mice transplanted with FXRKO bone marrow, including increased IL17A$^+$ ILC3s and serum IL17 levels (Fig. 3 *G–J* and *SI Appendix,* Fig. S8*F*). Furthermore, in an inducible FXRflox/Rorc-cre model where FXR is selectively reduced in Th17 and ILC3 cells, FexD treatment failed to block DSS-induced changes in cytokine and ILC signature gene expression in LP immune cells, the increases in ILC2s and ILC3s, or the increase in IL17A$^+$ILC3s (Fig. 3 *K–P* and *SI Appendix,* Fig. S8 *G–L*). Given the limited FXR expression in Th17 cells (*SI Appendix,* Fig. S6*N*), these findings support FXR signaling in ILCs in the regulation of intestinal inflammation. Importantly, the protective effects of FXR agonism were evident in an intervention model, where normalization of serum IL17 and IL6 levels was associated with a reduction in ILC3s in the LP in a chronic DSS model (*SI Appendix,* Fig. S9 *A–L*).

ILC3 Intrinsic FXR Regulates Th17 Function via Cellular Crosstalk. To provide insights into the cellular crosstalk underlying the in vivo changes in T cells, the ability of ILC-secreted factors to affect Th17 cell differentiation/function was determined. Conditioned media from ILCs, isolated from ADSS-challenged mice and treated ex vivo with FexD or T-βMCA, affected the in vitro differentiation of naive CD4$^+$ T cells, most notably the secretion of IL17 (*SI Appendix,* Fig. S10 *A–C*). Moreover, the correlation between the opposing effects of the FXR agonist and antagonist on the secretion of IL2 and IL10 from ILCs (34) with the effects of ILC conditioned media on Th17 cell functionality implicated these cytokines as potential mediators of in vivo cellular crosstalk (33) (*SI Appendix,* Fig. S10 *A–D*).

FXR Regulates ILC3 Development and Functional Maturation. The ILC compositional changes seen in FexD-treated IBD models implicated FXR as an intrinsic regulator of ILC development and functional maturation. To explore this effect, single-cell transcriptomic analyses (scRNA-seq) were performed on ILCs isolated from ADSS mice. The expression of signature genes revealed 9 discrete cell clusters, including 2 ILC2 and 2 ILC3 clusters (Fig. 4*A* and *SI Appendix*, Figs. S11–S13 and Table S1) (7, 16, 30, 35). In addition, a small proportion of precursor-like (preILC-like) cells was evident (Fig. 4*A*), consistent with the recruitment of circulating preILCs upon an inflammatory stimulus (6, 35–37). Treatment with FexD reduced the relative proportions of the ILC subsets including a 3-fold reduction in ILC3s and lowered the expression of *Il17f* and *Il22* in the ILC3 clusters (Fig. 4 *B* and *C* and *SI Appendix*, Figs. S14–S16 and Table S2). These population changes were accompanied by decreased expression of signature cytokines, transcription factors, and metabolic genes, as well as commitment markers (Fig. 4*D* and *SI Appendix*, Figs. S17*A* and S18 *A–D*). Moreover, a preILC-like population, expressing higher levels of the precursor marker *Tox,* was increased more than 10 fold in treated mice, consistent with a role for FXR in the functional maturation of ILCs (Fig. 4 *B* and *D* and *SI Appendix,* Figs. S17*D* and S18*D*) (6, 13, 36–39).

To support FXR as a determining factor in the functional maturation of intestinal ILCs, a differentiation trajectory was constructed from the single-cell transcriptional data using precursor (*Tox* and *Tox2*) and differentiated cell signature genes (e.g., *Tcf7*, *Gjb2*, *Batf3*, *Rorc,* and *Nr1d1* in ILC3s) (37, 39–42) (*SI Appendix,* Fig. S18 *E–I*). The resultant lineage trajectory confirmed the pre-ILC-like cluster as a precursor population with multilineage potential (6) (Fig. 4*E*). In addition, the projection of RNA velocities (calculated based on the ratio of unspliced to spliced mRNA) onto the tSNE map revealed altered developmental trajectories in FexD-treated cells (40) (*SI Appendix,* Fig. S17 *B* and *C*). Moreover, these *in silico* analyses were consistent with the changes in marker gene expression in LP immune cells and the increase in PLZF$^+$ and ID2$^+$ preILCs in FexD-treated mice (6, 9, 36, 43–45) (Fig. 4 *F* and *G* and *SI Appendix,* Fig. S17 *E* and *F*). Of note, FexD inhibited the in vitro differentiation of PLZF^{high} innate lymphoid common precursors (ILCPs) isolated from the bone marrow of PLZF^{GFPcre} mice (GFP$^+$CD45.2$^+$Lin$^-$CD127$^+$ cells[6]), phenocopying the in vivo effects (Fig. 4 *H–K* and *SI Appendix,* Fig. S17 *G–I*).

Expression of *Il17* in Th17 and ILC3 cells is transcriptionally regulated by the opposing actions of RORγτ (Rorc, transcriptional activator) and REVERBα (*Nr1d1,* transcriptional repressor) (46), (39, 41, 42). To provide insights into how FexD treatment suppresses *Il17* expression, we initially utilized an *Il17* luciferase reporter (2.0 kb core promoter containing a known RORgt binding site (47)) to establish a cell-intrinsic effect of FXR (Fig. 4*L*). Of note, the REVERBα agonist SR9009 used as a positive control in these studies has been reported to have effects independent of REVERBα Given the induction of *Nr1d1* by FexD in ADSS mice and ex vivo treated ILC3s (Figs. 2*D* and 4*G*), and the presence of FXR binding sites in both the gene body and regulatory regions of *Nr1d1* in mouse liver and intestinal tissues (48) (*SI Appendix,* Fig. S17*J*), we posited that REVERBα is a downstream effector of FXR in ILCs. In support of this notion, FXR induced dose- and ligand-dependent activation of a luciferase reporter under the control of the REVERBα promoter in HEK293T cells cotransfected with human or mouse FXR, while having no effect in comparable experiments with the RORc promoter (Fig. 4*M* and *SI Appendix,* Fig. S17 *K–M*).

In combination, the above findings support a key role for FXR signaling in the differentiation and functional maturation of intestinal ILCs in response to an inflammatory challenge and, combined with the secondary effects on T cell responses, point to the potential of FXR as a therapeutic target in intestinal inflammatory diseases such as IBD (6, 31, 36, 49, 50).

Discussion

As early dietary sensors and genetic effectors, BA have emerged as pleiotropic signaling molecules important in gut epithelial regeneration and mucosal immunity (25–27, 51). Notably, bile acid levels are increased in IBD, in concert with decreased FXR signaling in the ileum of Crohn's disease patients (28). Reciprocally, FXR agonists reduce intestinal inflammation and epithelial permeability (20, 25, 52). Here, we show in an aggressive DSS-induced inflammation model that attenuation of the innate immune cell response contributes to the protective effects of FXR activation.

Tissue-specific ILC subtypes develop from circulating precursor cells in response to local environmental cues (10, 11, 50, 53). In the gut, ILCs function as early effectors, secreting cytokines that activate and regulate both the innate and adaptive immune

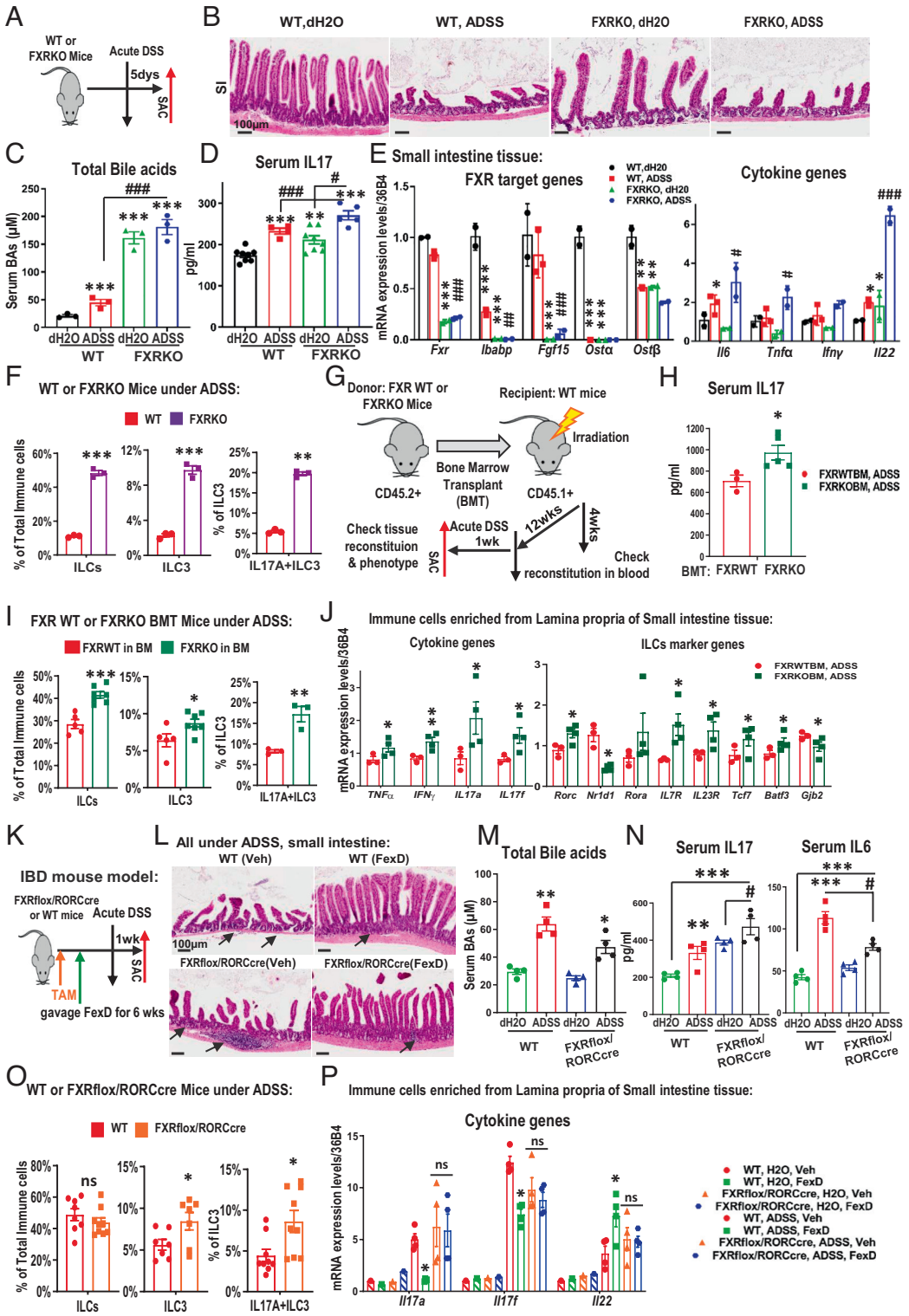

Fig. 3. FXR attenuates intestinal ILC3 responses to inflammatory insults. (*A*) Experimental scheme of WT and whole-body FXRKO mice subjected to ADSS administration, for panels *B–F*. (*B*) Representative H&E staining of small intestine (Scale bar, 100 μm.) (*C* and *D*) Serum bile acid (*C*, n = 3 per arm) and IL17 levels (*D*, n = 8 H_2O-treated WT and FXRKO; n = 4 and 5 DSS-treated WT and FXRKO mice). (*E*) Expression of FXR target and cytokine genes in small intestine tissue, measured by qRT-PCR (n = 3 to 5). (*F*) ILC and ILC3 (percentages of total immune cells) and $IL17A^+$ ILC3 (percentage of ILC3s) cell numbers in ADSS-treated WT and FXRKO mice (n = 3 per arm). (*G*) Scheme of bone marrow transplants (BMT) from WT and FXRKO donor mice prior to ADSS challenge, for panels *I–K*. (*H*) Serum IL17 levels (n = 3 FXRWT, n = 5 FXRKO). (*I*) ILC and ILC3 (percentages of total immune cells) and $IL17A^+$ ILC3 (percentage of ILC3s) cell numbers in mice receiving WT and FXRKO BMTs (n = 5 and 7, respectively). (*J*) Expression of cytokine and ILC lineage commitment marker genes in small intestine tissue, measured by qRT-PCR. (*K*) Experimental scheme of WT and FXR conditional knockout (RORC+ cells) mice challenged by ADSS, for panels *M–Q*. (*L*) Representative H&E staining of small intestine. Arrows indicate neutrophil infiltration (Scale bar, 100 μm.) (*M* and *N*) Serum bile acid (*M*, n = 4 per arm), and IL17 and IL6 levels (*N*, n = 4 per arm). (*O*) Total ILC, ILC3, and $IL17A^+$ILC3 cell numbers in small intestine lamia propria (n = 8 per arm). (*P*) Expression of cytokine genes in immune cells isolated from the LP of the small intestine, measured by quantitative RT-PCR (n = 4 per arm). Experiments were independently replicated 2 to 6 times, with representative data shown as the mean ± SEM. * ADSS versus vehicle; # FexD versus vehicle; *, # $P < 0.05$; **, ## $P < 0.01$; and ***, ### $P < 0.005$. Student's unpaired *t* test was used for two-way comparisons; one-way ANOVA test followed by multiple comparisons was used to compare multiple groups.

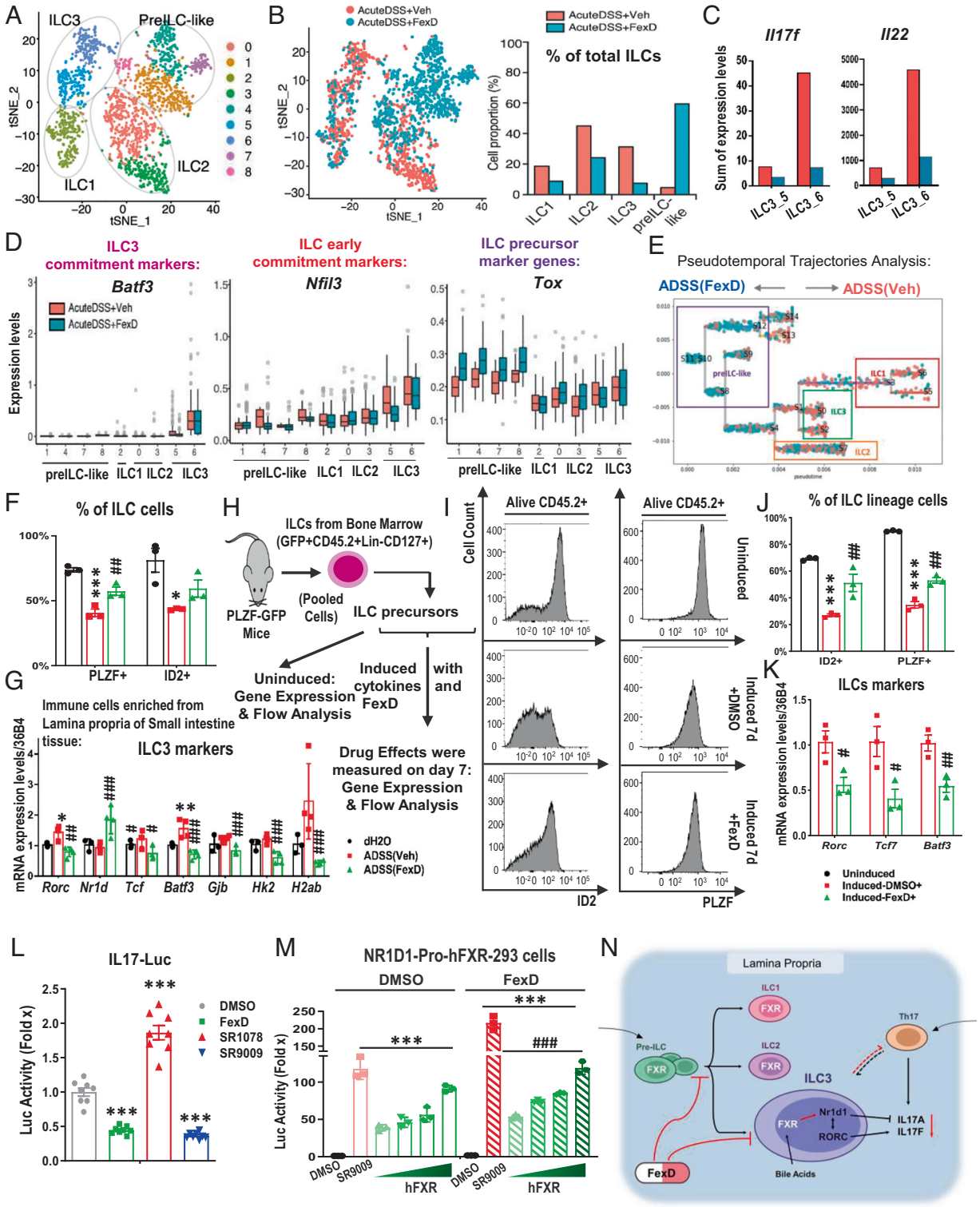

Fig. 4. FXR regulates ILC3 development and functional maturation. (*A* and *B*) tSNE plots of ILC clusters (*A*) and FexD-induced changes (*B*) identified from single-cell RNA sequencing (n = 4 pooled). (*C*) ILC subtype composition (*Left*) and cumulative *Il17f* and *Il22* expression in ILC3 clusters (*Right*). (*D*) Box plots of ILC commitment markers *Batf3Nfil3*, and *Tox* expression. (*E*) Pseudotemporal trajectories of ILCs based on scRNA-seq data. (*F*) PLZF$^+$ and ID2$^+$ ILC progenitor cells in the LP of WT mice, treated as indicated in *G*. (*G*) Expression of ILC3 commitment markers in immune cells enriched from small intestine LP of indicated models (measured by RT-qPCR: n = 3, control; n = 4 ADSS ± FexD). (*H*) Experimental scheme for in vitro differentiation of ILCs, for panels *I*–*K*. (*I*) Histograms of ID2$^+$ and PLZF^{high} cell numbers before and 7 d after differentiation with and without FexD treatment. (*J*) ID2$^+$ and PLZF$^+$ cell numbers, expressed as percentage of ILC lineage cells, measured by flow cytometry. (*K*) Expression of signature ILC marker genes measured by RT-qPCR (n = 3, per arm). (*L*) Effects of FXR agonist (FexD), RORγτ agonist (SR1078), and REVERBα agonist (SR9009) on *Il17* expression, as measured by luciferase reporter activity in HEK293T cells (2.0 kb core promoter). (*M*) Effects of increasing levels of human FXR on *Nr1d1* expression with and without FexD treatment, as measured by luciferase reporter activity in HEK293T cells, with DMSO as negative control and Nr1d1 agonist (SR9009) as positive control. (*N*) Schematic model depicting the beneficial effects of FXR activation in intestinal immune cells. Experiments were independently replicated three times, with representative data shown as the mean ± SEM; * and #, $P < 0.05$; ** and ##, $P < 0.01$; and *** and ###, $P < 0.005$. Student's unpaired *t* test was used for two-way comparisons; one-way ANOVA test followed by multiple comparisons was used to compare multiple groups.

responses (32, 53). In particular, ILC3s respond to extracellular bacteria and maintain tolerance to intestinal commensals (4, 49, 54). While an increase in T_{eff} cells is a hallmark of IBD (17), we show that FexD reduces inflammatory cytokines by directly affecting the differentiation and functional maturation of ILCs (36, 37). Indeed, FexD fails to affect naive T cells, in agreement with the negligible levels of FXR expression. In contrast, our single-cell analyses of ILCs from the inflamed gut indicated that activation of FXR not only sharply reduced total ILC numbers but also decreased the signature transcriptional factors (including *Tcf7*, *Batf3*, *Rorα,* and *Gata3*) and functional cytokines (most notably, IL17F and IL22, Fig. 4 and *SI Appendix,* Fig. S18), particularly in ILC3 and ILC2 (30). Strikingly, FexD increased the population of preILC-like cells (Fig. 4*B*). In accordance, FexD coordinately upregulated the expression of key transcriptional factors associated with early ILC progenitors (ILCP; including *Id2*, *Tox, and Tox2*) while downregulating ILC early commitment transcriptional factors (*Nfil3* and *IL2ra*) (Fig. 4 and *SI Appendix*, Figs. S17 and S18) (6, 36, 37, 50). Our findings suggest that FXR signaling regulates the commitment of ILC precursors into functional ILCs and implicates the transcriptional repressor REVERBα as a downstream effector of FXR (Fig. 4*N* and *SI Appendix,* Fig. S17). While the functional impact of an increased precursor population is currently unknown, the influx of preILC-like cells is expected to significantly decrease in the absence of an inflammatory insult. Recently, the clinical effects of FXR agonists such as obeticholic acid (55) have been explored extensively in liver steatosis and cirrhosis (29, 56). By extension, pharmacologic FXR activation may offer a new integrative strategy offering synergistic effects in both intestinal epithelial cells and innate immune cells.

Data, Materials, and Software Availability. The accession number for the single-cell RNA-seq data reported in this paper is NCBI SRA: PRJNA557472.

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