JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225

JPET #170225

Therapeutic Role of Rifaximin in Inflammatory Bowel Disease: Clinical Implication of Human Pregnane X Receptor Activation

Jie Cheng, Yatrik M. Shah, Xiaochao Ma, Xiaoyan Pang, Toshiya Tanaka, Tatsuhiko Kodama, Kristopher W. Krausz, Frank J. Gonzalez

Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 (J.C., K.W.K. and F.J.G.). Department of Molecular and Integrative Physiology, Department of Internal Medicine, Division of Gastroenterology, University of Michigan School of Medicine, 7712B Med Sci II, 1301 E Catherine St, Ann Arbor, MI 48109-0622 (Y.M.S.). Department of Pharmacology, Toxicology and Therapeutics, The University of Kansas Medical Center, 4089 KLSIC, MS 1018, 3901 Rainbow Boulevard, Kansas City, Kansas 66160 (X.M.). Laboratory for Systems Biology and Medicine, RCAST #35, The University of Tokyo, Tokyo Japan (T.T. and T.K.)

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Running title:

Rifaximin protects against IBD via human PXR

Correspondence:

Frank J. Gonzalez, Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, Building 37, Room 3106, Bethesda, MD 20892. email: fjgonz@helix.nih.gov; fax: (301) 496-8419.

Counts :

No. of text pages: 29

No. of tables: 0

No. of figures: 9

No. of references: 34

No. of words in Abstract: 236

No. of words in Introduction: 606

No. of words in Discussion: 788

Abbreviations: Rifax, rifaximin, 4-deoxy-4'-methylpyrido[1',2'-1,2]imidazo[5,4-*c*]rifamycin SV; RIF, rifampicin, 3-(4-methylpiperazinyliminomethyl) rifamycin SV; IBD, inflammatory bowel disease; DSS, dextran sulphate sodium; TNBS, trinitrobenzene sulfonic acid; PXR, pregnane X receptor; NF-κB, nuclear factor kappa B; hPXR mice, humanized PXR mice; WT, wild-type mice; DSS/RIF, co-administration of rifampicin and DSS; DSS/Rifax, co-administration of rifaximin and DSS; TNBS/Rifax, co-administration of rifaximin and TNBS.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT

Human pregnane X receptor (PXR) has been implicated in the pathogenesis of inflammatory bowel disease (IBD). Rifaximin, a human PXR activator, is in clinical trials for treatment of IBD and has demonstrated efficacy in Crohn's disease and active ulcerative colitis. In the current study, the protective and therapeutic role of rifaximin in IBD and its respective mechanism were investigated. PXR-humanized (hPXR), wild-type, and *Pxr*-null mice were treated with rifaximin in the dextran sulfate sodium (DSS)-induced and trinitrobenzene sulfonic acid (TNBS)-induced IBD models to determine the protective function of human PXR activation in IBD. The therapeutic role of rifaximin was further evaluated in DSS-treated hPXR and *Pxr*-null mice. Results demonstrated that pre-administration of rifaximin ameliorated the clinical hallmarks of colitis in DSS-treated and TNBS-treated hPXR mice as determined by body weight loss and assessment of diarrhea, rectal bleeding, colon length, and histology. Additionally, higher survival rates and recovery from colitis symptoms were observed in hPXR mice, and not in *Pxr*-null mice when rifaximin was administered after the onset of symptoms. NF-κB target genes were markedly down-regulated in hPXR mice by rifaximin treatment. *In vitro* NF-κB reporter assays demonstrated inhibition of NF-κB activity following rifaximin treatment in colon-derived cell lines expressing hPXR. These findings demonstrated the preventive and therapeutic role of rifaximin on IBD through human PXR-mediated inhibition of the NF-κB signaling cascade, thus suggesting that human PXR may be an effective target for the treatment of IBD.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

Rifaximin (Xifaxan), a non-systemic rifamycin-derived antibiotic that exhibits low gastrointestinal (GI) absorption while retaining potent antibacterial activity (Koo and DuPont, 2010), was approved in 2004 for the treatment of traveler's diarrhea (Laustsen and Wimmett, 2005). Clinical trials have also indicated the potential role for rifaximin in inflammatory bowel disease (IBD) (Day and Gearry, 2010). Rifaximin appears to have more efficacy in the therapy of irritable bowel syndrome (IBS) than do other antibiotics such as neomycin, doxycycline, amoxicillin/clavulanate, and ciprofloxacin(Yang et al., 2008). Other studies revealed that two-thirds of adult Crohn's disease (CD) patients entered remission after rifaximin therapy (Shafran and Burgunder, 2010). A retrospective review revealed a well-tolerated and favorable role for rifaximin in pediatric IBD (Muniyappa et al., 2009; Trehan et al., 2009). However, the role of rifaximin in IBS and IBD therapy and its mechanisms of action are not understood.

A previous study demonstrated that rifaximin is a gut-specific human pregnane X receptor (PXR) agonist (Ma et al., 2007b). PXR is a ligand-activated transcription factor important for its induction of drug transport and metabolism, in particular induction of the cytochrome P450 CYP3A4 involved in the metabolism of many clinically used drugs. However, PXR exhibits a species difference in ligand activation between humans and mice (Ma et al., 2008). Recent data indicate that PXR may be involved in IBD. Gene expression analysis of colon tissues from UC and CD patients demonstrated a significant inhibition of PXR and its target genes as compared to normal intestinal samples (Langmann et al., 2004). Specific polymorphisms in the *PXR* locus, which is associated with a decrease in PXR activity, are correlated with an increased susceptibility to IBD (Dring et al., 2006). PXR inhibits the pro-inflammatory transcription factor NF-kappa B (NF-κB), providing a potential molecular

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

mechanism that links PXR signaling and inflammation (Xie and Tian, 2006; Zhou et al., 2006a). For example, activation of mouse PXR ameliorates dextran sulfate sodium (DSS)-induced colitis via inhibition of NF-κB (Shah et al., 2007). However, PXR ligands are structurally diverse and as noted above display species specificity. Rifampicin is an agonist for human PXR but does not activate rodent PXR, while pregnenolone-16α-carbonitrile (PCN), a rodent-specific PXR agonist, does not activate human PXR (Zhou et al., 2009). Due to specific activation of rifaximin on human PXR, the role of this drug in therapy of IBD has been a focus of interest.

In the current study, the preventive and therapeutic role of rifaximin was assessed in the DSS-induced or TNBS-induced IBD models. Rifampicin (Ma et al., 2007b), as another rifamycin-derived antibiotic and human PXR agonist (Bertilsson et al., 1998), was compared to rifaximin. Since rodent models cannot accurately predict inducers and potential drug-drug interactions mediated by human PXR due to different responses to PXR ligands (Jones et al., 2000), PXR-humanized mice (hPXR) mice were used to investigate the potential role human PXR activation in the mouse IBD models. The hPXR mice express human PXR in the *Pxr*-null background and only respond to human PXR-specific ligands (Ma et al., 2007a). Wild-type and *Pxr*-null mouse lines were also assessed to distinguish the antibiotic role from its PXR ligand effect. Rifampicin treatment demonstrated no protection on mouse IBD, and exacerbated the severity of IBD. However, the hPXR mice treated with rifaximin demonstrated significant protection and a potential therapeutic role of human PXR in IBD. No protection of rifaximin was observed in wild-type and *Pxr*-null mice, thus demonstrating that rifaximin functions through activating human PXR and not through its gut-specific antibiotic effects. In addition, rifaximin inhibited the NF-κB signaling cascade in a human PXR-dependent manner. These data provide the first mechanistic evidence by which rifaximin and human PXR influence experimental IBD.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Methods

Animals. hPXR, wild-type, and *Pxr*-null male mice were housed in temperature- and light-controlled rooms and were given water and pelleted chow ad libitum. The hPXR mice express the human PXR in the *Pxr*-null background. All animal experiments were carried out in accordance with the Institute of Laboratory Animal Resources guidelines and approved by the National Cancer Institute Animal Care and Use Committee.

Experimental Design. Two- to three-month-old hPXR, wild-type, and *Pxr*-null male mice were subjected to the DSS-induced IBD and TNBS-induced IBD models. Mice were placed into four groups (n>=6 per group) in DSS-induced IBD study: control, DSS, rifaximin or rifampicin, and DSS treatment following rifaximin or rifampicin pre-treatment. The control and DSS groups received a control diet for 11 consecutive days, and mice in the rifaximin (Salix Pharmaceuticals, Inc., Morrisville, NC) or rifampicin (Sigma-Aldrich, St. Louis, MO) group received rifaximin (1 mg/kg/day) or rifampicin (3 mg/kg/day or 10 mg/kg/day) in the diet for 11 consecutive days. Dosages of rifaximin or rifampicin were calculated based on mouse daily dietary intake. Mice in the rifaximin and DSS or rifampicin and DSS group received the compounds in the diet for 11 days, and on the 5th day of treatment the mice were administered 2.5% DSS (MP Biomedicals, Aurora, OH) in drinking water (wt/vol), and all mice were killed 11 days following treatment with rifaximin or rifampicin diet or the control diet. Serum samples were collected by retro-orbital bleeding. hPXR and *Pxr*-null mice were used for the therapeutic studies (n>=10 per group) in which mice were treated with 2.5% DSS and control diet for 5 consecutive days, then DSS was withdrawn and rifaximin was administered and compared to vehicle-treated mice. The mice were observed for 7 consecutive days after

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

the onset of rifaximin treatment. The TNBS-induced IBD protocol was also used to confirm the role of rifaximin and human PXR in IBD (n>=6 per group). A 2 mg aliquot of TNBS in 50% ethanol was administered intrarectally to anesthetized mice via a round bottom (50.8 mm) needle (Popper, New York, USA), followed by control diet or rifaximin diet treatment for 6 consecutive days. The mice were killed 5 days after TNBS administration and the colons were flushed and resected. All above IBD studies were repeated twice. All mice were killed by CO_2 asphyxiation after final administration, and tissue samples were harvested and stored at -80°C before analysis.

Colitis Evaluation. Daily changes of body weight, diarrhea, rectal bleeding and bloody stool were assessed and reported as a score from 0 to 4. For assessment of macroscopic colon damage, the colon was opened longitudinally, flushed with PBS and fixed in 10% buffered formalin. Colitis was measured by blinded analysis on a routine hematoxylin and eosin-stained section according to the morphological criteria previously described (Cooper et al., 1993).

RNA Analysis. RNA was isolated and mRNA expression was assessed by qPCR as previously described (Cheng et al., 2009).

Cell lines and Luciferase Assays. Human epithelial colorectal adenocarcinoma cells Caco-2 and human colonic epithelial cells HT-29 were grown at 37°C with 5% CO_2 in DMEM (Invitrogen, Grand Island, NY) supplemented with 10% FBS (Gemini Bio-Products, Woodland, CA) and 1% penicillin streptomycin (Invitrogen). Caco-2 and HT-29 cells were seeded at density of 5×10^4 cells/well in 24-well plates. Expression vectors for human PXR, RXR and NF-κB luciferase reporters were transfected into cells using the Fugene transfection reagent (Roche, Indianapolis, IN). Twenty four hours post-

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

transfection, the cells were incubated with DMSO (vehicle) or rifaximin for 24 h, followed by incubation with TNFα (10 ng /ml) for 24 h. A standard dual luciferase assay was used and normalized to a cotransfected control reporter plasmid (Promega, Madison, WI).

Protein Analysis of Human PXR and Microsomal SCD-1. Fresh colon tissues were collected and epithelial cell were isolated. Nuclear protein was isolated using the NE-PER kit (Thermo Science, Rockford, IL). Liver tissues were collected and homogenized in ice-cold buffer (1.15% KCl, 50 mM Tris-HCl, and 1 mM EDTA, pH 7.4) and microsomes were prepared by differential centrifugation. Pellets were resuspended in 100 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 0.1 mM DTT, 0.15 M KCl, and 20% v/v glycerol, aliquoted, and stored at -80°C. Primary antibodies to human PXR (H5017) and SCD-1 (Santa Cruz Biotechnology) diluted 1:1000 with TBST respectively, followed by peroxidase-conjugated anti-mouse IgG diluted 1:10,000 with TBST, were used for western blot analysis. Nuclear hepatocyte nuclear factor 4 alpha (HNF4α) and microsomal glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as loading controls.

Metabolomics Analysis of Serum. A 1-µl aliquot of diluted serum samples were injected into a Waters UPLC-TOFMS system. An Acquity UPLC^{TM} BEH C18 column (Waters) was used to separate chemical components at 35°C. The mobile phase flow rate was 0.5 ml/min with an aqueous acetonitrile gradient containing 0.1% formic acid over a 10-min run (0% acetonitrile for 0.5 min to 20% acetonitrile by 5 min to 95% acetonitrile by 9 min, then equilibration at 100% water for 1 min before the next injection). The QTOF Premier^{TM} mass spectrometer was operated in the positive electrospray ionization

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

mode. Capillary voltage and cone voltage were maintained at 3 kV and 20 V, respectively. Source temperature and desolvation temperature were set at 120°C and 350°C, respectively. Nitrogen was used as both cone gas (50 l/h) and desolvation gas (600 l/h), and argon was used as collision gas. For accurate mass measurement, the TOFMS was calibrated with sodium formate solution (range m/z 100–1000) and monitored by the intermittent injection of the lock mass sulfadimethoxine ($[M+H]^+$ = 311.0814 m/z) in real-time. Mass chromatograms and mass spectral data were acquired and processed by MassLynx software (Waters) in centroid format.

Principal Components Analysis (PCA) of Serum Metabolomic Data. Chromatographic and spectral data were deconvoluted by MarkerLynx software. A multivariate data matrix containing information on sample identity, ion identity (retention time and m/z) and ion abundance was generated through centroiding, deisotoping, filtering, peak recognition, and integration. The intensity of each ion was calculated by normalizing the single ion counts versus the total ion counts in the whole chromatogram. The data matrix was further exported into SIMCA-P^{TM} software (Umetrics, Kinnelon, NJ) and transformed by mean-centering and Pareto scaling, a technique that increases the importance of low abundance ions without significant amplification of noise. Principal components of serum were generated by PCA analysis, to represent the major latent variables in the data matrix and were described in a scores scatter plot.

Data Analysis. Experimental values are expressed as mean ± standard deviation (SD). Statistical analysis was performed with two-tailed Student's t tests, except percent survival was analyzed by Prism 5.0 with survival curve statistical analysis, both with a *p* value of <0.05 considered statistically significant.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Results

Rifampicin Does Not Protect hPXR Mice in the DSS-induced Colitis Model. Rifampicin is a derivative antibiotic of rifamycin SV, which is a systemic drug widely studied as a typical human PXR agonist. Long-term treatment in tuberculosis therapy is thought to increase hepatotoxicity (Tostmann et al., 2008). Here, rifampicin was investigated for the treatment of DSS-induced colitis. Effective dosages of 3 mg/kg/day (Xie et al., 2000) and 10 mg/kg/day were selected (Cheng et al., 2009). hPXR mice pre-treated with rifampicin followed by DSS treatment demonstrated no protection against DSS-induced colitis when compared to DSS treatment alone. No significant changes were observed in control and rifampicin (3 or 10 mg/kg/day) treated mice in the absence of DSS. Interestingly, high dose rifampicin, combined with DSS, led to more severe colitis as compared to co-administration of low dosage of rifampicin with DSS as revealed by a decrease of body weight and increased diarrhea and rectal bleeding scores (Fig 1). Expression of hepatic SCD-1, a lipogenic enzyme regulated by PXR (Zhou et al., 2006b), which is protective in IBD (Chen et al., 2008), was repressed at the mRNA and protein levels after DSS alone and while 3 mg/kg/day rifampicin treatment combined with DSS did not alter these low levels, expression was markedly more reduced with 10 mg/kg/day of the drug (Supplementary Fig 1). This reduction of SCD-1 was associated with a reduction of unsaturated lysophosphatidylcholine (LPC) in plasma. Human PXR target genes in liver and colon were induced and proinflammatory factors were unchanged by rifampicin in the DSS IBD model (Supplementary Fig 2).

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Rifaximin Protects hPXR Mice in the DSS-induced Colitis Model. Different from rifampicin, rifaximin is a non-systemic antibiotic not significantly absorbed into the circulation. The preventive role of rifaximin was assessed in the DSS-induced colitis model. No significant changes were observed in the colon following rifaximin treatment alone in hPXR, WT and *Pxr*-null mice compared to the control groups (Supplementary Fig 3). In contrast, DSS treatment increased crypt and epithelial cell damage, infiltration of granulocytes and mononuclear immune cells, and tissue edema in hPXR mice. Notably, rifaximin pre-treatment significantly attenuated DSS-induced colon damage in hPXR mice compared to DSS treatment alone (Fig 2A), as noted by an observed decrease in epithelial loss and mild inflammation. While, rifaximin did not protect DSS-induced colitis in wild-type and *Pxr*-null mice as assessed by colon histology. In addition, several clinical parameters of colitis were statistically improved with rifaximin pre-treatment in hPXR mice, such as daily body weight loss, rectal bleeding, diarrhea, and colon length compared to DSS administration alone (Fig 2B). Meanwhile, there was no protection in WT and *Pxr*-null mice treated with DSS and rifaximin compared to DSS alone as revealed by clinical indexes of colitis, including body weight, rectal bleeding scores and diarrhea scores as well as colon length (Supplementary Fig 4). These results suggest that the beneficial effects of rifaximin may be attributed to its specific activation of human PXR, its high bioavailability in the intestine, and its decreased toxicity as compared to rifampicin. To ensure that the protective role of rifaximin in acute colitis is not via inducing hepatic SCD-1, western blot analysis of hepatic microsomal SCD-1 was performed. Rifaximin alone had no effect on SCD-1 expression in hPXR, wild-type or *Pxr*-null mice, whereas, 2.5% DSS administration clearly repressed SCD-1 expression in

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

hPXR, wild-typr or *Pxr*-null mice when compared to control (Supplementary Fig 5A). Moreover, serum metabolomics analysis demonstrated that rifaximin-treated hPXR, wild-type and *Pxr*-null mice have similar profiles of score plots and loading plots as determined by principle components analysis (Supplementary Fig 5B), suggesting that rifaximin may be acting locally in the colon and not systemically to decrease the severity of DSS-induced colitis.

Rifaximin Protects TNBS-Induced IBD in hPXR mice. To confirm results from the DSS-induced colitis model, an additional acute colitis models was used. The TNBS-induced IBD model, which is driven by an initial IL-12 and TNF-α response of lymphoid cells (Strober et al., 2009). TNBS-induced IBD model was initiated in hPXR mice by intrarectal administration of TNBS following rifaximin pre-treatment. TNBS triggered weight loss, bloody diarrhea, rectal prolapse and large bowel wall thickening; rifaximin significantly improved colon histology (Fig 3A) and body weight (Fig 3B), as well as maintaining colon length (Fig 3C) in hPXR mice, whereas no protection was observed in WT and *Pxr*-null mice (data not shown). Thus, using a second mouse IBD model, these data further indicate that rifaximin protects against IBD in hPXR mice.

Rifaximin Exerts Therapeutic Efficacy in the DSS-induced colitis Model in hPXR Mice. In addition to a preventive role of rifaximin, a therapeutic study was performed in DSS-induced hPXR and *Pxr*-null mice. Acute colitis was induced by 2.5% DSS treatment for 5 days. Rifaximin (1mg/kg/day) was administrated to hPXR and *Pxr*-null mice after the onset of IBD. Survival rates were markedly increased in hPXR mice (P=0.011) compared to *Pxr*-null mice (P=0.730) administered rifaximin following withdrawal of DSS (Fig 4A). Clear signs of recovery from colitis were noted in hPXR mice

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

administered rifaximin (Fig 4B), such as increased colon length (P=0.002), increased body weight compared to controls. A significant resolution of colon damage was observed in hPXR mice treated with DSS alone compared to colons from rifaximin-treated hPXR mice (Fig 4C). Whereas no significant protection was afforded in *Pxr*-null mice treated with rifaximin with respect to survival (Fig 4D) or improvement of clinical signs (Fig 4E) as compared to hPXR mice.

Rifaximin Activates hPXR Target Genes in the Colon. Human PXR expression in colon was measured with quantitative real-time PCR (qPCR) (Fig 5A) and Western blot (Fig 5B). Mouse β-actin served as an internal control for qPCR mRNA analysis and HNF4α for western blot analysis. Human PXR was expressed in the colons of hPXR mice, however the expression was significantly less than that observed in the liver. Previous work demonstrated that rifaximin is a human PXR agonist in the small intestine (Ma et al., 2007b). To assess the potential function of rifaximin as a PXR ligand in the colon, qPCR analysis of mouse *Cyp3a11*, *Cyp3a13*, *Gsta1*, and *Mdr1a* genes in colon were investigated. The expression of Cyp3a11, Cyp3a13, Gsta1 and Mdr1a mRNAs were increased in hPXR mice following rifaximin treatment when compared to the control group (Fig 6), whereas, there was no induction of Cyp3a11, Cyp3a13, Gsta1, and Mdr1a mRNAs in wild-type and *Pxr*-null mice following rifaximin treatment. Together, these data demonstrate that rifaximin is a human PXR agonist in the colon.

Activation of hPXR by Rifaximin Inhibits NF-κB Target Gene Expression. NF-κB is the central transcription factor in the regulation of pro-inflammatory cytokines and chemokines. Previous studies demonstrated that activation of PXR can attenuate NF-κB signaling (Xie and Tian, 2006). To elucidate the potential function of NF-κB on

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

protection of DSS-induced colitis by rifaximin, qPCR analysis of several NF-κB regulated pro-inflammatory cytokines and chemokines in colon were evaluated. The results demonstrated that mRNAs encoding the inducible nitric oxide synthase (iNOS), chemokine motif receptor 2 (CCR2), tumor necrosis factor (TNFα), interferon-a (IFNα), intercellular adhesion molecule 1 (ICAM-1), monocyte chemoattractant protein 1 (MCP-1), and interleukin-10 (IL-10), interleukin-6 (IL-6), interleukin-1β (IL-1β) mRNA were induced in colonic tissue following 5-day DSS treatment of hPXR mice, compared to the control group (Fig 7). The increase in inflammatory mediators (iNOS, CCR2, TNFα, IFNα, ICAM-1, MCP-1, IL-10, IL-6 and IL-1β) following DSS treatment were significantly decreased in hPXR mice pretreated with rifaximin. These data suggest that rifaximin activated human PXR may protect DSS-induced colitis through inhibiting the NF-κB mediated pro-inflammatory response.

Direct Inhibition of NF-κB by Rifaximin. To determine the direct role of human PXR in repression of NF-κB signaling by rifaximin, luciferase activities of an NF-κB reporter construct were measured. In HT-29 and Caco-2 cells, TNFα (10 ng/ml) induced NF-κB activation, which was significantly attenuated following co-transfection with human PXR and RXR constructs. These data revealed that human PXR could inhibit TNFα-induced inflammation. The addition of 1 μM and 100 μM rifaximin potentiated the repression of NF-κB activation following TNFα treatment (Fig 8A). The significant inhibition of NF-κB activity following rifaximin treatment in the absence of human PXR transfection might be due to basal expression of human PXR in HT-29 and Caco-2 cells as assessed by western blot analysis (data not shown). Moreover, a dose response of rifaximin on NF-κB inhibition was evaluated in HT-29 cells. These results showed that the optimal

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

inhibition dosage of rifaximin was between 0.01 μM-1 μM (Fig 8B). Consistent with the *in vivo* results, the *in vitro* luciferase assay indicated that rifaximin protected hPXR mice from acute colitis through NF-κB inhibition via gut-specific human PXR activation.

Rifaximin Inhibits NF-κB Target Genes Through a hPXR-mediated Mechanism. NF-κB target genes iNOS, CCR2, TNFα, IFNα, ICAM-1, MCP-1, IL-10, IL-6, IL-1β mRNAs were measured in wild-type and *Pxr*-null mice following DSS treatment. A significant induction of all pro-inflammatory mediators was observed in wild-type and *Pxr*-null mice compared to control group. However, rifaximin did not decrease inflammatory mediators following DSS treatment (Fig 9), but actually increased TNFα and CCR2 mRNAs in *Pxr*-null and TNFα, IL-1β, IL-10 and MCP-1 in wild-type mice when compared to DSS and rifaximin co-treatment to DSS treated alone, which might result from a differential inflammation response of these above factors in *Pxr*-null and wild-type mice. These data demonstrate that rifaximin affords protection mainly through a gut specific hPXR-dependent mechanism and not through its antibiotic properties.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

The etiology of IBD is still not completely understood, however it is widely accepted that the development of IBD is associated with the interplay of genetic, bacterial, and environmental factors, and dysregulation of the intestinal immune system (Kaser et al., 2010). Among the IBD models, chemically induced animal models are commonly used, particularly with DSS colitis and hapten-induced colitis employing either DNBS/TNBS or oxazolone. These two models produce robust and immediate inflammation in the colon, and have high reproducibility (Hoffmann et al., 2002). In the present study, acute colitis was induced by DSS and TNBS administration. Despite the acute nature of these models, they clearly show the impact of human PXR and rifaximin and suggest its clinical utility.

The treatment of IBD is highly individualized, however in most cases the first-line therapy utilizes immune-suppressing drugs, which act systemically. Rifaximin, an efficient gut-specific antibiotic due to its zwitterionic property which inhibits absorption of rifaximin into the systemic circulation (Marchi et al., 1985), has been shown to be beneficial in specific cases of IBD (Gionchetti et al., 2006). Using mouse models that measure accurately the species-specific role of human PXR, the present study clearly demonstrated that rifaximin protects through a PXR-dependent mechanism and not as a general antibiotic both in DSS-induced IBD and TNBS-induced IBD. In the therapeutic study, no mice in the DSS group survived while rifaximin post-treatment resolved the colitis symptoms and led to 60% survival. The resolution of inflammation in acute models of colitis are not completely understood, however these data provide evidence that rifaximin and hPXR might be worthwhile for treatment of human IBD.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Interestingly, a well-known human PXR agonist, rifampicin, which is a systemic antibiotic used to treat tuberculosis, demonstrated an increase in hepatotoxicity when administered before treatment with DSS and provided no significant protection of hPXR mice in the DSS-induced colitis model. Rifampicin treatment in the colitis model actually slightly increased the severity of symptoms such as body weight loss and diarrhea score. This is quite unexpected since PCN, which also induces PXR target genes in liver and colon, protects against DSS-induced IBD (Shah et al., 2007). The reason for the differences between the protective effect of rifaximin and rifampicin in experimental IBD is not understood. Since both drugs can induce PXR target genes in the intestine, the difference is not due to the increase in availability of rifaximin in the gut as compared to rifampicin. Further experimentation is required to determine the reason for the lack of effect of rifampicin on DSS-induced colitis in the hPXR mouse. It was reported that rifampicin led to several pseudomembranous colitis cases (Akbar et al., 2003; Mazokopakis et al., 2008; Chen et al., 2009), as well as being associated with adverse effects in patients with chronic inflammatory diseases (Yuhas et al., 2009). It is noteworthy that rifampicin administered with DSS markedly lowered the already suppressed SCD-1 protein and the serum levels of unsaturated fatty acids as compared to DSS alone. The current results in the mouse IBD model suggest that these clinical observations could be the result of the lowering of SCD-1 expression and associated anti-inflammatory unsaturated fatty acids (Chen et al., 2008).

Previous studies have shown that PXR is remarkably divergent across mammalian species with the ligand binding domains sharing 70-80% identity compared

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

with the 90% typically exhibited by other nuclear receptors (Zhou et al., 2009). Since mouse PXR was reported to ameliorate DSS-induced IBD by NF-κB inhibition, it was critical to investigate the mechanism by which human PXR was protective in an acute colitis model. *In vivo* data demonstrated several NF-κB target genes to be significantly attenuated in hPXR mice following rifaximin pre-treatment and DSS treatment as compared to DSS treatment alone, and the attenuation of NF-κB signaling by rifaximin was not observed in wild-type and *Pxr*-null mice. Consistent with the *in vivo* data, *in vitro* evaluation of rifaximin on NF-κB inhibition suggested a direct role for human PXR in the inhibition of NF-κB signaling. Analysis of NF-κB response in colon-derived cancer cells using the NF-κB response element demonstrated a significant induction with TNFα treatment, which was inhibited following over-expression of human PXR and the inhibition was further potentiated in the presence of rifaximin. It was assumed that transrepression of NF-κB target genes might be mediated by SUMOylation of PXR (Zhou et al., 2006b), and, NF-κB disrupted binding of the PXR-RXR complex to its binding motif (Gu et al., 2006).

In summary, the hPXR mice were used to demonstrate the beneficial effects of rifaximin in acute models of colitis through activation of human PXR, and a critical role for PXR in IBD. In addition, these data provides a mechanistic basis for novel therapies targeting human PXR in the treatment of inflammatory diseases of the gut.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Acknowledgements. We thank John Buckley for assistance with the animal studies and Salix Pharmaceuticals, Inc., for supplying rifaximin.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

References

Akbar DH, Al-Shehri HZ, Al-Huzali AM and Falatah HI (2003) A case of rifampicin induced pseudomembraneous colitis. *Saudi Med J* **24**:1391-1393.

Bertilsson G, Heidrich J, Svensson K, Asman M, Jendeberg L, Sydow-Backman M, Ohlsson R, Postlind H, Blomquist P and Berkenstam A (1998) Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci U S A* **95**:12208-12213.

Chen C, Shah YM, Morimura K, Krausz KW, Miyazaki M, Richardson TA, Morgan ET, Ntambi JM, Idle JR and Gonzalez FJ (2008) Metabolomics reveals that hepatic stearoyl-CoA desaturase 1 downregulation exacerbates inflammation and acute colitis. *Cell Metab* **7**:135-147.

Chen TC, Lu PL, Lin WR, Lin CY, Wu JY and Chen YH (2009) Rifampin-associated pseudomembranous colitis. *Am J Med Sci* **338**:156-158.

Cheng J, Ma X, Krausz KW, Idle JR and Gonzalez FJ (2009) Rifampicin-activated human pregnane X receptor and CYP3A4 induction enhance acetaminophen-induced toxicity. *Drug Metab Dispos* **37**:1611-1621.

Cooper HS, Murthy SN, Shah RS and Sedergran DJ (1993) Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* **69**:238-249.

Day AS and Gearry RB (2010) Rifaximin and Crohn's disease: a new solution to an old problem? *Dig Dis Sci* **55**:877-879.

Dring MM, Goulding CA, Trimble VI, Keegan D, Ryan AW, Brophy KM, Smyth CM, Keeling PW, O'Donoghue D, O'Sullivan M, O'Morain C, Mahmud N, Wikstrom AC, Kelleher D and McManus R (2006) The pregnane X receptor locus is

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

associated with susceptibility to inflammatory bowel disease. *Gastroenterology* **130**:341-348.

Gionchetti P, Rizzello F, Lammers KM, Morselli C, Tambasco R and Campieri M (2006) Antimicrobials in the management of inflammatory bowel disease. *Digestion* **73 Suppl 1**:77-85.

Gu X, Ke S, Liu D, Sheng T, Thomas PE, Rabson AB, Gallo MA, Xie W and Tian Y (2006) Role of NF-kappaB in regulation of PXR-mediated gene expression: a mechanism for the suppression of cytochrome P-450 3A4 by proinflammatory agents. *J Biol Chem* **281**:17882-17889.

Hoffmann JC, Pawlowski NN, Kuhl AA, Hohne W and Zeitz M (2002) Animal models of inflammatory bowel disease: an overview. *Pathobiology* **70**:121-130.

Jones SA, Moore LB, Shenk JL, Wisely GB, Hamilton GA, McKee DD, Tomkinson NC, LeCluyse EL, Lambert MH, Willson TM, Kliewer SA and Moore JT (2000) The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Mol Endocrinol* **14**:27-39.

Kaser A, Zeissig S and Blumberg RS (2010) Inflammatory bowel disease. *Annu Rev Immunol* **28**:573-621.

Koo HL and DuPont HL (2010) Rifaximin: a unique gastrointestinal-selective antibiotic for enteric diseases. *Curr Opin Gastroenterol* **26**:17-25.

Langmann T, Moehle C, Mauerer R, Scharl M, Liebisch G, Zahn A, Stremmel W and Schmitz G (2004) Loss of detoxification in inflammatory bowel disease: dysregulation of pregnane X receptor target genes. *Gastroenterology* **127**:26-40.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Laustsen G and Wimmett L (2005) 2004 drug approval highlights: FDA update. *Nurse Pract* **30**:14-29.

Ma X, Idle JR and Gonzalez FJ (2008) The pregnane X receptor: from bench to bedside. *Expert Opin Drug Metab Toxicol* **4**:895-908.

Ma X, Shah Y, Cheung C, Guo GL, Feigenbaum L, Krausz KW, Idle JR and Gonzalez FJ (2007a) The Pregnane X receptor gene-humanized mouse: a model for investigating drug-drug interactions mediated by cytochromes P450 3A. *Drug Metab Dispos* **35**:194-200.

Ma X, Shah YM, Guo GL, Wang T, Krausz KW, Idle JR and Gonzalez FJ (2007b) Rifaximin is a gut-specific human pregnane X receptor activator. *J Pharmacol Exp Ther* **322**:391-398.

Marchi E, Montecchi L, Venturini AP, Mascellani G, Brufani M and Cellai L (1985) 4-Deoxypyrido[1',2':1,2]imidazo[5,4-c]rifamycin SV derivatives. A new series of semisynthetic rifamycins with high antibacterial activity and low gastroenteric absorption. *J Med Chem* **28**:960-963.

Mazokopakis EE, Giannakopoulos TG and Christias EG (2008) Acute brucellosis as a cause of infective colitis. *Mil Med* **173**:1145-1147.

Muniyappa P, Gulati R, Mohr F and Hupertz V (2009) Use and Safety of Rifaximin in Children With Inflammatory Bowel Disease. *J Pediatr Gastroenterol Nutr*. **49**:400-410.

Shafran I and Burgunder P (2010) Adjunctive antibiotic therapy with rifaximin may help reduce Crohn's disease activity. *Dig Dis Sci* **55**:1079-1084.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Shah YM, Ma X, Morimura K, Kim I and Gonzalez FJ (2007) Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-kappaB target gene expression. *Am J Physiol Gastrointest Liver Physiol* **292**:G1114-1122.

Strober W, Kitani A, Fichtner-Feigl S and Fuss IJ (2009) The signaling function of the IL-13Ralpha2 receptor in the development of gastrointestinal fibrosis and cancer surveillance. *Curr Mol Med* **9**:740-750.

Tostmann A, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ and Dekhuijzen R (2008) Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. *J Gastroenterol Hepatol* **23**:192-202.

Trehan I, Shulman RJ, Ou CN, Maleta K and Manary MJ (2009) A Randomized, Double-Blind, Placebo-Controlled Trial of Rifaximin, a Nonabsorbable Antibiotic, in the Treatment of Tropical Enteropathy. *Am J Gastroenterol*. **104**:2326-2333.

Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, Neuschwander-Tetri BA, Brunt EM, Guzelian PS and Evans RM (2000) Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature* **406**:435-439.

Xie W and Tian Y (2006) Xenobiotic receptor meets NF-kappaB, a collision in the small bowel. *Cell Metab* **4**:177-178.

Yang J, Lee HR, Low K, Chatterjee S and Pimentel M (2008) Rifaximin versus other antibiotics in the primary treatment and retreatment of bacterial overgrowth in IBS. *Dig Dis Sci* **53**:169-174.

Yuhas Y, Berent E, Cohen R and Ashkenazi S (2009) Roles of NF-kappaB activation and peroxisome proliferator-activated receptor gamma inhibition in the effect of

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

rifampin on inducible nitric oxide synthase transcription in human lung epithelial cells. *Antimicrob Agents Chemother* **53**:1539-1545.

Zhou C, Tabb MM, Nelson EL, Grun F, Verma S, Sadatrafiei A, Lin M, Mallick S, Forman BM, Thummel KE and Blumberg B (2006a) Mutual repression between steroid and xenobiotic receptor and NF-kappaB signaling pathways links xenobiotic metabolism and inflammation. *J Clin Invest* **116**:2280-2289.

Zhou J, Zhai Y, Mu Y, Gong H, Uppal H, Toma D, Ren S, Evans RM and Xie W (2006b) A novel pregnane X receptor-mediated and sterol regulatory element-binding protein-independent lipogenic pathway. *J Biol Chem* **281**:15013-15020.

Zhou C, Verma S and Blumberg B (2009) The steroid and xenobiotic receptor (SXR), beyond xenobiotic metabolism. *Nucl Recept Signal* **7**:e001.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Footnotes

This work was supported by the National Cancer Institute Intramural Research Program.

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Legends for figures:

Fig. 1. Colitis assessment of hPXR mice treated with 2.5% DSS following rifampicin (RIF) pre-treatment. hPXR male mice were placed into six groups (n>=6 per group): control group (Cont), DSS treatment alone (DSS), RIF (3 mg/kg/day) treatment alone (RIF3), RIF (10 mg/kg/day) treatment alone (RIF10), DSS treatment following RIF (3 mg/kg/day) pre-treatment (DSS/RIF3) and DSS treatment following RIF (10 mg/kg/day) pre-treatment (DSS/RIF10). Each bar represents the mean ± standard deviations, n>=6 (* for $p < 0.05$, ** for $p < 0.01$).

Fig. 2. Rifaximin protects hPXR mice in a DSS-induced colitis model. (A) Representative hematoxylin and eosin (H&E)-stained colon sections of hPXR, wild-type (WT) and *Pxr*-null treated with DSS alone (DSS) or DSS/rifaximin (Rifax). Magnification: 200 ×. (B) Colitis assessment of hPXR mice treated with 2.5% DSS (DSS) or 2.5 % DSS following rifaximin pre-treatment (DSS/Rifax). Each bar represents the mean ± standard deviations, n>=6 (* for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$).

Fig. 3. Rifaximin protects hPXR mice in a TNBS-induced colitis model (A) Representative hematoxylin and eosin (H&E)-stained colon sections of hPXR treated with control (left upper), rifaximin (right upper), TNBS (left below), and TNBS/rifaximin (right below). Magnification: 200 ×. (B) Body weight loss of hPXR mice treated with control (Cont), rifaximin (Rifax), TNBS, and TNBS/rifaximin (TNBS/Rifax). (C) Colon length of hPXR mice treated with control (Cont), rifaximin (Rifax), TNBS, and TNBS/rifaximin (TNBS/Rifax). Each bar represents the mean ± standard deviations, n>=6 (* for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$).

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 4. Therapeutic role of rifaximin on DSS-induced IBD in hPXR mice. (A) Survival curve of hPXR mice comparing rifaximin post-treatment to no rifaximin treatment. (B) Colitis assessment of hPXR mice comparing rifaximin post-treatment to no rifaximin treatment. (C) Representative hematoxylin and eosin (H&E)-stained colon sections of hPXR treated with DSS (left) or with rifaximin after DSS-induced colitis (middle) (Magnification: 200 ×) and macroscopic observation of colon length (left: DSS; right: DSS/Rifax). (D) Survival curves of *Pxr*-null mice comparing rifaximin post-treatment to no rifaximin treatment. (E) Colitis assessment of *Pxr*-null mice comparing rifaximin post-treatment to no rifaximin treatment. Each bar represents the mean ± standard deviations, n>=10 (* for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$).

Fig. 5. Basal expression of human PXR in the colon of hPXR mice. (A) qPCR analysis of huma*n* PXR expression in colon and liver of hPXR, mice and in colon of wild type and *Pxr*-null mice. Mouse *β*-actin mRNA served as an internal control. (B) Western blot analysis of human PXR expression in colon and liver of hPXR mice and in colon of wild-type and *Pxr*-null mice. Pooled nuclear protein (30 μg) were loaded for each sample (n=3 per group). The monoclonal antibody against human PXR (H5017) specifically recognizes human PXR but not mouse PXR or other liver proteins. HNF4α was used as a loading control.

Fig. 6. Messenger RNA analysis of hPXR target genes in colon tissue. Expression of mRNAs encoding Cyp3a11, Cyp3a13, Gsta1 and Mdr1a were determined by qPCR from colon epithelial cells isolated from hPXR, wild-type and *Pxr*-null mice treated with control (Cont) and rifaximin (Rifax). Data was normalized to *β*-actin. Each bar represents the mean ± standard deviations, n>=6 (* for $p < 0.05$).

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 7. Messenger RNA analysis of pro-inflammatory mediators in colon tissue from hPXR mice. Colon RNA was isolated from hPXR mice treated with control (Cont), DSS, rifaximin (Rifax) and DSS/rifaximin (DSS/Rifax). Expression of mRNAs encoding iNOS, CCR2, TNFα, IFNα, ICAM-1, MCP-1, IL-10, IL-6 and IL-1β were determined by qPCR**.** Data was normalized to *β*-actin. Each bar represents the mean ± standard deviations, n>=6 (* for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$).

Fig. 8. Rifaximin inhibited TNFα-activated NF-κB luciferase reporter through human PXR. (A) HT-29 cells and Caco-2 cells (5×10^4 cells/well) were cotransfected with NF-κB luciferase reporter (0.2 mg/well) and mouse RXR (0.04 μg/well) or cotransfected with NF-κB luciferase reporter (0.2 mg/well), mouse RXR (0.04 μg/well) and hPXR (0.04 μg/well). 24 h after transfection cells were incubated with vehicle (Veh, DMSO), rifaximin (Rifax, 1μM or 100 μM, dissolved in DMSO), or TNFα (10 ng/ml), or co-incubated with TNFα and rifaximin for 24 h (TNFα+ Rifax). (B) HT-29 cell were cotransfected with NF-κB luciferase reporter (0.2 mg/well) and mouse RXR (0.04 μg/well) or cotransfected with NF-κB luciferase reporter (0.2 mg/well), mouse RXR (0.04 μg/well) and hPXR (0.04 μg/well). 24 h after transfection Cells were incubated with vehicle (Veh), rifaximin (Rifax, 100 μM), TNFα (10 ng/ml), or co-incubated with rifaximin (0.001, 0.01, 0.1, 1, 100μM) and TNFα (10 ng/ml) for 24 h (TNFα+ Rifax). Standard dual luciferase assays were performed on cell extracts. Each bar represents the mean ± standard deviation, n>=6 (* for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$)

JPET Fast Forward. Published on July 13, 2010 as DOI: 10.1124/jpet.110.170225
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 9. Messenger RNA analysis of pro-inflammatory factors in colon tissue from wild-type and *Pxr*-null mice. Colon RNA was isolated from wild-type (WT) and *Pxr*-null mice treated with control (Cont), DSS, rifaximin (Rifax) and DSS/rifaximin (DSS/Rifax). Expression of mRNAs encoding iNOS, CCR2, TNFα, IFNα, ICAM-1, MCP-1, IL-10, IL-6 and IL-1β were determined by qPCR**.** Data was normalized to *β*-actin. Each bar represents the mean ± standard deviations, n>=6 (* for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$).

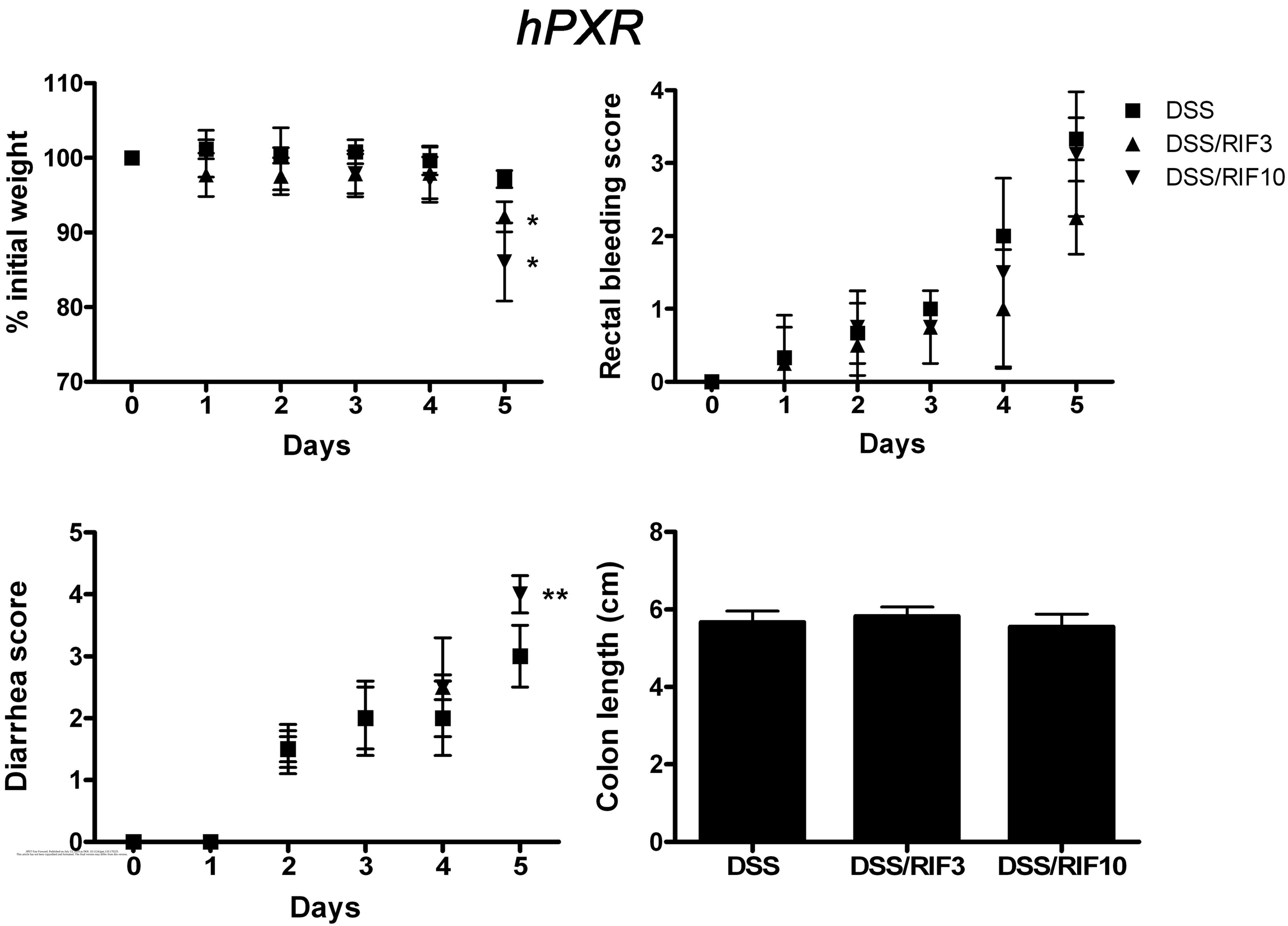


Figure 1

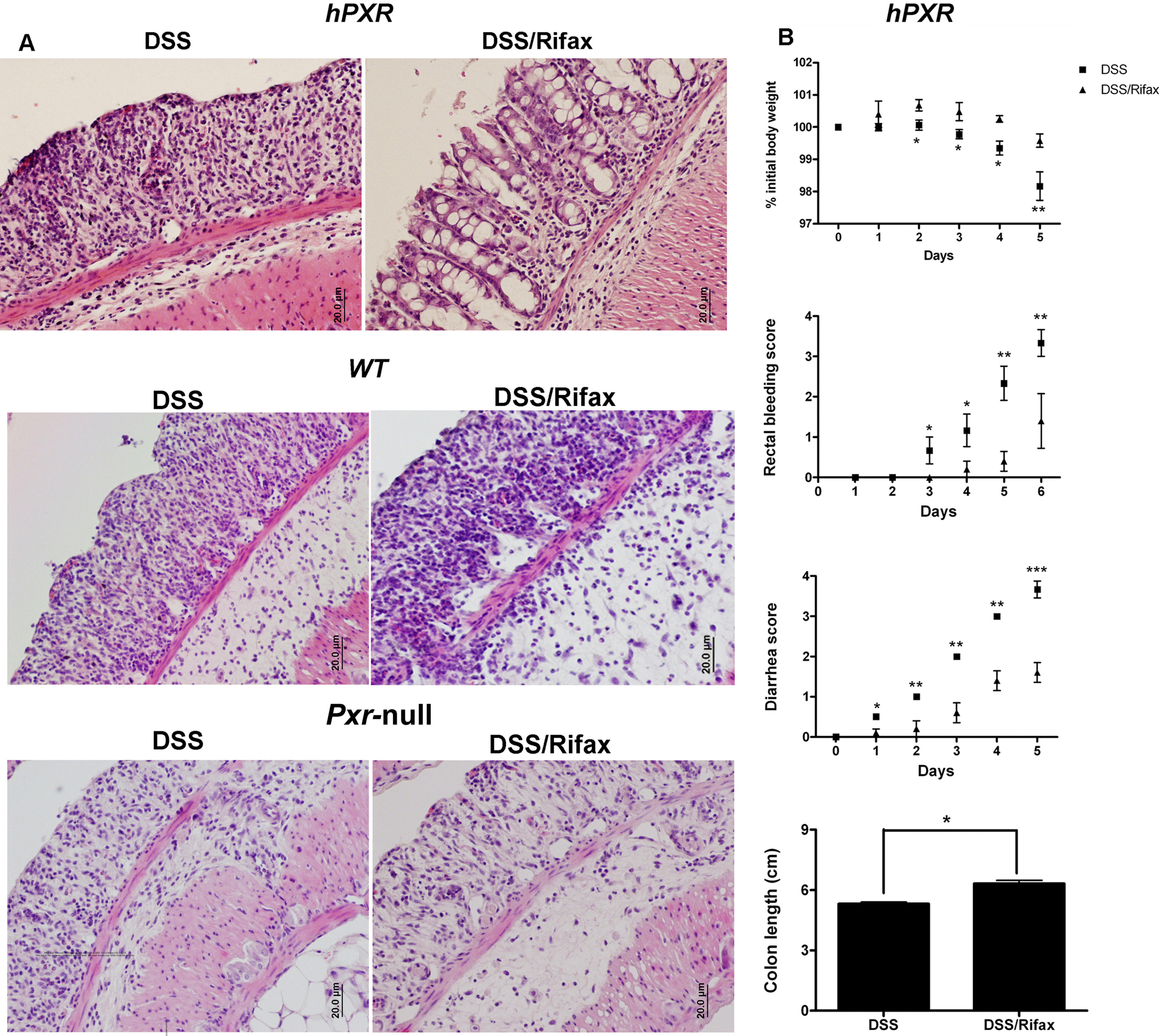

hPXR
A
DSS
DSS/Rifax
20.0 µm
WT
DSS
DSS/Rifax
Pxr-null
DSS
DSS/Rifax
hPXR
B
DSS
DSS/Rifax
% initial body weight
Days
Rectal bleeding score
Days
Diarrhea score
Days
Colon length (cm)
DSS
DSS/Rifax

Figure 2

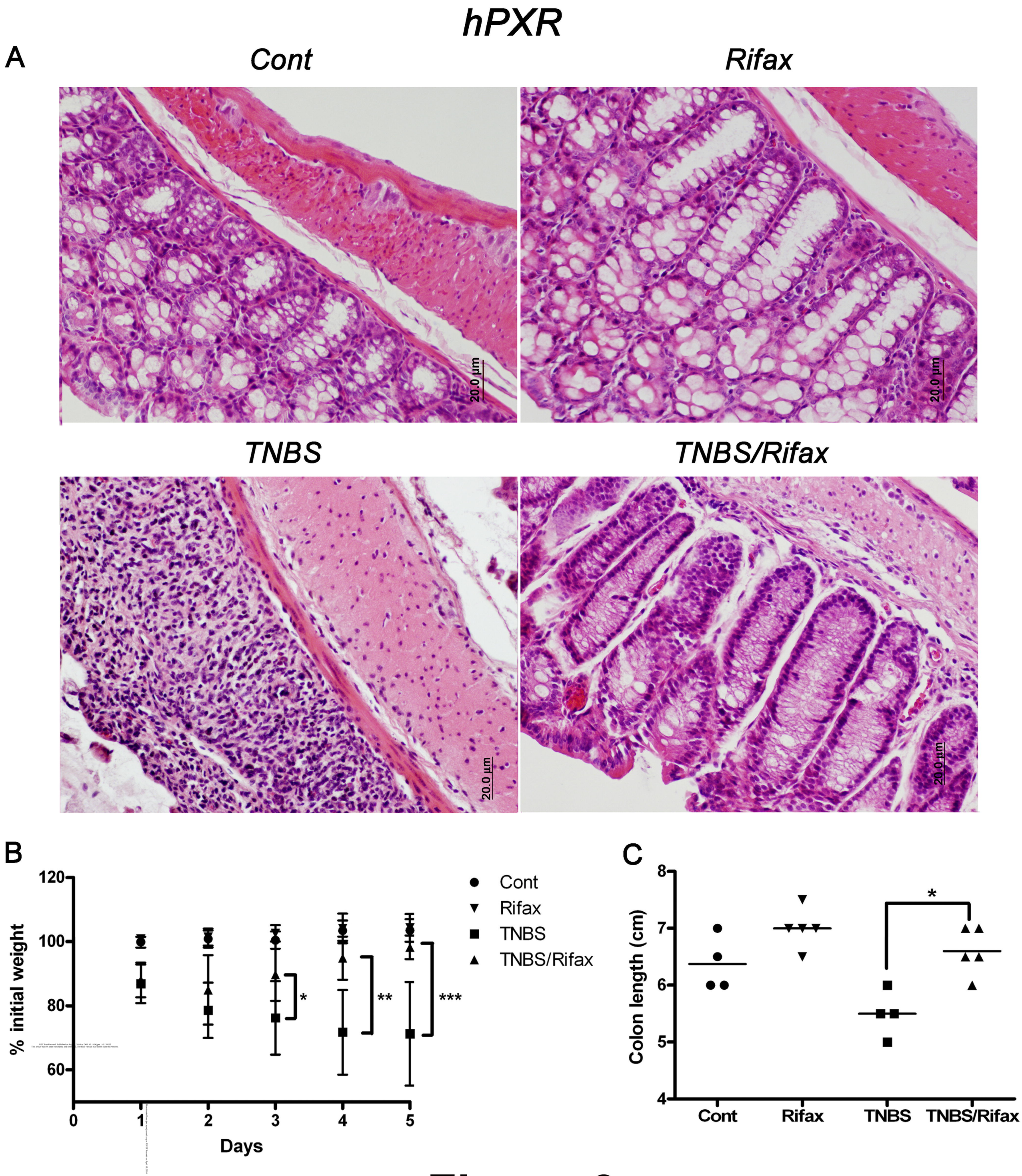

hPXR
A
Cont
Rifax
TNBS
TNBS/Rifax
20.0 µm
B
% initial weight
Days
Cont
Rifax
TNBS
TNBS/Rifax
*
**
***
C
Colon length (cm)
Cont
Rifax
TNBS
TNBS/Rifax
*

Figure 3

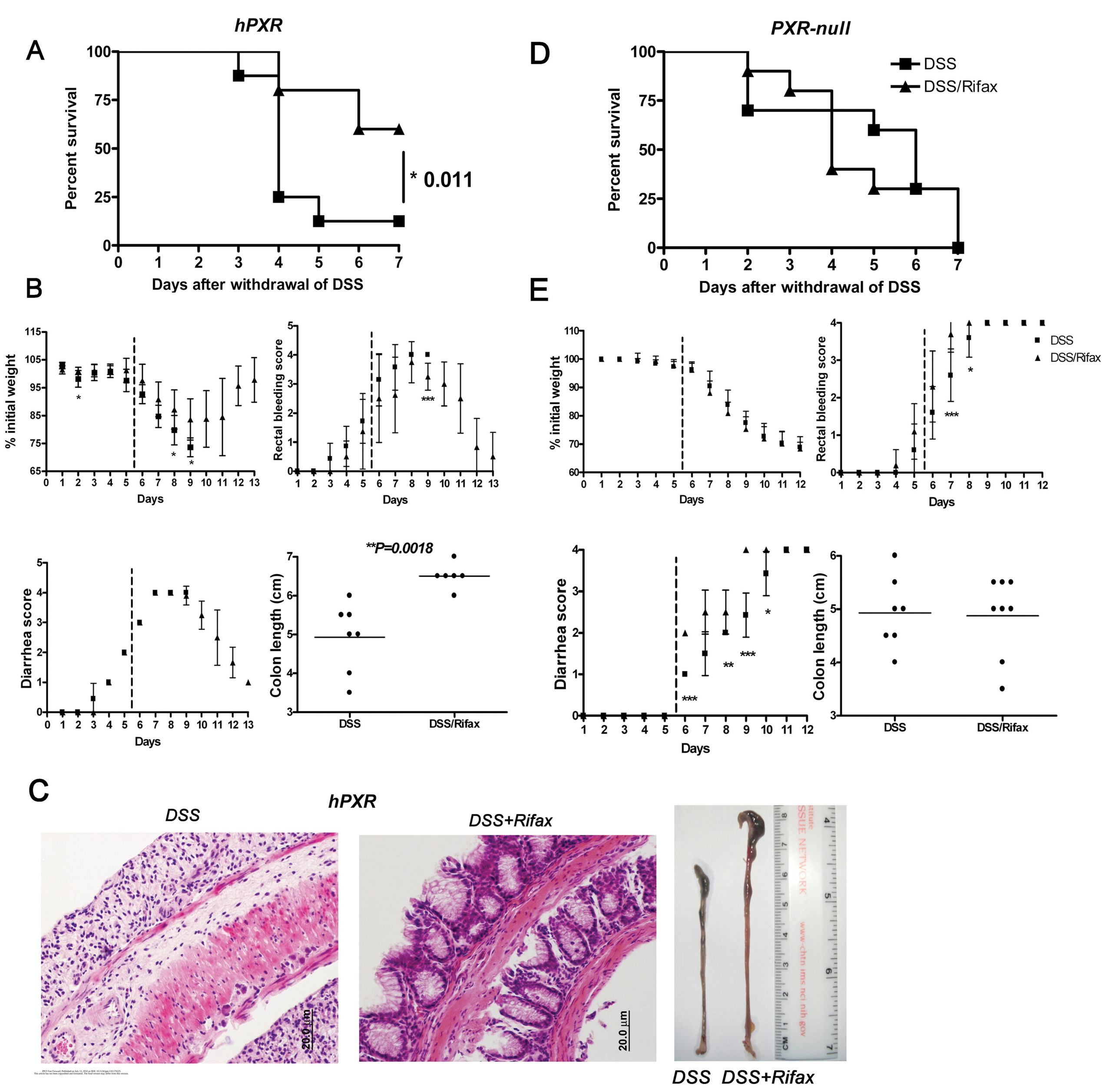
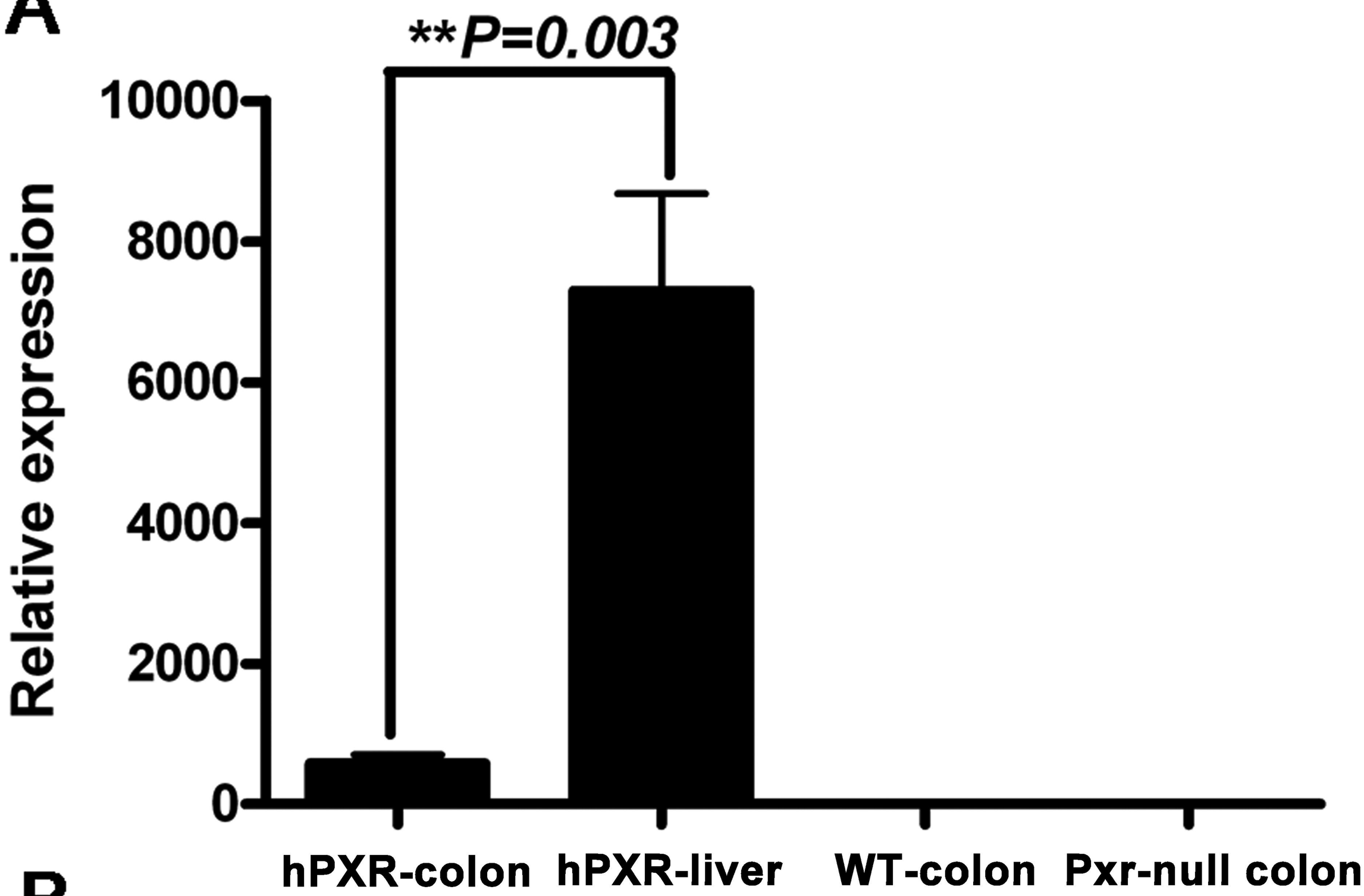


Figure 4

A



B

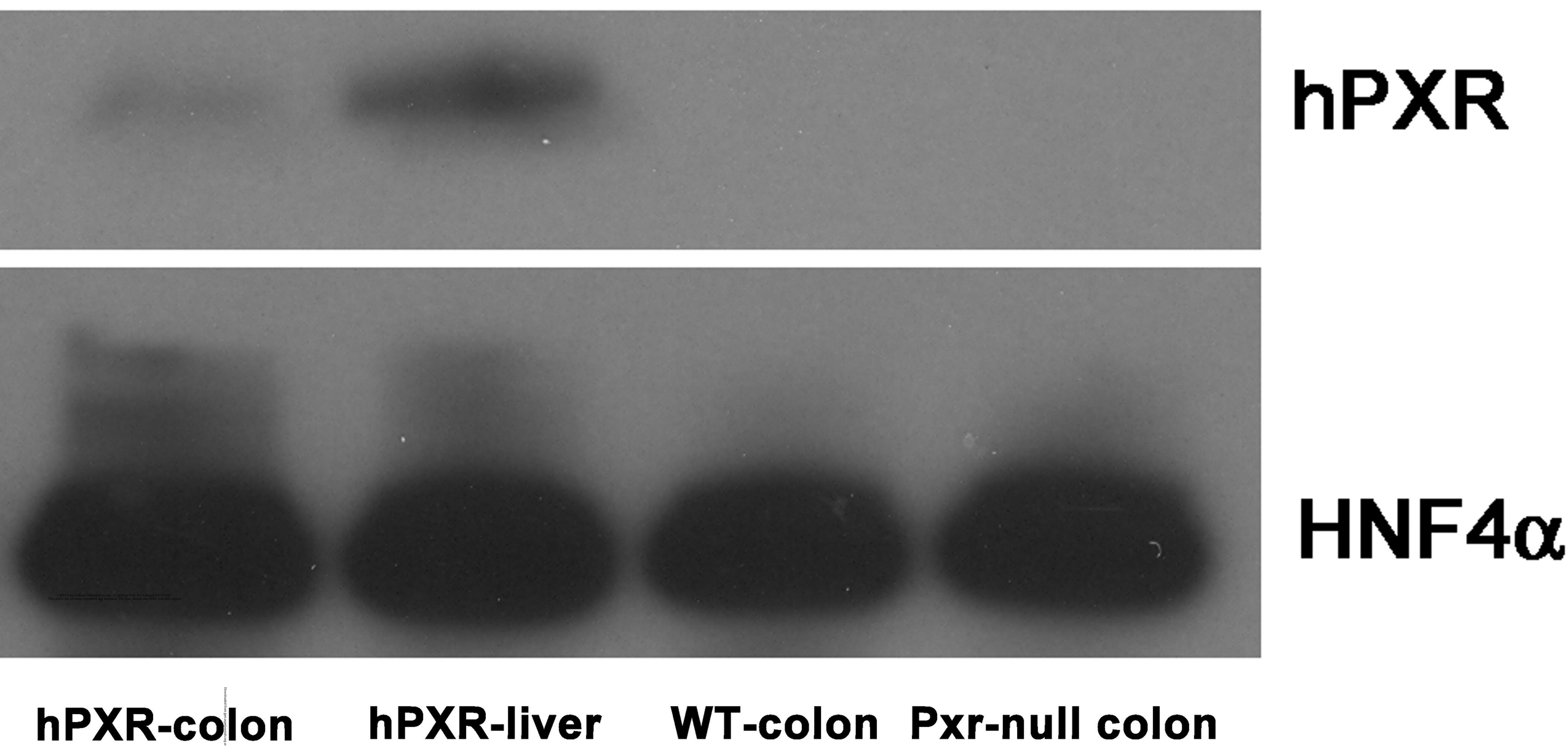


Figure 5

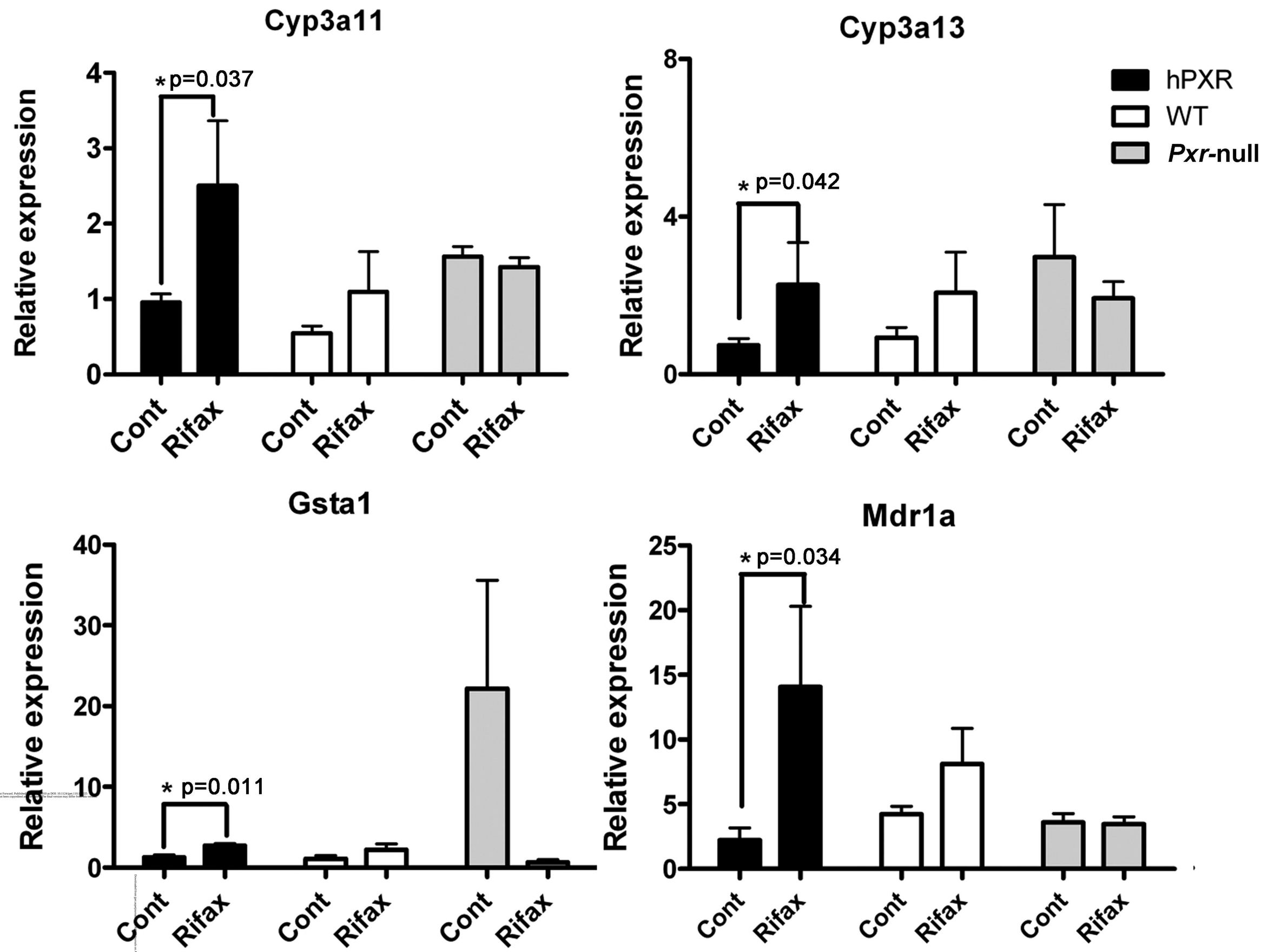


Figure 6

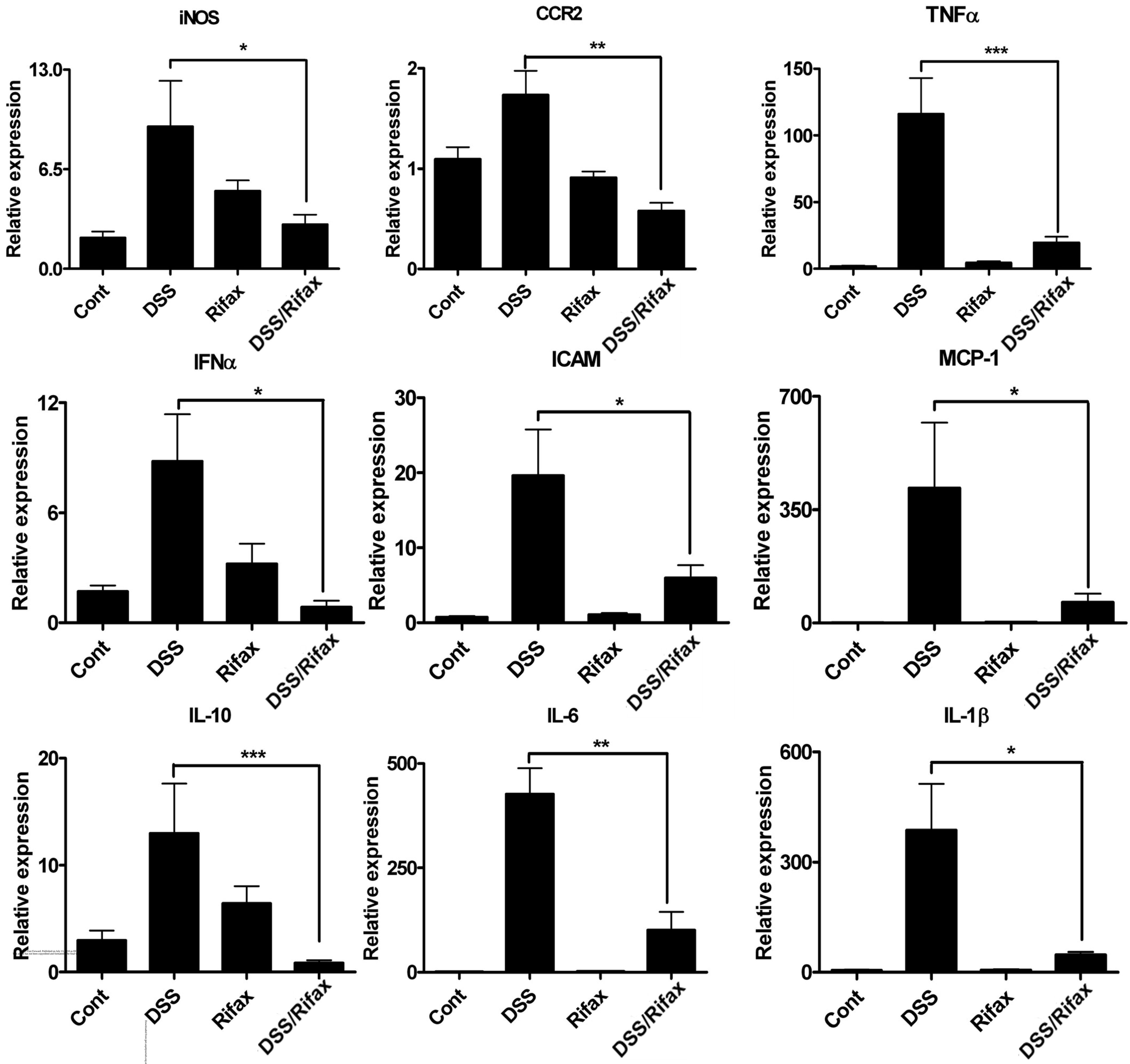


Figure 7

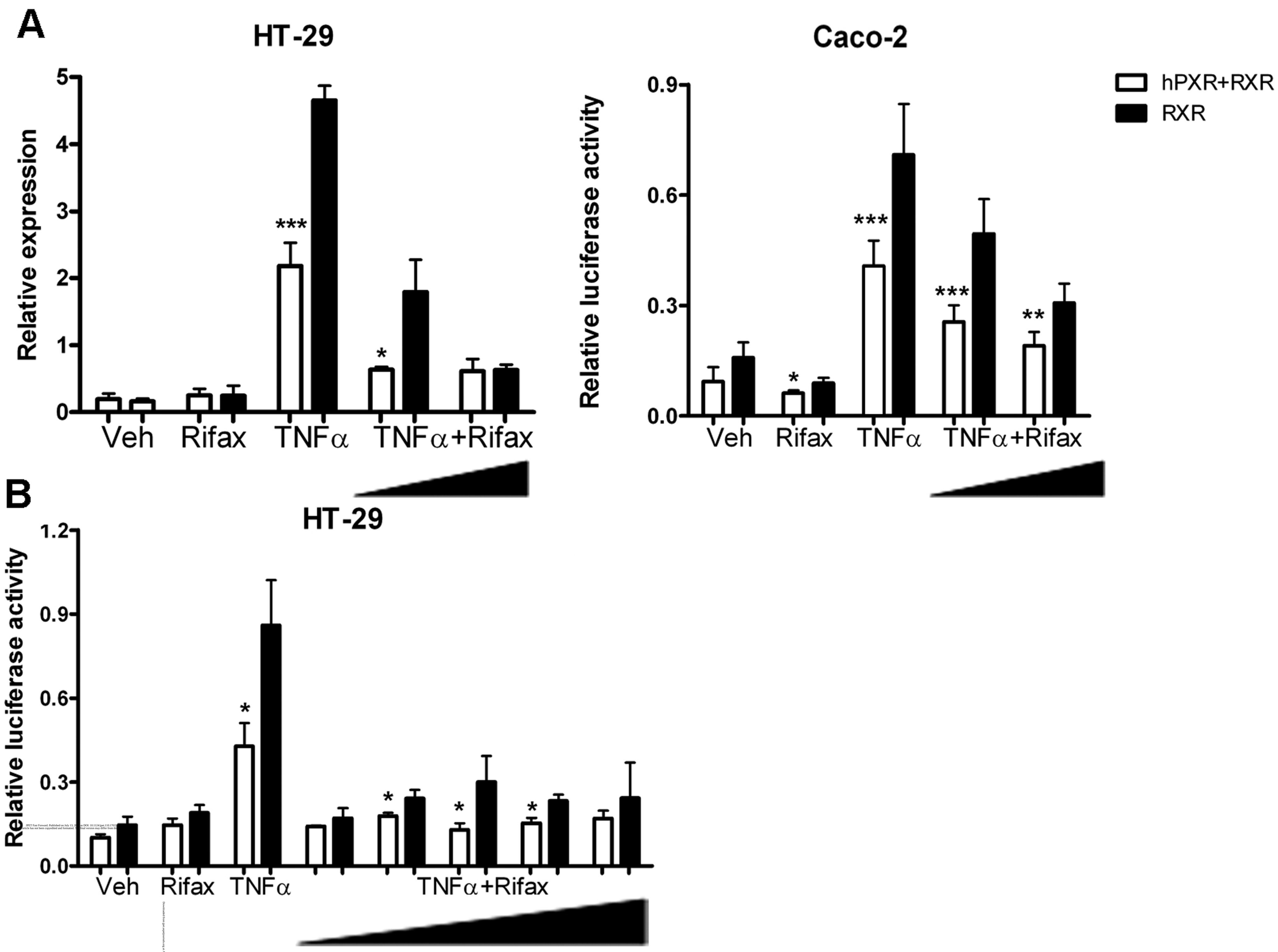


Figure 8

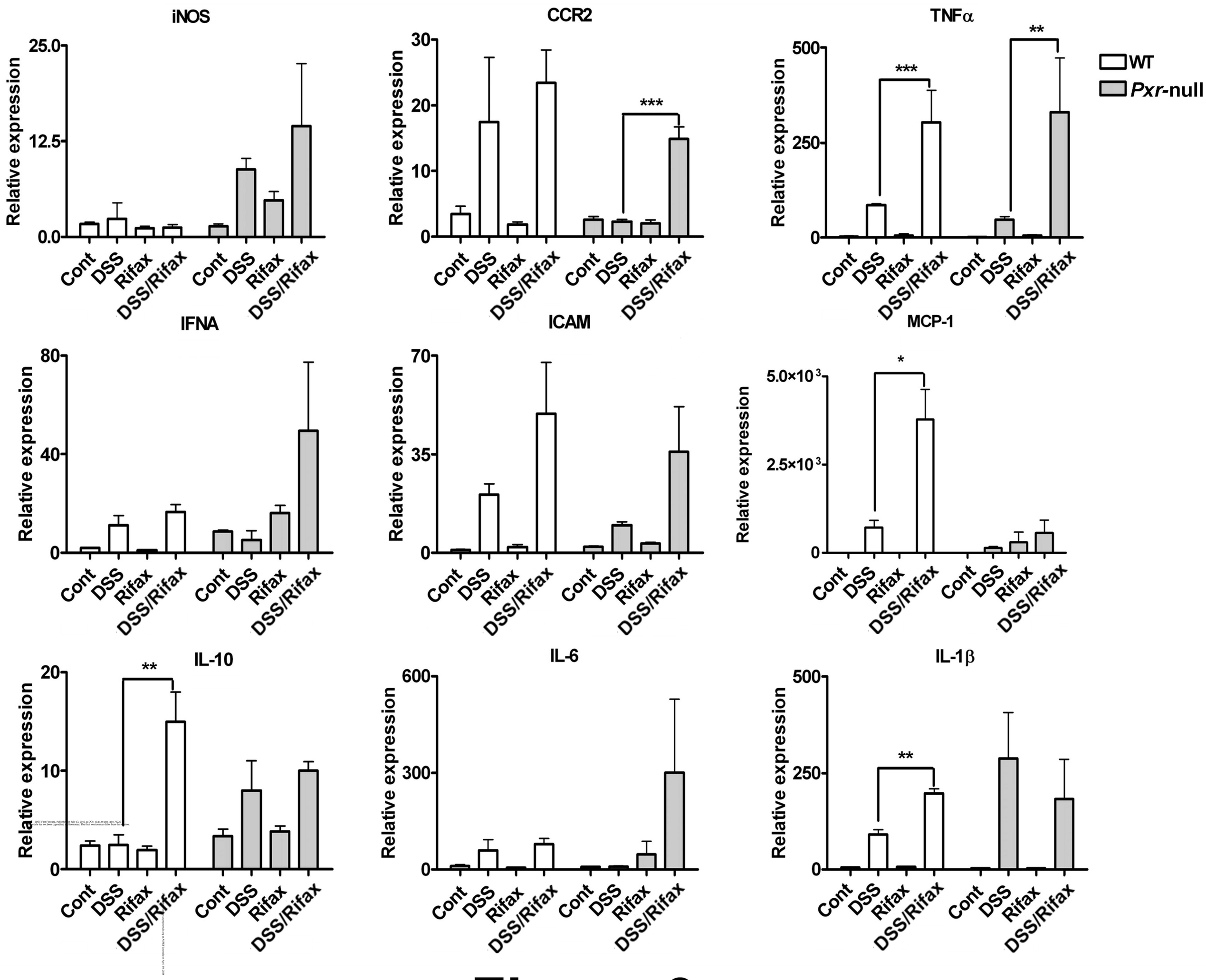

iNOS
CCR2
TNFα
IFNA
ICAM
MCP-1
IL-10
IL-6
IL-1β
Relative expression
Cont
DSS
Rifax
DSS/Rifax
WT
Pxr-null
***
**
*

Figure 9