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Activation of bile salt nuclear receptor FXR is repressed by pro-inflammatory cytokines activating NF- κ B signalling in the intestine.

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ABSTRACT.

Hyperactivation of NF- κ B is a key factor in the pathophysiology of inflammatory bowel disease (IBD). We previously showed that the bile salt nuclear Farnesoid X Receptor (FXR) counter-regulates intestinal inflammation, possibly via repression of NF- κ B. Here, we examine whether mutual antagonism between NF- κ B and FXR exists.

FXR and its target genes IBABP and FGF15/19 expression were determined in HT29 colon carcinoma cells and *ex vivo* in intestinal specimens of wild type (WT) and *Fxr-ko* mice, treated with/without FXR ligands (GW4064/INT-747) and inflammatory stimuli (TNF α /IL-1 β). In addition, FXR activation was studied *in vivo* in WT and *Fxr-ko* mice with DSS-colitis. The involvement of NF- κ B in decreasing FXR activity was investigated by reporter assays and Glutathione S-transferase pulldown assays.

FXR target gene expression was highly reduced by inflammatory stimuli in all model systems, while FXR mRNA expression was unaffected. In line with these results, reporter assays showed reduced FXR transcriptional activity upon TNF α /IL-1 β stimulation. We show that this reduction in FXR activity is probably mediated by NF- κ B, since overexpression of NF- κ B subunits p50 and/or p65 also lead to inhibition of FXR activity. Finally, we report that p65 and p50 physically interact with FXR *in vitro*.

Conclusions: Together, these results indicate that intestinal inflammation strongly reduces FXR activation, probably via NF- κ B-dependent tethering of FXR. Therefore, FXR not only inhibits inflammation, but also is targeted by the inflammatory response itself. This could result in a vicious cycle where reduced FXR activity results in less repression of inflammation, contributing to development of chronic intestinal inflammation.

1. INTRODUCTION.

Inflammatory bowel disease (IBD), including Crohn disease (CD) and ulcerative colitis (UC), is characterized by chronic intestinal inflammation, with potentially severe complications and even mortality.[1] Although the exact aetiology is unclear, it is thought to result from a combination of dysregulation of the mucosal immune system, hyper-reactive against the intestinal microbiota, and compromised intestinal epithelial barrier function in genetically predisposed individuals.[2] In IBD patients, the nuclear transcription Factor kappa B (NF- κ B) was identified as a key factor in the pro-inflammatory response, resulting in strongly enhanced expression of pro-inflammatory genes and recruitment of excess inflammatory cells to the intestinal wall.[3] The NF- κ B complex is a heterodimeric protein predominantly consisting of p65 and p50 sub-units.[4] In the absence of activating signals, the inhibitor protein I κ B α retains NF- κ B in the cytosol. Presence of pro-inflammatory cytokines (e.g. TNF α , IL-1 β), reactive oxygen species or viral products, initiates phosphorylation and subsequent degradation of I κ B α , allowing NF- κ B to translocate to the nucleus and directly regulate the expression of specific target genes.[5]

The bile salt nuclear Farnesoid X Receptor (FXR) is a member of the superfamily of nuclear receptors. Nuclear receptors are ligand-activated transcription factors that, in response to lipophilic ligands (e.g. hormones, vitamins and dietary lipids), regulate many aspects of mammalian physiology, including development, reproduction and metabolism.[6,7] FXR is mainly expressed in the ileum and liver. Once activated by bile salts, it regulates transcription of genes involved in bile salt synthesis, transport and metabolism.[8] FXR binds as a heterodimer with Retinoid X Receptor (RXR) to the FXR responsive elements on the promoters of target genes, such as the small heterodimer partner (SHP), intestinal bile acid binding protein (IBABP) and fibroblast growth factor 15/19 (FGF15/19 in mouse and human, respectively), involved in bile salt homeostasis. We have recently found that pharmacological

FXR activation decreases the severity of inflammation and preserves the intestinal barrier integrity in two well-established murine colitis models.[9] In recent years, reciprocal repression between several nuclear receptors (NR) and inflammatory pathways has been described. Activation of several NRs (e.g. Glucocorticoid Receptor, Androgen Receptor and Estrogen Receptor) inhibits inflammation, whereas activation of these nuclear receptors is highly reduced in inflamed tissues, suggesting that repression of nuclear receptors might be a mechanism required for inflammation to progress.[10] Also, FXR-mediated gene expression was shown to be suppressed during hepatic inflammation[11] and FXR mRNA expression was shown to be reduced in inflamed colonic mucosa in a small cohort of Crohn's patients.[12]

In the current work, we provide *in vitro*, *ex vivo* and *in vivo* evidence that the inflammatory response reciprocally inhibits activation of FXR and its target genes in the intestine. These complementary findings may have important implications for intestinal inflammation and regulation of bile salt homeostasis in patients with inflammatory bowel disease.

2. METHODS.

2.1 Cell culture

Human embryonic kidney cells HEK293T and human colon carcinoma HT29 (passage number 10-20) cells were grown at 37°C with 5% CO₂ in Dulbecco Modified Eagle's Medium (DMEM) GlutaMax™ (4.5 g/L D-Glucose and Pyruvate; Gibco BRL, Breda, the Netherlands), supplemented with 10% heat-inactivated fetal calf serum and 1% penicillin/streptomycin (Gibco BRL). HT29 cells were maintained in culture for 7 days in order to allow differentiation into mature enterocyte-like cells. Both HT29 and HEK293 cells were treated with DMSO, the synthetic FXR ligand GW4064 (1μM for 24 hours), Tumor Necrosis Factor α (TNFα; 500U/mL, for 1, 6 or 24 hours, as indicated) or Interleukin 1β (IL-1β; 20ng/mL for 24 hours). GW4064 is a synthetic FXR agonist which binds to FXR with an EC 50 of 15 nM.[13]

2.2 Reporter assays

HEK293T cells were grown in 96-multiwell plates and co-transfected with empty pGL3, pGL3-IBABP or pGL3-SHP reporters, tK-Renilla and either empty pcDNA or pcDNA-hFXRα2 together with pcDNA-RXRα using the standard calcium phosphate method, as described elsewhere.[14] After 24 hours, cells were incubated with vehicle (DMSO) or 1 μM GW4064 for 24 hours, in presence or absence of either TNFα (500U/ml) or IL-1β (20ng/mL). Alternatively, cells were co-transfected with NF-κB sub-units pcDNA-p50 and/or pcDNA-p65 (kind gift by Dr. E. Kalkhoven, Utrecht, The Netherlands). Cells were lysed and Firefly and Renilla luciferase activity were measured according to manufacturer's instructions (Promega Dual-Luciferase Reporter Assay System, Promega, Madison, Wisconsin, USA) with the Centro LB 960 luminometer (Berthold Technologies, Vilvoorde, Belgium).

2.3 Glutathione S-Transferase (GST) Pull-down assay

GST-pull down experiments were performed as described elsewhere.[15] Briefly, p50 and p65 or FXR were transcribed and translated *in vitro* in the presence of [³⁵S] methionine and incubated with GST and either GST-hFXR α 2 or GST-p65 fusion proteins, respectively. Samples were subsequently washed and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. Coomassie brilliant blue was used to visualize GST proteins. [³⁵S]-labelled proteins were detected by autoradiography and analyzed with Storm 820 apparatus (Molecular dynamics, Pharmacia Biotechnology, Amersham Biosciences, Diegem, Belgium).

2.4 Animals

Nine-to-twelve weeks old male wild type (WT) C57BL/6J mice were obtained from Charles River Laboratories. Pure strain male C57BL/6J *Fxr-ko* mice (Dr. D.J. Mangelsdorf, Southwestern Medical Center, Dallas, TX) were kindly provided by Dr. Frank Gonzalez (NIH, Bethesda, MD). Mice were fed *ad libitum* and housed in a temperature- and light-controlled room. All experiments were approved by the Ethical Committee of the Consorzio Mario Negri Sud and by the Italian Ministry of Health.

2.5 Ex-vivo culturing of murine ileal specimens

Ileal samples of C57Bl/6 wild type (WT; n=4) and *Fxr-ko* (n=4) mice were collected, washed with PBS, divided into 6 pieces of approximately equal size and kept in culture for 24 hours at 37°C with 5% CO₂ in Dulbecco Modified Eagle's Medium (DMEM) GlutaMax™ (4.5 g/L D-Glucose and Pyruvate; Gibco BRL, Breda, the Netherlands), supplemented with 10% heat-inactivated fetal calf serum and 1% penicillin/streptomycin (Gibco BRL). The specimens were treated with DMSO, GW4064 (1 μ M) and/or either TNF α (500U/mL) or IL-1 β (20ng/mL). After 24 hours mRNA was isolated (see below).

2.6 FXR activation and induction of murine colitis

Pharmacological activation of Fxr in mice was accomplished by daily oral gavage with 5mg/kg/day INT-747 (6-ethyl-chenodeoxycholic acid, Obeticholic acid, Intercept Pharmaceuticals Inc, New York, NY)[16] or vehicle (1% wt/vol methyl-cellulose) from 3 days prior to induction of colitis, and continued until the end of the experiments. INT 747 is a semi-synthetic FXR ligand activating the receptor with an EC 50 of approximately 100 nM.[16] Colitis was induced by administration of 2.5% (wt/vol) Dextran Sodium Sulphate (DSS; MW 36-50 kDa, MP Biochemicals Inc., The Netherlands) in drinking water for 10 days (n=8-10 mice per group).[17]

2.7 mRNA extraction and qRT-PCR analysis

Ileal specimens were homogenized (Omni TH tissue homogenizer, Omni International, Kennesaw, USA) and RNA was isolated using RNeasy Micro kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The quantity, quality and integrity of isolated mRNA were confirmed by absorption measurement and RNA gel electrophoresis. Subsequently cDNA was generated from 500 ng of total RNA using SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and random hexamers (Roche, Basel, Switzerland). qRT-PCR analysis was carried out using SYBR green PCR master mix (Biorad, Veenendaal, The Netherlands) and a MyIQ real time PCR cycler (Biorad). Values were quantified using the comparative threshold cycle method and FXR and target gene mRNA expression was normalized to GAPDH. Primers are listed in supplementary Table 1.

2.8 Statistical analysis

Results are expressed as means \pm SEM or \pm SD as indicated in the figure legends. ANOVA or Student t test was performed as appropriate with GraphPad Software (GraphPad Software,

Inc., Avenida de la Playa, La Jolla, USA), as appropriate. Two-sided p-values <0.05 are considered statistically significant and are expressed as * $p<0.05$; ** $p<0.01$ and *** $p<0.001$.

ACCEPTED MANUSCRIPT

3. RESULTS

3.1 TNF α inhibits FXR target gene expression in differentiated HT29 cells

To study the effects of an inflammatory stimulus on FXR target gene expression, we treated undifferentiated and differentiated HT29 colon carcinoma cells with TNF α in presence or absence of the FXR synthetic ligand GW4064. In undifferentiated enterocytes, none of the treatments exerted any effects on FXR, IBABP or SHP expression. FXR is expressed and functional exclusively in fully differentiated HT29 cells (Figure 1A), in line with its *in vivo* intestinal expression, which is limited to the villi of the enterocyte.[18] There was no significant difference in FXR mRNA expression between the various experimental conditions in differentiated cells (Figure 1A). GW4064 markedly induced the FXR target genes *IBABP* and *FGF19*, only in differentiated cells (Figure 1B and C). Co-treatment with TNF α dramatically reduced *IBABP* and *FGF19* expression in a time-dependent manner, indicating that in the presence of a pro-inflammatory stimulus, FXR activation is inhibited (Figure 1B and C). TNF α treatment alone did not affect *IBABP* and *FGF19* mRNA expression (Figure 1B and C).

3.2 The pro-inflammatory cytokines TNF α and IL-1 β inhibit FXR transcriptional activity

In order to investigate whether pro-inflammatory agents (TNF α and IL-1 β) decrease FXR target gene expression by inhibiting FXR transcriptional activity, HEK293 cells were transiently transfected with IBABP or SHP reporter constructs alone or in combination with FXR and RXR expression plasmids, and incubated with GW4064 and either TNF α or IL-1 β . FXR transcriptional activity of IBABP and SHP promoters was markedly increased by GW4064, while neither TNF α nor IL-1 β had any effect on basal luciferase expression. Strikingly, TNF α or IL-1 β in combination with GW4064 markedly decreased the IBABP- and

SHP-responsive luciferase expression compared to GW4064 alone (Figure 2A and B), suggesting that the inhibition of FXR target gene expression by these pro-inflammatory cytokines is due to a decrease in FXR transcriptional activity.

3.3 Overexpression of the NF- κ B subunits p50 and p65 inhibits FXR transcriptional activity

Since TNF α and IL-1 β activate NF- κ B, we next explored whether NF- κ B subunits (p50 and p65) directly affect FXR transcriptional activity. HEK293 cells were transfected with either pGL3-IBABP or pGL3-SHP promoter reporter constructs together with FXR and RXR in combination with either p50, p65 or both. The p50 subunit decreased GW4064-dependent IBABP and SHP promoter activity (by 42% and 85%, respectively) and p65 decreased GW4064-dependent IBABP and SHP promoter activities by 97% and 98%, respectively. Co-transfection of p50 and p65 completely abolished GW4064-dependent IBABP and SHP promoter activity (Figure 3A and B). These results suggest that the inhibition of FXR transcriptional activity is mediated by NF- κ B.

3.4 FXR interacts with the NF κ B subunits p50 and p65 in a GST-pull down assay

To investigate whether the decrease of FXR transcriptional activity is due to physical interaction between FXR and the NF- κ B subunits p50 and p65, a GST-pull down assay was performed. The NF- κ B subunits p65 and p50 bind to FXR, in a ligand independent fashion (Figure 3C). Also, GST-pull down assays performed with GST-p65 fusion proteins and [35S]-labeled FXR, revealed interaction between FXR and the NF- κ B subunit p65 (Figure 3D).

3.5 Fxr activation is inhibited by TNF α and IL-1 β in ex-vivo ileal samples of WT and Fxr-ko mice

To extrapolate our *in vitro* findings, we prepared ileal specimens from WT and *Fxr-ko* mice, and cultured them *ex vivo* in the presence or absence of GW4064 and either TNF α or IL-1 β . *Fxr* expression in the ileal specimens did not change under any of the described conditions (Figure 4A). TNF α and IL-1 β treatment alone did not affect *Fxr* target gene mRNA expression. GW4064 induced the *Fxr* target genes *Ibabp* and *Fgf15*, only in WT mice. TNF α or IL-1 β treatment in combination with GW4064 dramatically reduced *Ibabp* and *Fgf15* expression compared to GW4064 treatment alone (Figure 4B and C), indicating that *Fxr* activity is reduced.

3.6 *Fxr* activation is decreased in the ileum and colon of mice with DSS-induced colitis

To explore the inhibition of *Fxr* activity by inflammatory stimuli *in vivo*, we investigated whether DSS-induced inflammation inhibits *Fxr*-dependent gene expression in the ileal and colonic mucosa of WT and *Fxr-ko* mice. *Fxr* was expressed in WT mice, but not *Fxr-ko* mice. *Fxr* mRNA expression was not affected by DSS and/or INT747 (Figure 5A and C). Nevertheless, INT-747 activated *Shp* mRNA expression in WT, but not in *Fxr-ko* mice. Concomitant induction of colitis by DSS markedly reduced *Shp* mRNA expression induced by INT-747 in the ileum (Figure 5B). A similar trend was observed in the colon (Figure 5D).

4. DISCUSSION

We have recently found that FXR is an important player in the counter-regulation of intestinal inflammation. In particular, the potent semi-synthetic FXR agonist INT-747 improves clinical symptoms and histology in the DSS and TNBS murine models of colitis. These beneficial effects are only detected in WT mice, not in *Fxr-ko* mice. Also, Fxr activation inhibits the increase of epithelial permeability and pro-inflammatory cytokine mRNA expression in the murine intestinal mucosa, under these circumstances.[9] The multi-level protection against intestinal inflammation provides a clear rationale to further explore FXR agonists as a novel therapeutic strategy for IBD. Current treatment options for IBD are mainly aimed at suppressing the immune response. Although reasonably effective, significant side effects and treatment failures can occur, stressing the need for novel treatment options for IBD. Therefore, a therapeutic trial with currently available FXR agonists in patients with inflammatory bowel disease seems warranted, in analogy of currently performed trials with INT-747 in patients with the cholestatic liver disease primary biliary cirrhosis.[19]

In the present study, we show mutual crosstalk between FXR and proinflammatory stimuli, because not only does FXR inhibit inflammation, but also FXR activation is inhibited by pro-inflammatory stimuli in different model systems. Firstly, the pro-inflammatory cytokine TNF α decreases FXR target gene expression in enterocyte-like differentiated HT29 cells. This finding was confirmed in ileal specimens of WT and *Fxr-ko* mice cultured *ex vivo*, in which we also showed decreased Fxr target gene expression upon TNF α and IL-1 β stimulation. Moreover, in mice with severe intestinal inflammation induced by DSS, expression of Fxr target genes *Ibabp* and *Fgf15* was similarly reduced in ileum as well as colon. Although DSS administration leads primarily to colitis, effects on FXR target gene expression in the ileum of DSS-treated mice may be explained by increased levels of circulating pro-inflammatory cytokines originating from the colonic lesions. In line with the

data shown in this paper, we have preliminary data indicating that ileal expression of the FXR target gene SHP is markedly lower (50%) in patients with Crohn colitis in clinical remission (unpublished data). In Crohn's patients and the *in vitro* and *ex vivo* models presented in this paper FXR expression itself is not significantly changed by pro-inflammatory cytokines, indicating that the inhibition of FXR target gene expression is due to decreased FXR activity.

Based on these results, it can be anticipated that ablation of Fxr would lead to more severe intestinal inflammation. Indeed, we observed that Fxr-ko mice have severely impaired intestinal integrity compared to WT mice at baseline (Supplementary Figure 3 of [9]), suggesting that they are probably more susceptible to chronic inflammation. However, we did not find more severe colitis in Fxr-ko mice than in wild type mice treated with DSS. This may relate to the short duration of the DSS protocol, the young age of the mice and/or the micro-environmental conditions. It is also possible that the severe inflammation in the DSS model does not allow detecting subtle differences between Fxr-ko and wild type mice.

This study shows that FXR is not only an active player in inhibition of inflammation, but also is a target of the inflammatory response itself. This could result in a vicious cycle where reduced FXR activity results in less repression of intestinal inflammation, contributing to development of chronic intestinal inflammation. Together, these findings imply an important role for FXR in the protection against IBD. We also hypothesize that decreased FXR activity may lead to altered bile salt enterohepatic circulation and potentially relate to cholestatic liver disease, which often coexists in patients with inflammatory bowel disease.[20]

In recent years, a reciprocal regulation between several steroid NRs on the one hand and inflammatory pathways on the other hand has been described. Little is known about the exact mechanisms underlying these processes. For example, NF- κ B has been shown to suppress PXR regulated gene expression, by interfering with the binding of PXR to the PXR

responsive element.[21] In the current study, we also show direct involvement of NF- κ B in repression of FXR activity, since overexpression of NF- κ B subunits p50 and p65 inhibits FXR activity and has the ability to physically interact with FXR. Similarly to PXR, NF- κ B may inhibit FXR activation by preventing binding of FXR to its cognate FXR responsive element in the DNA. However, at this stage, we cannot rule out other potential mechanisms such as the competition between the nuclear receptor and the NF- κ B complex for a common transcriptional cofactor (“cofactor squelching”).

It has long been appreciated that inflammation affects bile homeostasis and that bile salts have immune suppressive actions. Although additional mechanisms may exist, our work provides insights in the mechanisms by which bile salts interact with the immune system via FXR.

5. CONCLUSION

In this paper we show that in addition to the role of FXR in counter-regulation of the intestinal inflammatory response,[9] there is reciprocal inhibition of the FXR pathway by the inflammatory response. We provide evidence for involvement of NF- κ B in this suppressive effect. The crosstalk between NF- κ B and FXR at the intestinal level indicates an interaction between lipid metabolism and inflammatory disease and leads to new insights in pathogenesis and potentially also to novel treatment strategies for inflammatory bowel disease.

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Abbreviations:

BDL, bile duct ligation; CD, Crohn disease; DSS, dextran sodium sulphate; EV, empty vector; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GST, glutathione S-transferase; IBABP, intestinal bile acid binding protein; IBD, inflammatory bowel disease; IL, interleukin; KO, knock-out; NF- κ B, nuclear factor kappa B; NR, nuclear receptor; RXR, retinoid X receptor; SHP, small heterodimer partner; TNBS, trinitrobenzene sulphonic acid; TNF α , tumor necrosis factor alpha; UC, ulcerative colitis; WT, wild type.

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Figure Legends

Figure 1

TNF α decreases Fxr target gene expression in colon carcinoma-derived HT29 cells.

HT29 cells were differentiated by culturing them 7 days post-confluency (black bars). Undifferentiated cells served as control (grey bars). mRNA expression of *FXR* (A), *IBABP* (B) and *FGF19* (C) in HT29 cells incubated with DMSO, the FXR agonist GW4064 (1 μ M, 24h), TNF α (500U/mL, 1h, 6h, 24h) or both. Each bar represents the mean value \pm SEM. *p<0.05, **p<0.01, ***p<0.001 compared to GW4064 treated cells.

Figure 2

The pro-inflammatory cytokines TNF α and IL-1 β inhibit FXR transcriptional activity.

Reporter assays of FXR transcriptional activity on *IBABP* (A) and *SHP* (B) promoter reporter constructs. Cells were transfected with either empty vector (EV; grey bars) or FXR-RXR (black bars) and treated with DMSO, GW4064 (1 μ M), TNF α (500U/mL) and IL-1 β (20ng/mL) for 24 hours, as indicated. Each bar represents the mean value \pm SD; ***p<0.001 compared to GW4064 treated cells.

Figure 3

Overexpression of the NF- κ B subunits p50 and p65 inhibit FXR transcriptional activity.

Reporter assays of FXR transcriptional activity on *IBABP* (A) and *SHP* (B) promoter reporter constructs transfected with either empty vector (EV; grey bars) or FXR-RXR (black bars) together with p50, p65 or both. Each bar represents the mean value \pm SD; ***p<0.001 compared to GW4064 treated cells. (C) GST-pull down experiment to analyze p50 and p65 binding to FXR. *In vitro* translated p50 and p65 were subjected to a pull-down experiment

with GST-FXR and incubated with either DMSO or GW4064, as indicated. The top panel is a radiograph of [³⁵S]-labelled p50 and p65; the lower panel is a Coomassie brilliant blue staining of GST proteins. (D) GST-pull down experiment to analyze FXR binding to p65. *In vitro* translated FXR was subjected to a pull-down experiment with GST-p65 and incubated with either DMSO or GW4064, as indicated. The top panel is a radiograph of [³⁵S]-labelled FXR; the lower panel is a Coomassie brilliant blue staining of GST proteins.

Figure 4

TNF α and IL-1 β decrease Fxr target gene expression in ex-vivo cultured mouse ileal mucosa.

Ileal specimens were prepared from WT and *Fxr-ko* mice, cultured *ex vivo* and treated for 24 hours with DMSO, GW4064, TNF α and IL-1 β , as indicated. mRNA expression of *Fxr* (A) and *Fxr* target genes *Ibabp* (B) and *Fgf15* (C) in the ileal mucosa of WT (black bars) and *Fxr-ko* mice (grey bars). Each bar represents the mean value \pm SEM. * $p < 0.05$ compared to GW4064-treated mice.

Figure 5

DSS-induced colitis decreases Fxr activation in WT mice.

DSS colitis was induced in WT and *Fxr-ko* mice pre-treated with or without INT-747. mRNA expression of *Fxr* and *Shp* in the ileal (A, B) and colonic mucosa (C, D) of WT (black bars) and *Fxr-ko* mice (grey bars). Each bar represents the mean value \pm SEM. ** $p < 0.01$, *** $p < 0.001$ compared to WT INT-747-treated mice.

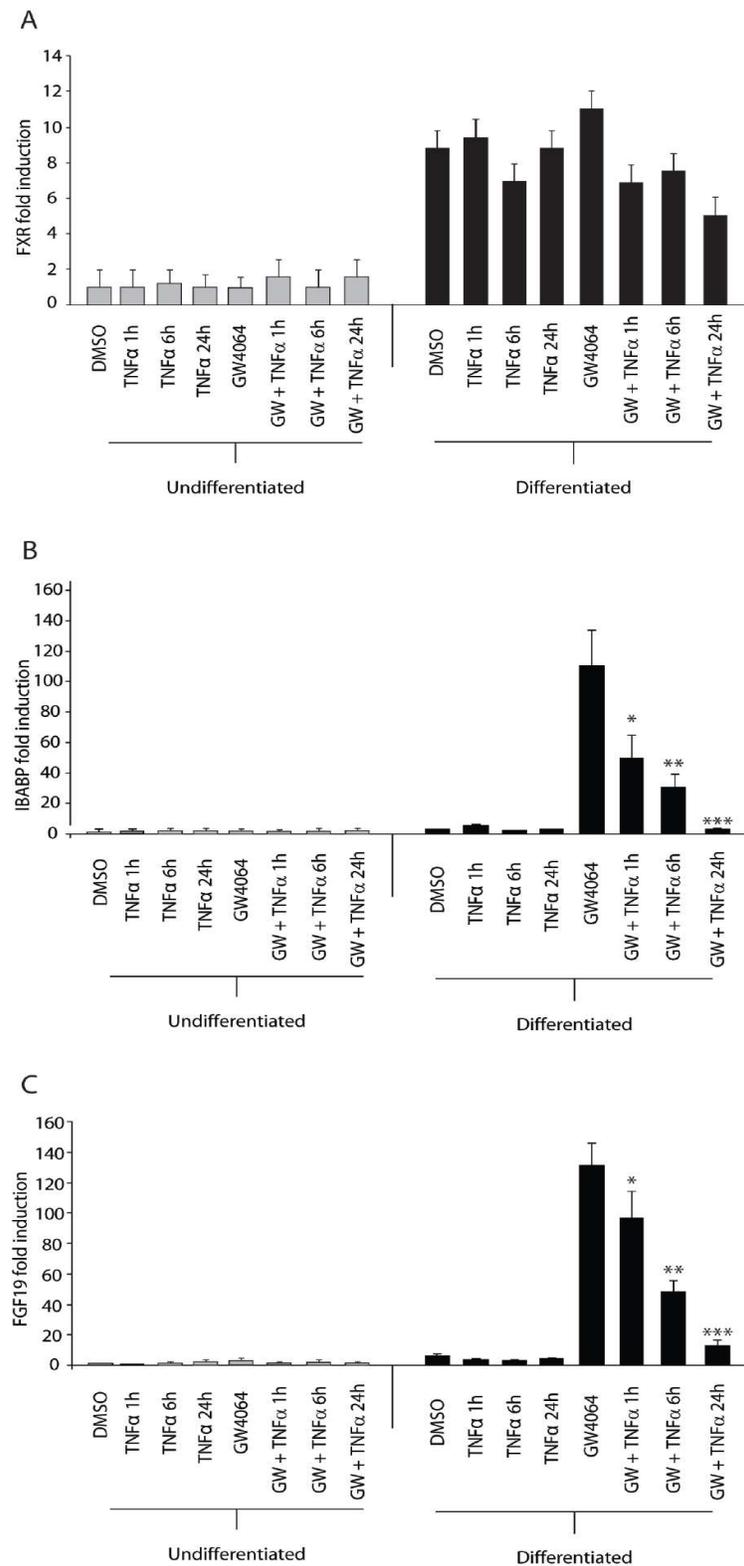


Fig. 1

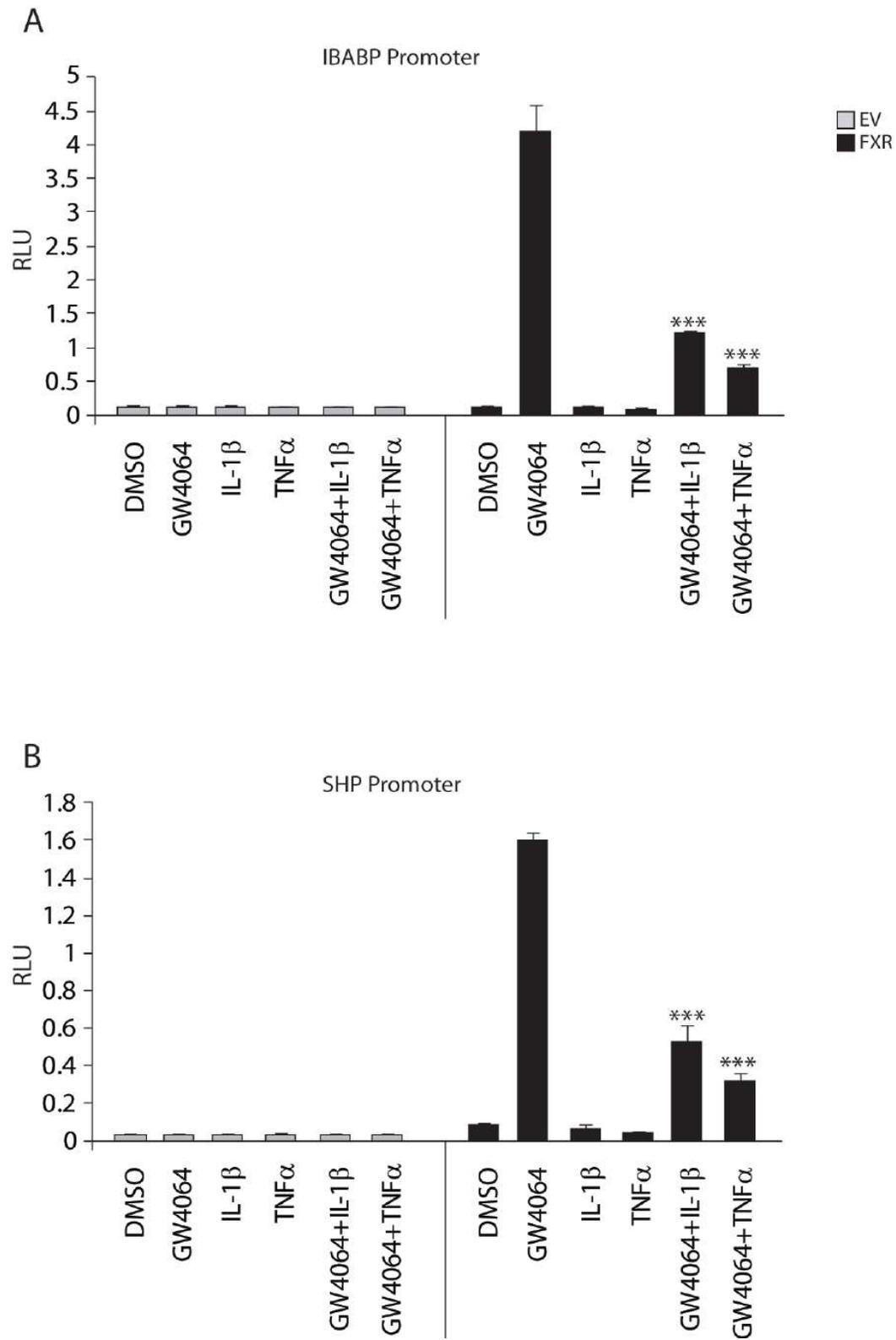


Fig. 2

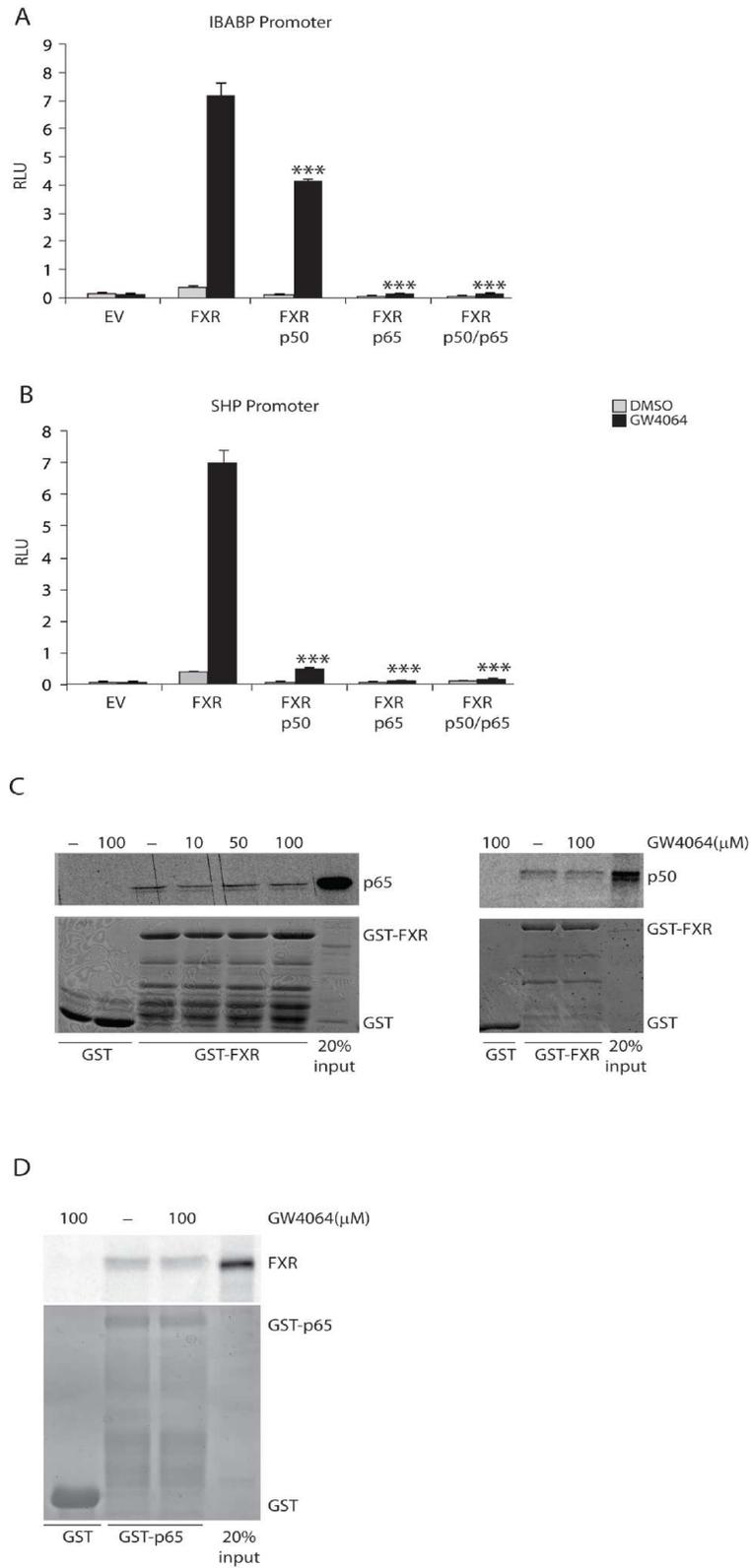


Fig. 3

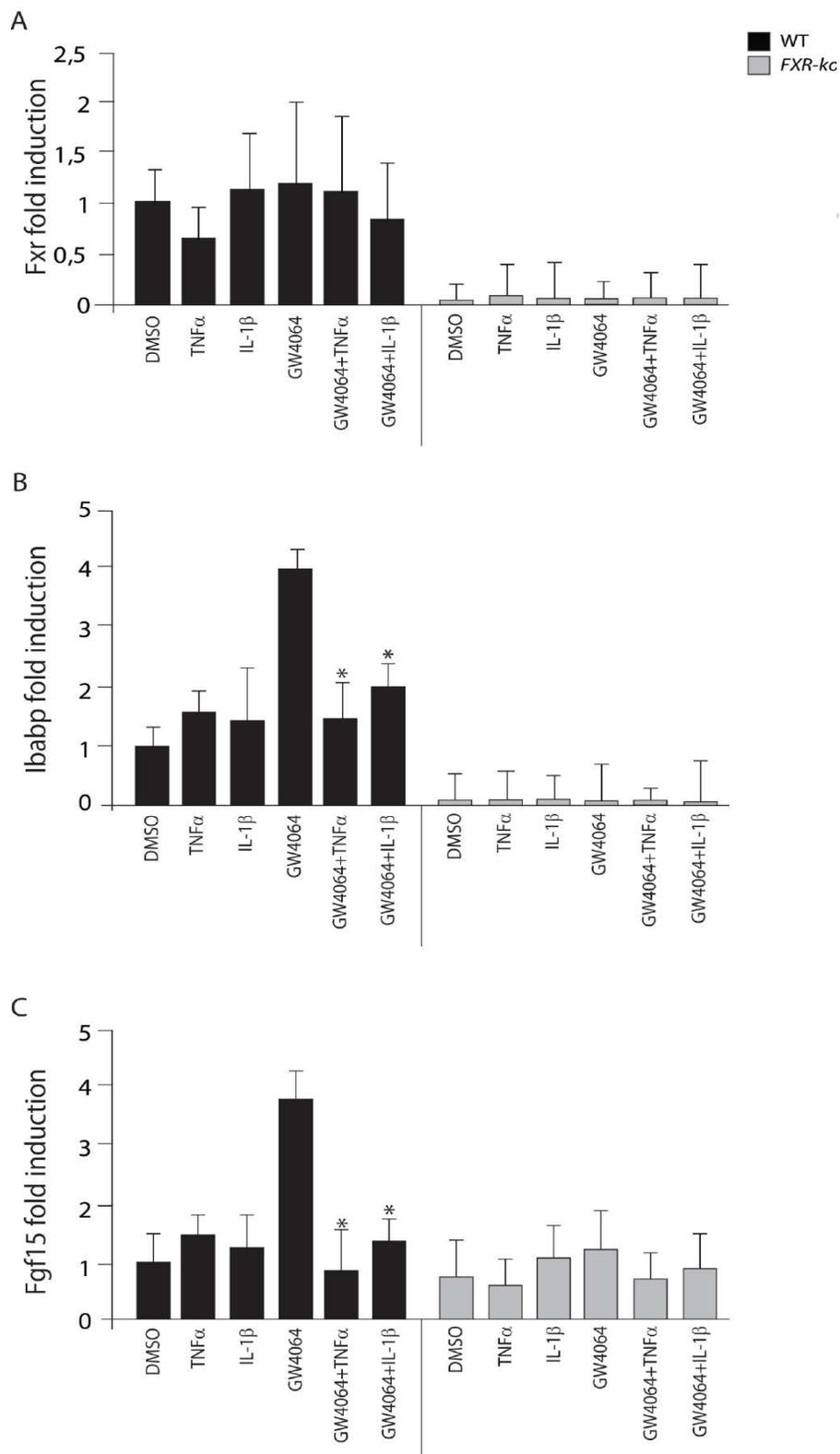


Fig. 4

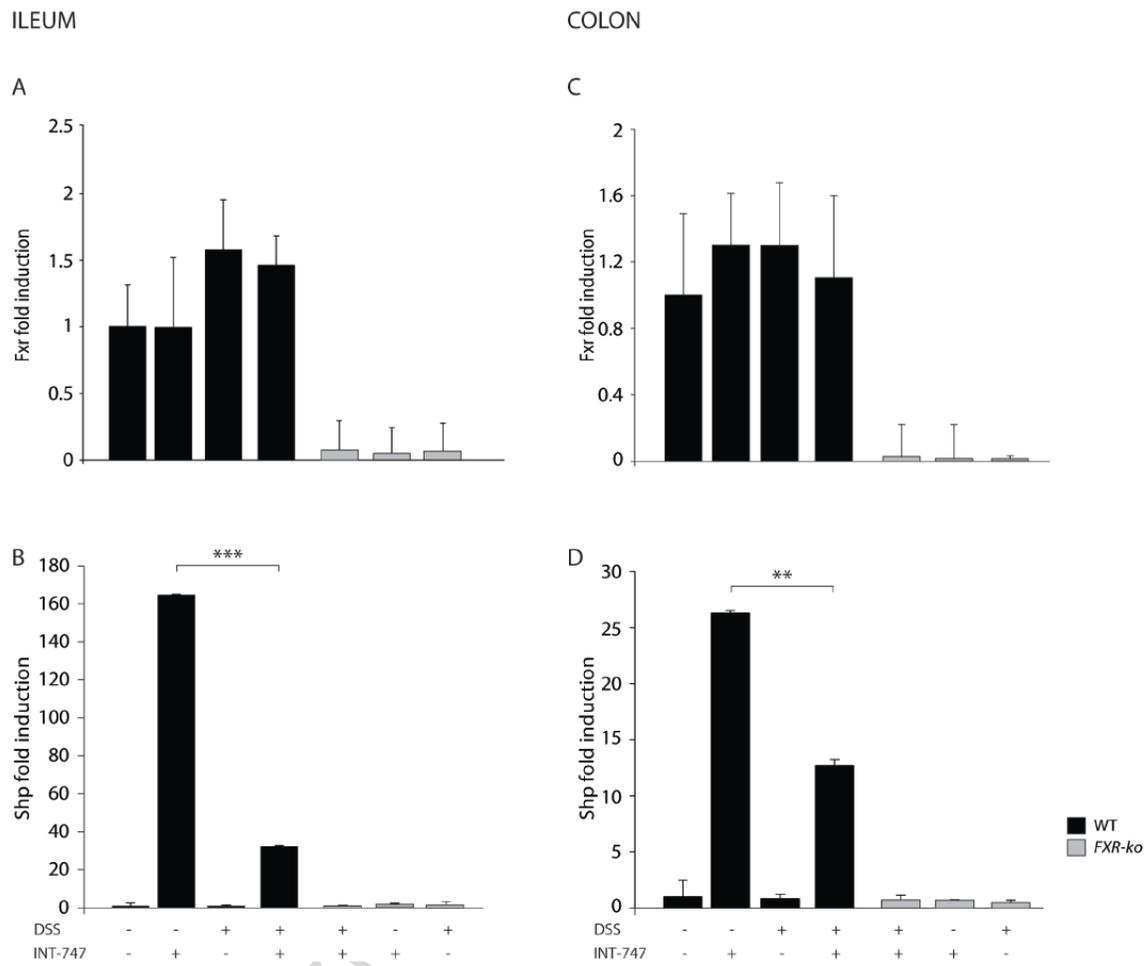


Fig. 5

Research Highlights

- TNF α decreases FXR target gene expression in differentiated HT29 intestinal cells
- TNF α and IL-1 β decrease ileal FXR target gene expression *ex vivo* in WT mice
- In the DSS model of murine colitis, FXR target gene expression is decreased
- TNF α , IL-1 β and overexpression of NF- κ B subunits P50/P65 decrease FXR transcriptional activity
- Intestinal inflammation inhibits FXR activation, probably via NF- κ B-dependent tethering of FXR

Supplementary Table 1**Human**

GAPDH F	TGTTGCCATCAATGACCCCTT
GAPDH R	CTCCACGACGTA CT CAGCG
FXR F	CTACCAGGATTT CAGACTTTGGAC
FXR R	GAACATAGCTTCAACCGCAGAC
SHP F	AGGGACCATCCTCTTCAACC
SHP R	TTCACACAGCACCCAGTGAG
IBABP F	TCAGAGATCGTGGGTGACAA
IBABP R	TCACGCGCTCATAGGTCA

Mouse

Gapdh F	GCAAAGTGGAGATTGTTGCCAT
Gapdh R	CCTTGACTGTGCCGTTGAATTT
Fxr F	TGAGAACCCACAGCATTTCG
Fxr R	GCGTGGTGATGGTTGAATGTC
Fgf15 F	AAAACGAACGAAATTTGTTGGAA
Fgf15 R	ACGTCCTTGATGGCAATCG
Shp F	CGATCCTCTTCAACCCAGATG
Shp R	AGGGCTCCAAGACTTCACACA