

Selective AhR Activation Suppresses Epithelial Chemokine Production and Promotes Barrier Repair Responses in Translational Models of Ulcerative Colitis

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Introduction

- The intestinal epithelium is a highly dynamic, single-cell barrier that maintains gut homeostasis by regulating permeability and coordinating mucosal defense. In ulcerative colitis (UC), disruption of epithelial barrier integrity leads to increased permeability, microbial translocation, chronic inflammation, and tissue injury¹.
- The Aryl Hydrocarbon Receptor (AhR) is a ligand-activated transcription factor that integrates environmental and microbial signals to regulate intestinal immune homeostasis and epithelial barrier integrity¹. Due to its role in gut barrier function, inflammation, and immune cell differentiation, AhR activation is currently being investigated as a potential therapeutic strategy for ulcerative colitis (UC).²⁻³
- Activation of AhR in intestinal epithelial cells induces canonical target genes such as CYP1A1, enhances epithelial repair through upregulation of IL22RA1 and IL-22 signaling, modulates tight junction pathways, and suppresses pro-inflammatory mediators driven by NF-κB and interferon signaling^{1,4}.
- Together, these activities support barrier integrity and mucosal healing⁵⁻⁶. Furthermore, preclinical studies indicate that AhR signaling promotes epithelial regeneration and barrier restoration in intestinal inflammation, at least in part through IL-22-dependent mechanisms⁷⁻⁸.

EQ504 is a potent and selective AhR modulator, a synthetic derivative of the endogenous AhR ligand ITE, designed to restore epithelial barrier function and promote mucosal homeostasis. In this study, we evaluated the effects of EQ504 across complementary in vitro and in vivo models, including human intestinal epithelial cells, human intestinal organoids, and DSS-induced colitis mice.

We demonstrate that EQ504 engages the AhR pathway, promotes epithelial repair and barrier restoration, and exhibits superior potency and efficacy compared with clinically validated AhR modulators.

Methods

Mouse DSS in vivo Study

Efficacy of EQ504 (0.1 and 1mg/kg) was tested by systemic delivery (i.p.) in a mouse DSS-colitis model together with Indirubin (10mg/kg). Body weight was measured daily. Colon weight and length was assessed at necropsy. Tissue analysis included colon histology and gene expression (CYP1A1, IL-10, IL-22) from colon, liver, spleen, and blood. Cyclosporin A (CSA) was used as the positive control. The study and endpoint analyses were performed at Inotiv.

Human Intestinal Organoids and FITC-Dextran (FD4 flow) assay

Human intestinal organoids (Sigma) were seeded as 2D monolayers and maintained in organoid growth medium (OGM) for 4 days before switching to organoid differentiation media (ODM) for another 4 days to induce differentiation. After 8 days, confluency was determined by TEER, and wells with values over 1000Ω were selected for treatment. Organoids were exposed to AhR agonists in ODM 1hour prior to the cytokine challenge. The polarized epithelium was then challenged with 20ng/ml TNFα and 10ng/ml IFNγ to induce barrier damage. FITC-dextran 1mg/ml is added at apical compartment and reading performed at 4, 24, and 48hour by taking aliquots from basolateral chamber, diluting 1/10 with PBS in black-walled, clear-bottom 96-well assay plate, and fluorescence read at 475nm/530nm. Genes expression assessed by SYBR Green qPCR after 48 hours of treatment.

RepliGut® System

Primary human transverse colon epithelial cells were plated in Altis RepliGut® Growth Media (RGM) using Altis-established protocols onto 1 x 96-well RepliGut® transwell plate coated with a thin layer of hydrogel. After 4 days, cells became confluent and were shifted to Maturation Media (RMM). After 48 hours in RMM the cells were pre-treated for 2 hours with the compounds before adding cytokines. At 48 hours after cytokine treatment, apical and basolateral media were recovered and the basolateral was tested for Meso Scale Discovery (MSD), while the cells were lysate for mRNA analysis (SYBR Green qPCR).

Transepithelial Electrical Resistance (TEER)

Caco2 cells were plated on 12mm- 0.4mm Polycarbonate membrane inserts and the EVOM instrument was used to record the TEER. The barrier was considered formed when the TEER values were above 800Ω. Polarized Caco2 were pre-treated with GNF-351 for 1 hours before adding the AhR agonists. 2h after the AhR agonists were added, the cells were treated with IL-6, IFNγ and TNFα for up to 72h and gene expression analyzed from cell lysate at the end of the study by SYBR Green qPCR.

Scratch assay

T84 cells were plated on 24-well TC treated plates for 10 days until confluence. Once confluent, the cell monolayer was scratched using a 20μl tip, and the cells were treated with EQ504, Indirubin or Vehicle. The cells were imaged at day 3 and collected at day 6 for gene expression analysis by SYBR Green qPCR.

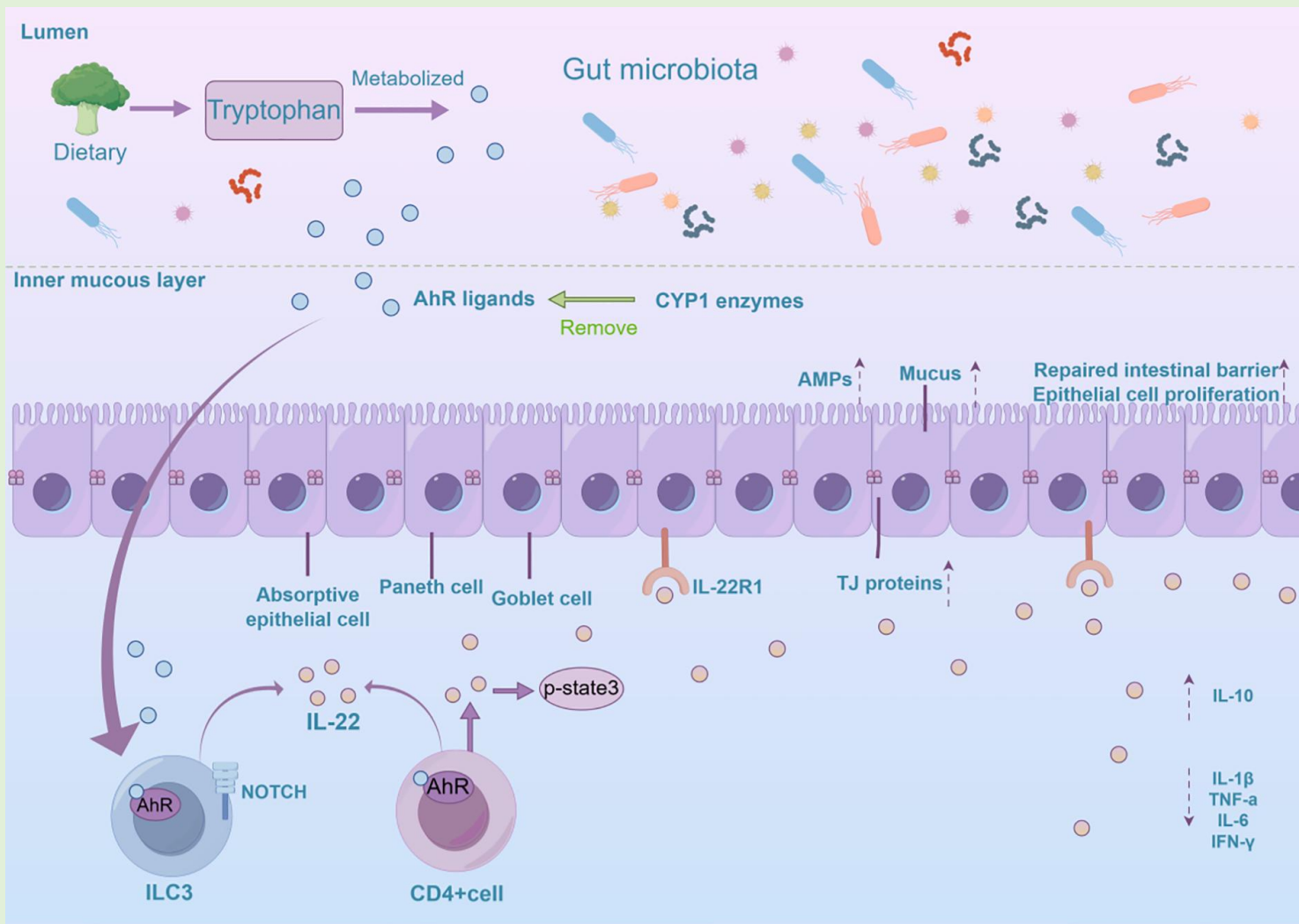


Figure 1. Mechanistic model of AhR signaling in intestinal homeostasis⁸.

Results

EQ504 protects barrier integrity in human intestinal organoids derived from both normal- and UC- patients

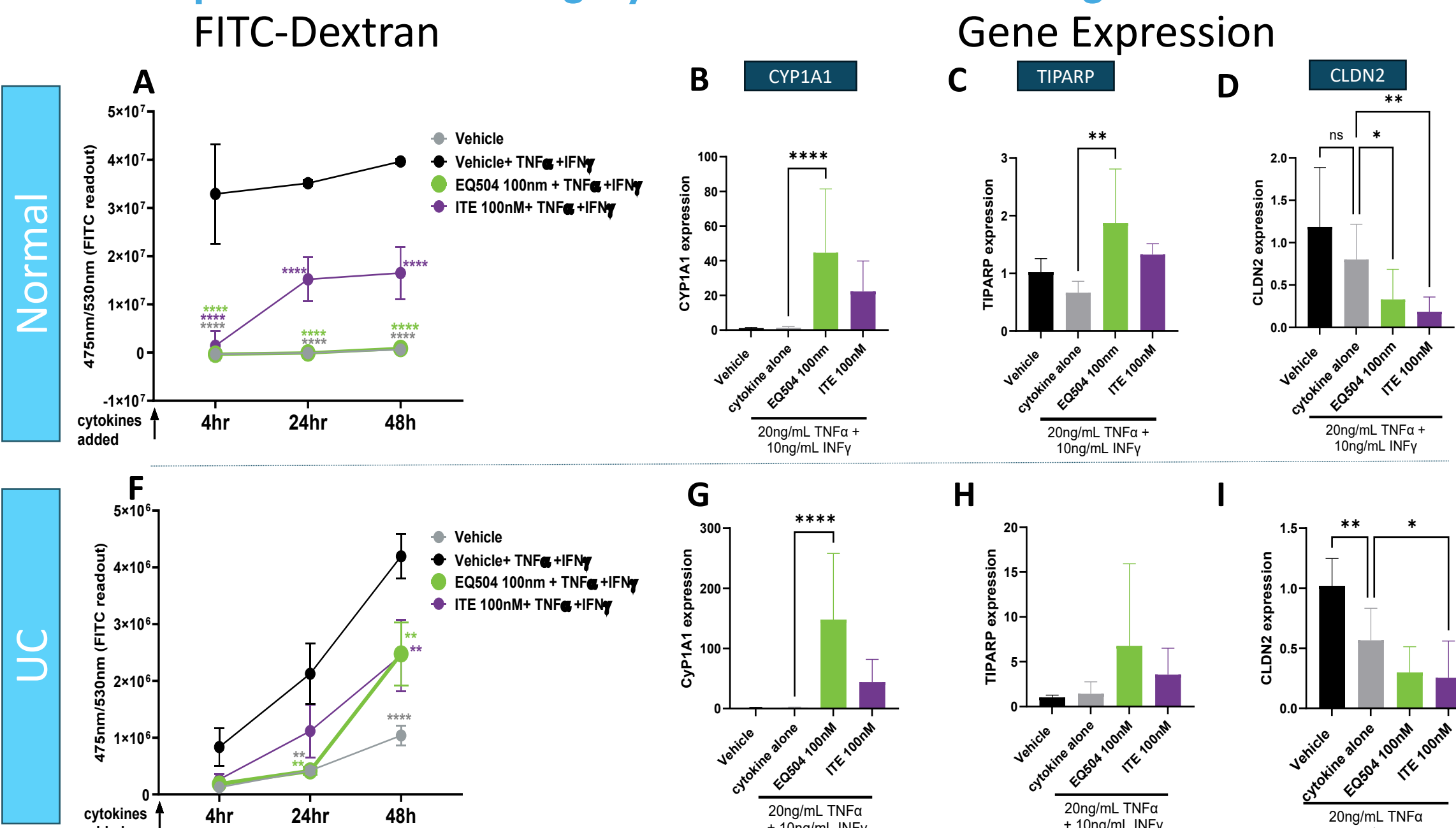


Figure 5. Intestinal Human-Derived Organoids (A,F) FD4 flow at 4, 24 and 48 hours after cytokine treatment, in Normal (A) and UC (F) intestinal organoids treated with 20ng/ml TNFα and 10ng/ml IFNγ 10ng/ml and pre-treated with EQ504 or ITE 100nM for 1h before the cytokine challenge. Gene expression analysis of CYP1A1 (B,G), TIPARP (C,H), and CLDN2 (D,I) after 48 hours of cytokine challenge from normal (B-E) and UC (G-L) organoids. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 vs Cytokines by One-way ANOVA.

EQ504 prevents CXCL11 and IL-8 production induced by TNF-alpha in a translational model of gut epithelium (RepliGut® System)

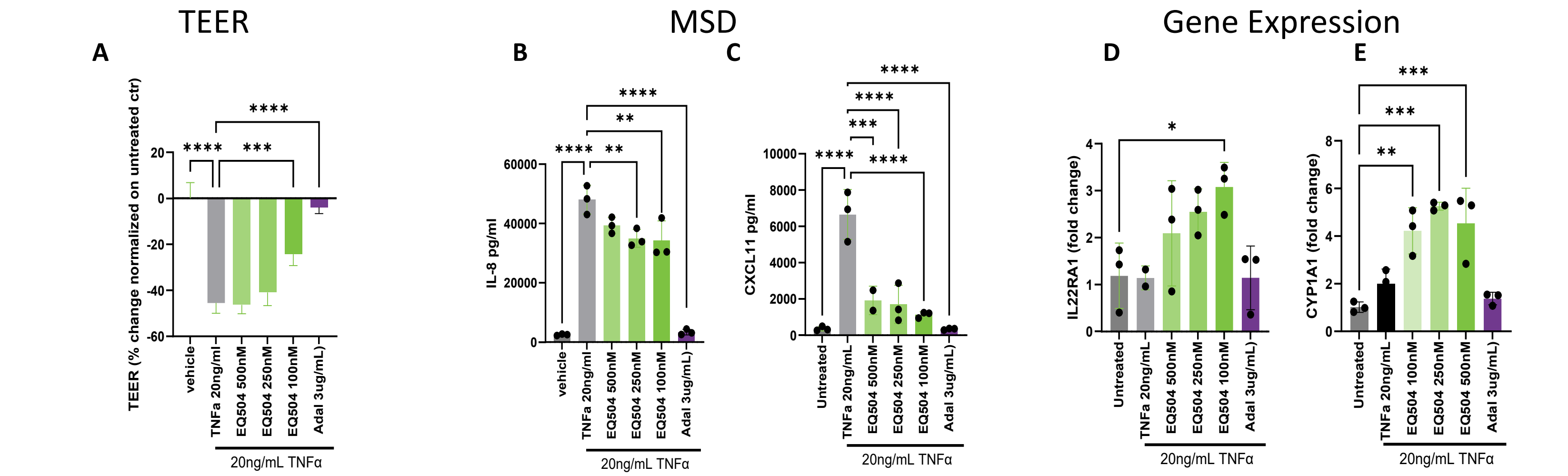


Figure 6. RepliGut (A) TEER Analysis after 48 hours of TNFα 20ng/ml treatment, alone or in the presence of different concentration of EQ504 or Adalimumab 30μg/mg. (B-C) MSD analysis of IL-8 and CXCL11 release in Apical media after 48h. (D-E) Gene expression analysis of IL22RA1 and CYP1A1 by qPCR after 48h of treatment. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 vs TNFα.

EQ504 protection of barrier integrity is AhR-dependent

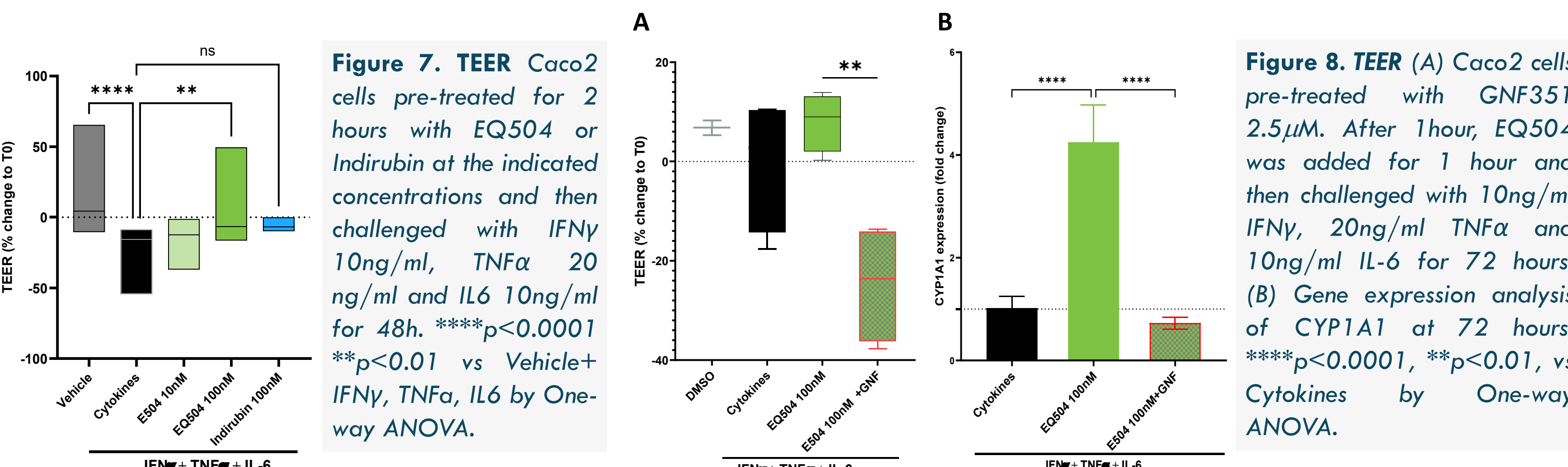


Figure 8. TEER (A) Caco2 cells pre-treated with GNF351 2.5μM. After 1hour, EQ504 was added for 1 hour and then challenged with 10ng/ml IFNγ, 20ng/ml TNFα and 10ng/ml IL-6 for 72 hours. (B) Gene expression analysis of CYP1A1 at 72 hours. ****p<0.0001, **p<0.01, vs Cytokines by One-way ANOVA.

EQ504 promotion of epithelial regeneration is AhR-dependent

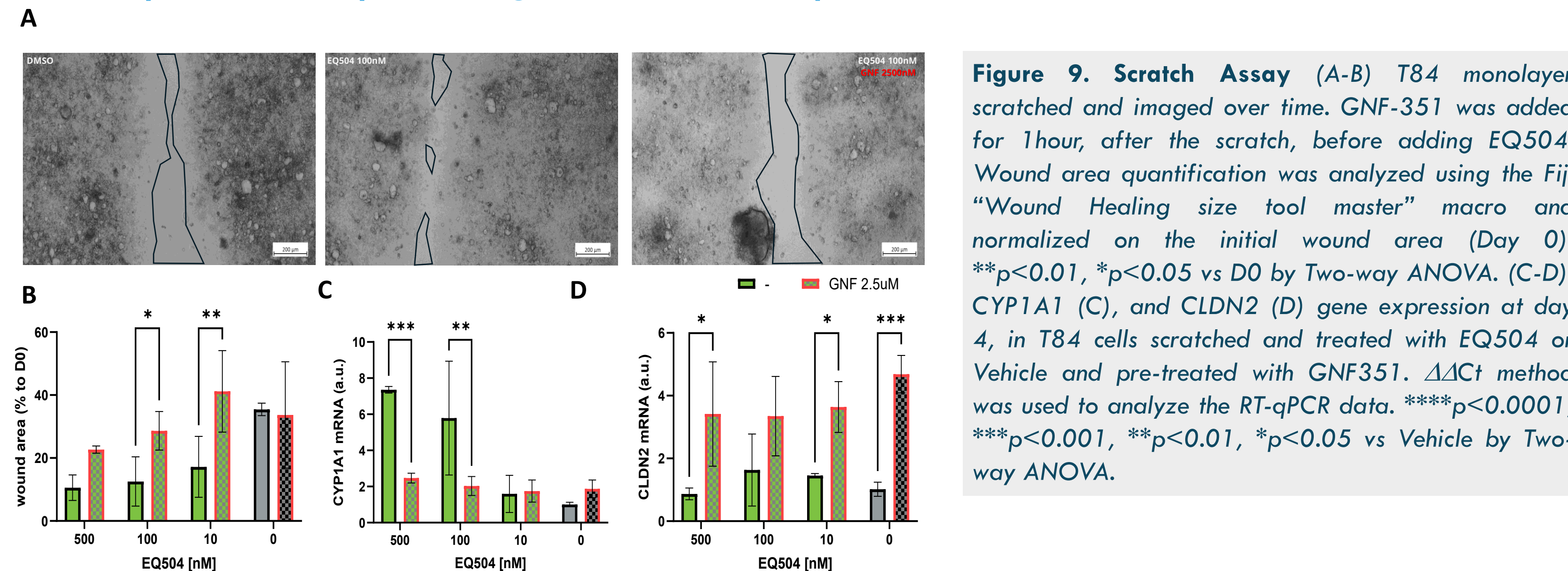


Figure 9. Scratch Assay (A-B) T84 monolayer scratched and imaged over time. GNF-351 was added for 1hour, after the scratch, before adding EQ504. Wound area quantification was analyzed using the Fiji "Wound Healing size tool master" macro and normalized on the initial wound area (Day 0). **p<0.01, *p<0.05 vs D0 by Two-way ANOVA. (C-D) CYP1A1 (C), and CLDN2 (D) gene expression at day 4, in T84 cells scratched and treated with EQ504 or Vehicle and pre-treated with GNF351. ΔΔCt method was used to analyze the RT-qPCR data. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05 vs Vehicle by Two-way ANOVA.

Results

EQ504 engages the AhR pathway and protects against body weight loss in DSS-induced colitis

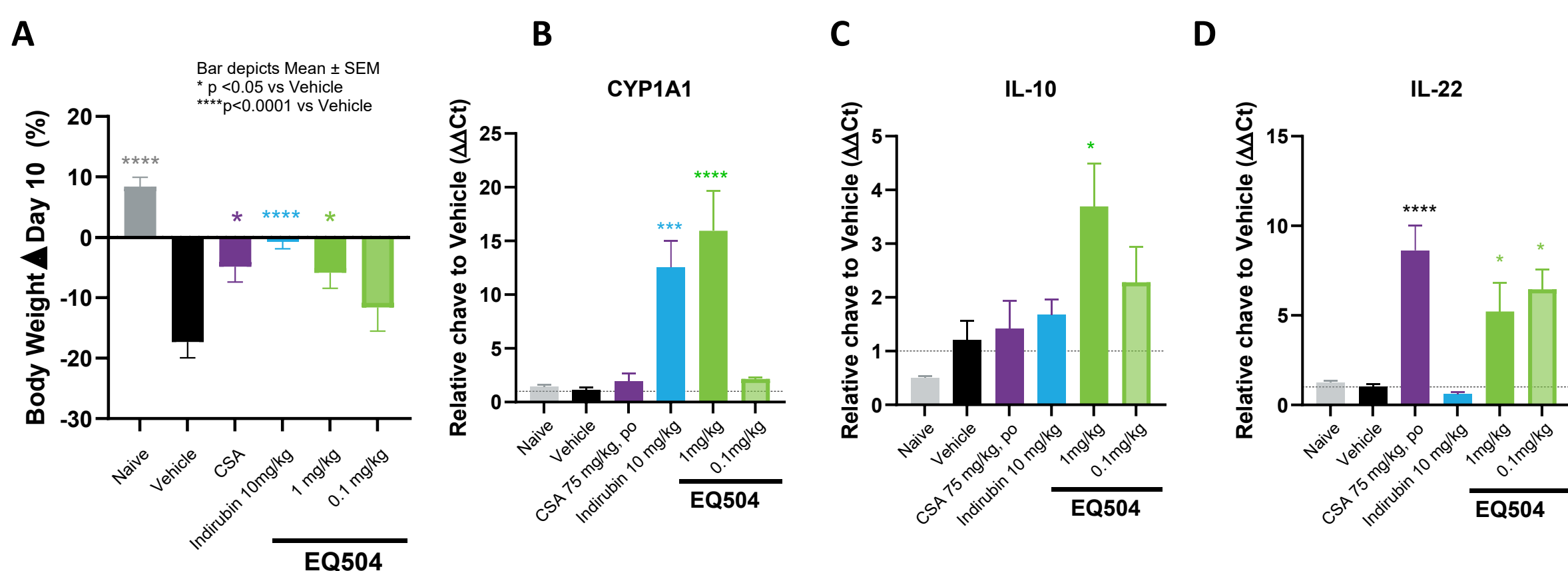


Figure 4. DSS Mouse Model (A) Treatment with 1mg/kg EQ504 protects against a significant reduction in body weight due to DSS-induced colitis, comparable to 75mg/kg Cyclosporin. (B-D) EQ504 at 1mg/kg significantly upregulated gene expression of CYP1A1, IL-10 and IL-22 in the colon compared to vehicle. (*p<0.05, ***p<0.0001 vs. vehicle by One-Way ANOVA.

EQ504 ameliorates pathology during DSS-induced colitis

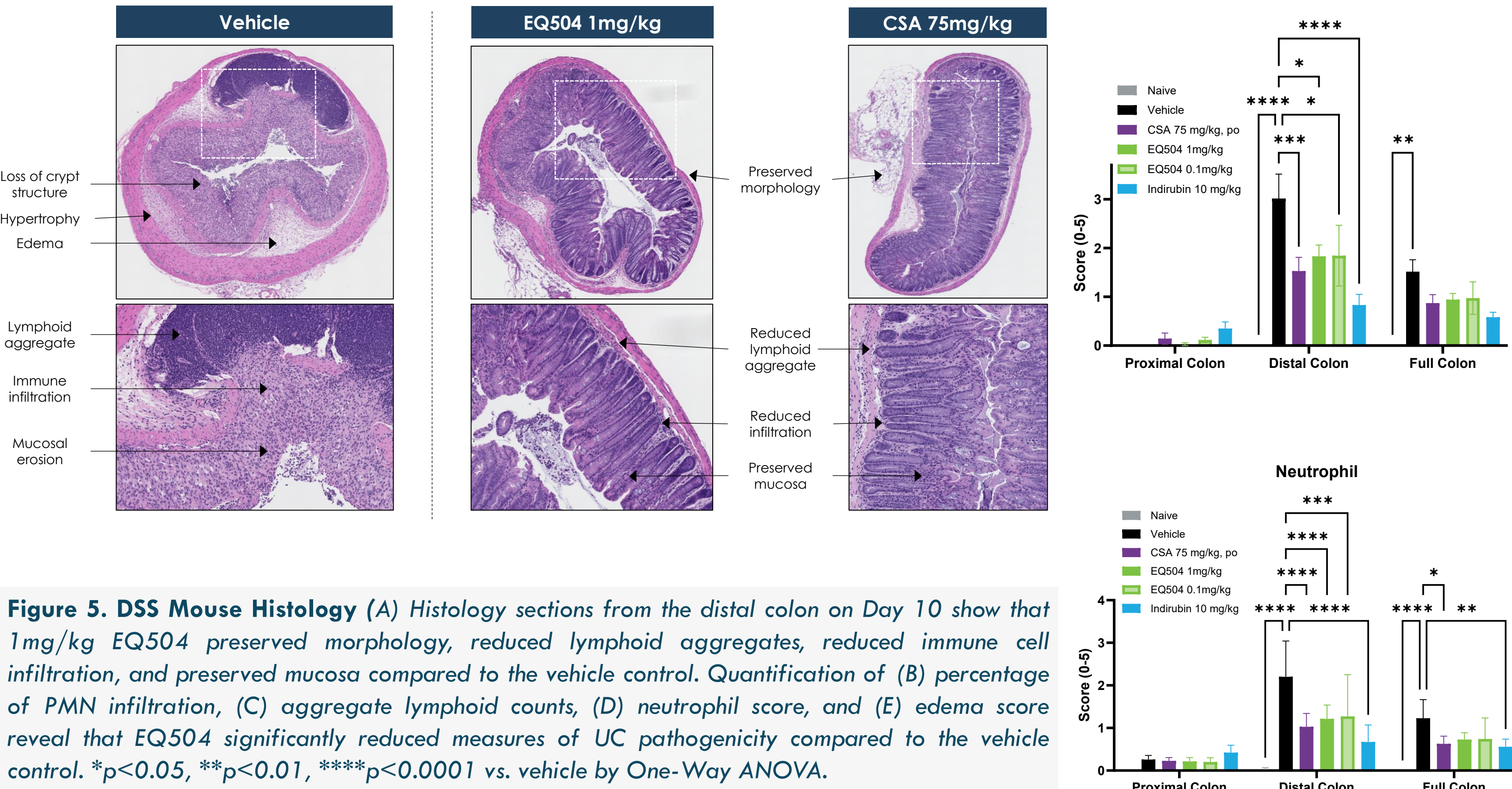


Figure 5. DSS Mouse Histology (A) Histology sections from the distal colon on Day 10 show that 1mg/kg EQ504 preserved morphology, reduced lymphoid aggregates, reduced immune cell infiltration, and preserved mucosa compared to the vehicle control. Quantification of (B) percentage of PMN infiltration, (C) aggregate lymphoid counts, (D) neutrophil score, and (E) edema score reveal that EQ504 significantly reduced measures of UC pathogenicity compared to the vehicle control. *p<0.05, **p<0.01, ****p<0.0001 vs. vehicle by One-Way ANOVA.

Conclusions

- EQ504 protects intestinal barrier integrity in the DSS mouse model of colitis.
- EQ504 induces IL-10 and IL-22 gene expression more strongly than indirubin, supporting modulation of immune pathways associated with intestinal homeostasis and repair.
- EQ504 protects against cytokine-induced barrier disruption in both normal and UC patient-derived intestinal organoids. This effect is associated with activation of the AhR pathway and downregulation of CLDN2, a pore-forming tight-junction protein associated with increased epithelial permeability.
- In the RepliGut system, EQ504 preserves epithelial barrier integrity while reducing the release of the inflammatory chemokines CXCL11 and IL-8.
- EQ504-mediated barrier protection is AhR-dependent, as demonstrated by loss/reduction of the protective effect in the presence of the AhR antagonist GNF-351.
- Together, these findings demonstrate that EQ504 engages AhR to promote intestinal barrier integrity through complementary epithelial and immune-modulatory mechanisms.
- The combined in vitro, human ex vivo, and in vivo data support the therapeutic potential of EQ504 in ulcerative colitis

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References

- Xiuchuan Hu et al., Nature Communications, 2023
- Goettel et al., Cell Reports, 2016
- Matsumoto et al., Intestinal Research, 2022
- Gustavo Henrique Olivera da Rocha et al., Int. J. Mol. Sci., 2024
- Julie P Saiki et al., BMJ Open Gastroenterol., 2021
- Carolina M. Polonio et al., Nature Reviews, 2025
- Marafini Irene et al., Internal Journal of Molecular Sciences, 2024
- Hiumin Kang et al., Front. Immunol., 2025

Disclosures

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