

AhR Modulation by EQ504 Restores Immune Balance by Suppressing Pathogenic Th17/M1 Responses While Enhancing Regulatory Pathways

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Introduction

- The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that maintains intestinal immune homeostasis through regulation of T cells and macrophages.
 - AhR activation promotes T_{reg} responses, supports anti-inflammatory macrophage phenotypes, and induces IL-10 and IL-22, which suppress inflammation and enhance epithelial repair^{1,2}.
 - Because AhR signaling is reduced in IBD, loss of AhR activity may contribute to excessive M1 macrophage cytokine production, pathogenic T_h17 responses, and chronic intestinal inflammation³.
 - Together, these activities make AhR a promising target for restoring immune balance in inflammatory diseases.
 - However, despite its therapeutic potential in inflammatory bowel disease (IBD), progress in developing AhR modulators has been constrained by the lack of potent, selective, drug-like compounds^{4,5}.
- We evaluated EQ504, a novel small-molecule AhR modulator derived from the endogenous ligand ITE. Human immune cells were treated with compound to assess AhR activation, T_{reg}/T_h17 cell differentiation, and macrophage polarization. Cytokine production of IL-10 and IL-22 and phenotypic markers were analyzed to define immunoregulatory outcomes.

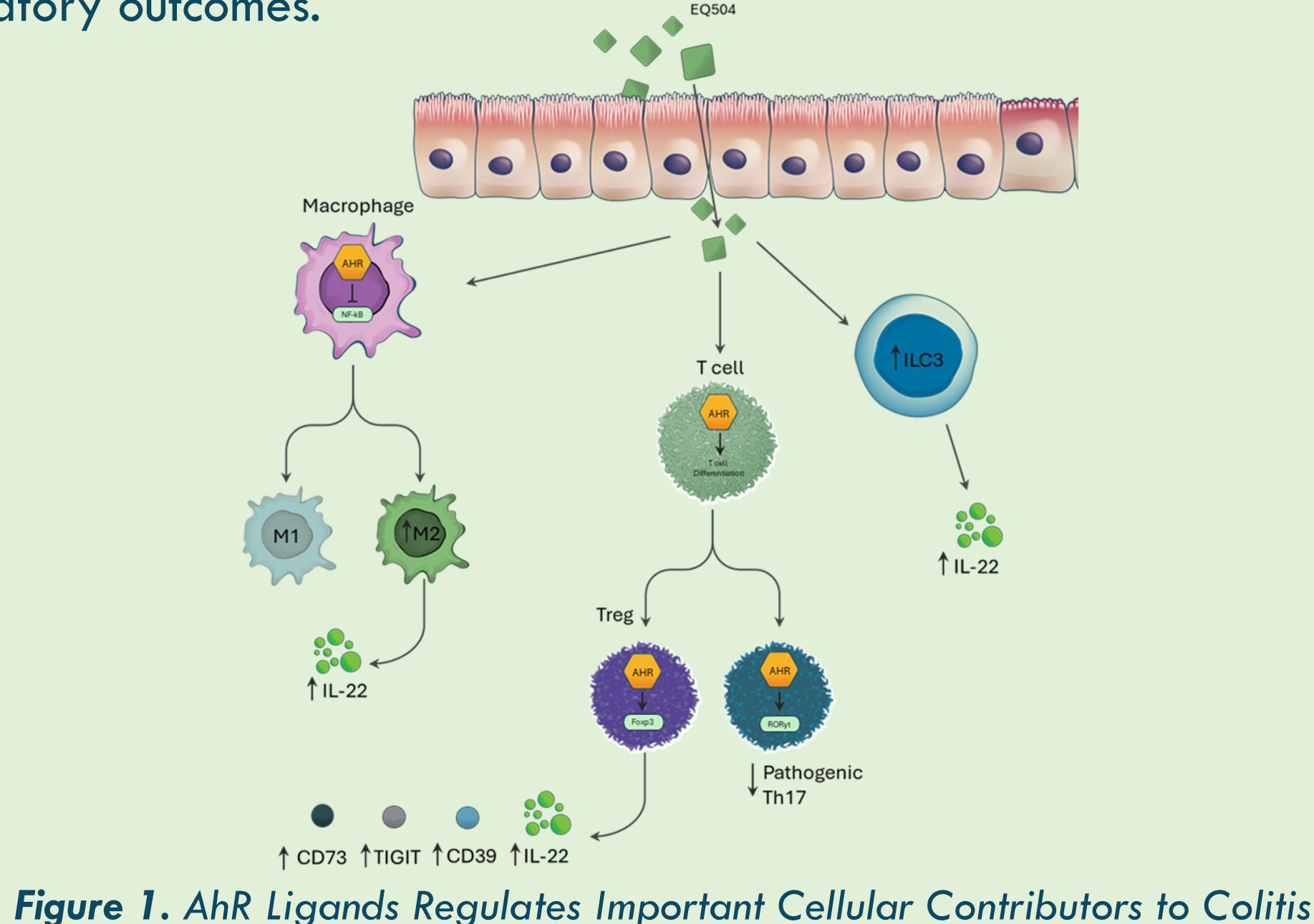


Figure 1. AhR Ligands Regulates Important Cellular Contributors to Colitis

Methods

Differentiated T_h17 and polarized M1 Macrophages: To differentiate T_h17 cells, isolated naïve T cells from total PBMCs were treated with IL-1β, IL-6, TGF-β, IL-23 and anti-CD3/CD28 stimulation for a total of 7 days with EQ504, vehicle control and other agonists. To induce M1 macrophage differentiation, monocytes were isolated from total PBMCs. GM-CSF was introduced along with EQ504 and Vehicle control for 5 days. At day 5 there was a media refresh and the addition of LPS and IFNγ to further drive polarization. Cells were then evaluated at day 8. M1s were assessed primarily by cytokine.

Differentiated Tregs (iT_{reg}s) and polarized M2 Macrophages: To differentiate iTregs, isolated naïve T cells were treated with a Treg differentiation cocktail (STEMCELL) and anti-CD3/CD28 stimulation for a total of 7 days with EQ504, Vehicle control and other agonists. To induce M2 macrophage differentiation, monocytes were isolated from total PBMCs. MCSF was introduced along with EQ504 and Vehicle control for 5 days. At day 5 there was a media refresh and the addition of IL-6, IL-4 and IL-13 to further drive polarization. Cells were then evaluated at day 8. M2s were assessed by IL-10 and IL-22 genes/protein expression and functional outcome by cytokine.

Results

EQ504 Decreases the Release of IL-17A While Inducing IL-22 of Differentiated T_h17

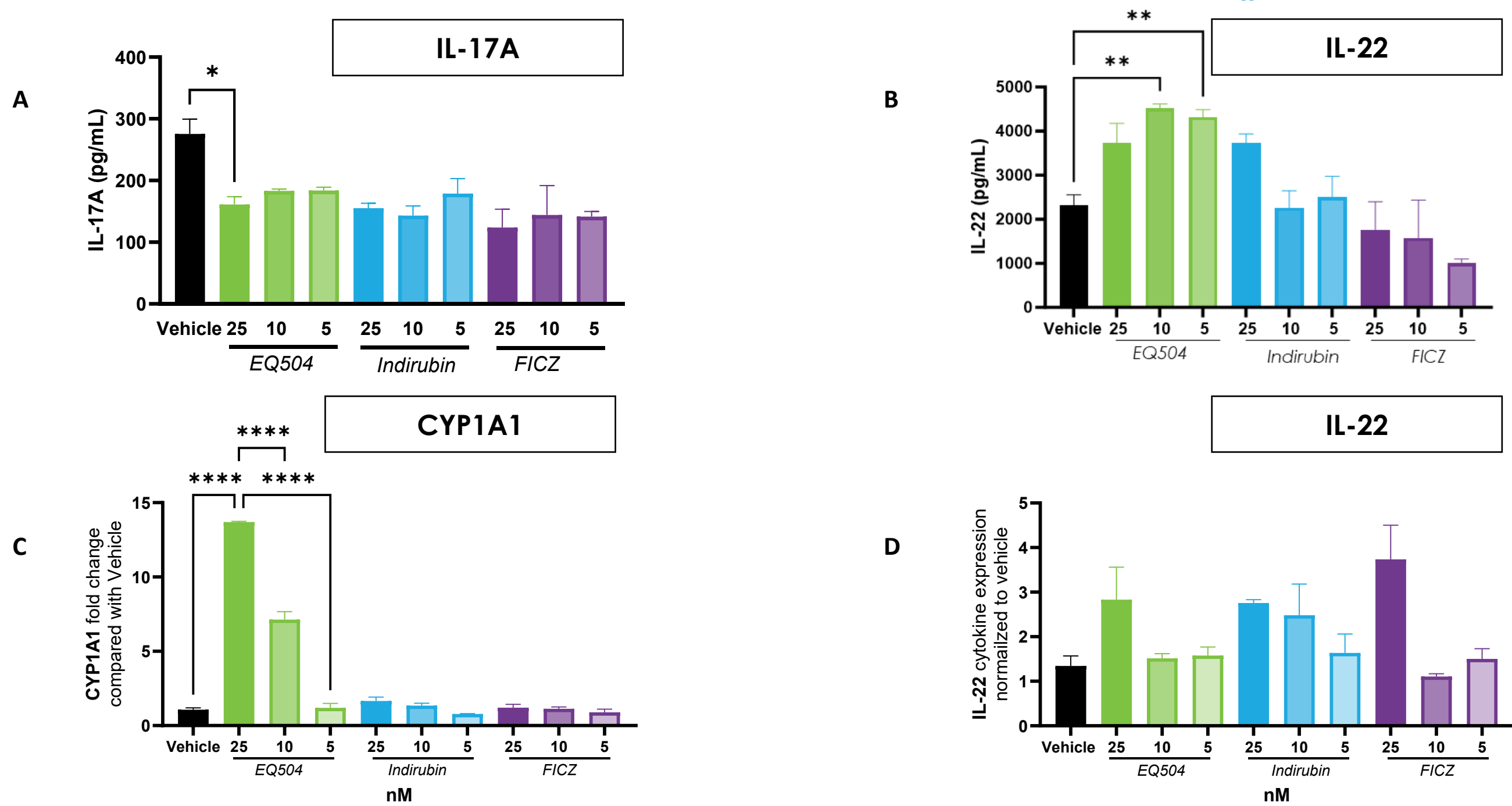


Figure 2. Differentiated naïve T cells treated with the IL-17 standard cocktail and anti-CD3/CD28 stimulation for 7 days in the presence of EQ504, Indirubin or FICZ. (A) EQ504 decreases IL-17A while inducing (B) IL-22 cytokine release and (D) gene expression. (C) EQ504 induces CYP1A1, a gene regulated by the AhR pathway. *****p*<0.00001, ****p*<0.0001, ***p*<0.001, **p*<0.005 by Ordinary one-way ANOVA.

EQ504 Significantly Inhibits Key Pro-inflammatory Cytokine Release in M1 Macrophages

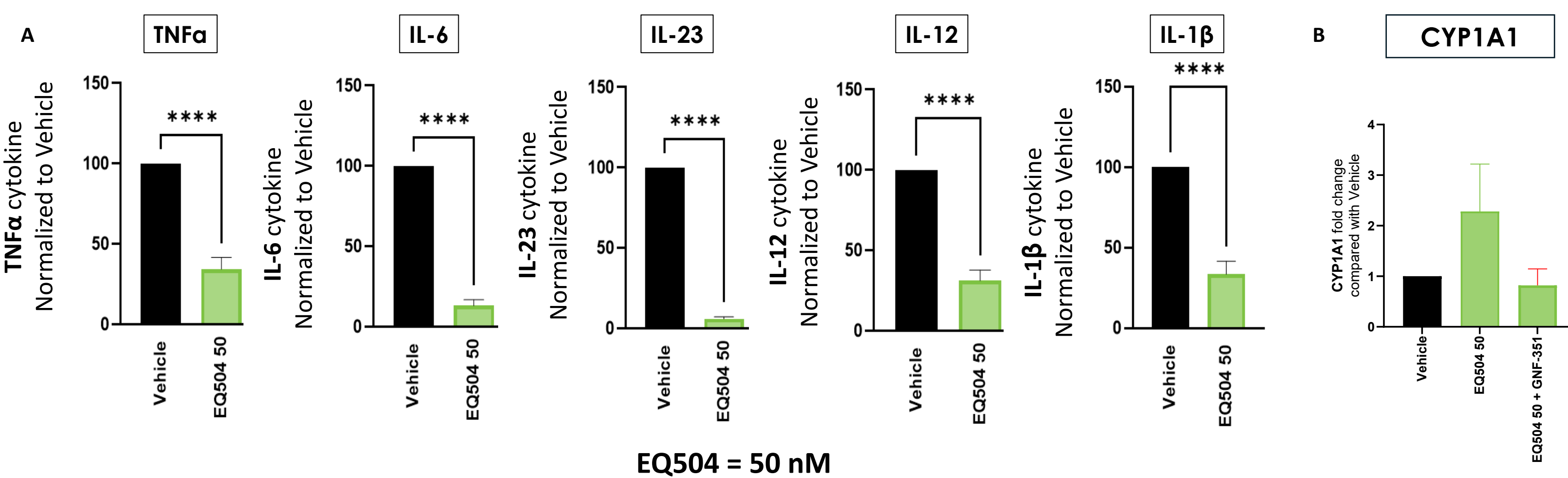


Figure 3. Isolated monocytes polarized towards an M1 phenotype with EQ504 for a total of 8 days. (A) EQ504 decreased several pro-inflammatory cytokines, thereby attenuating downstream T_h1/T_h17 responses that sustain chronic intestinal inflammation in IBD. (B) Target engagement by CYP1A1 is AhR dependent. Test article plus AhR antagonist GNF-351 shown in red. *****p*<0.00001 by Welch's *t*-test.

Results

EQ504 Modulates Cytokine and AhR Signaling in M2 Macrophages

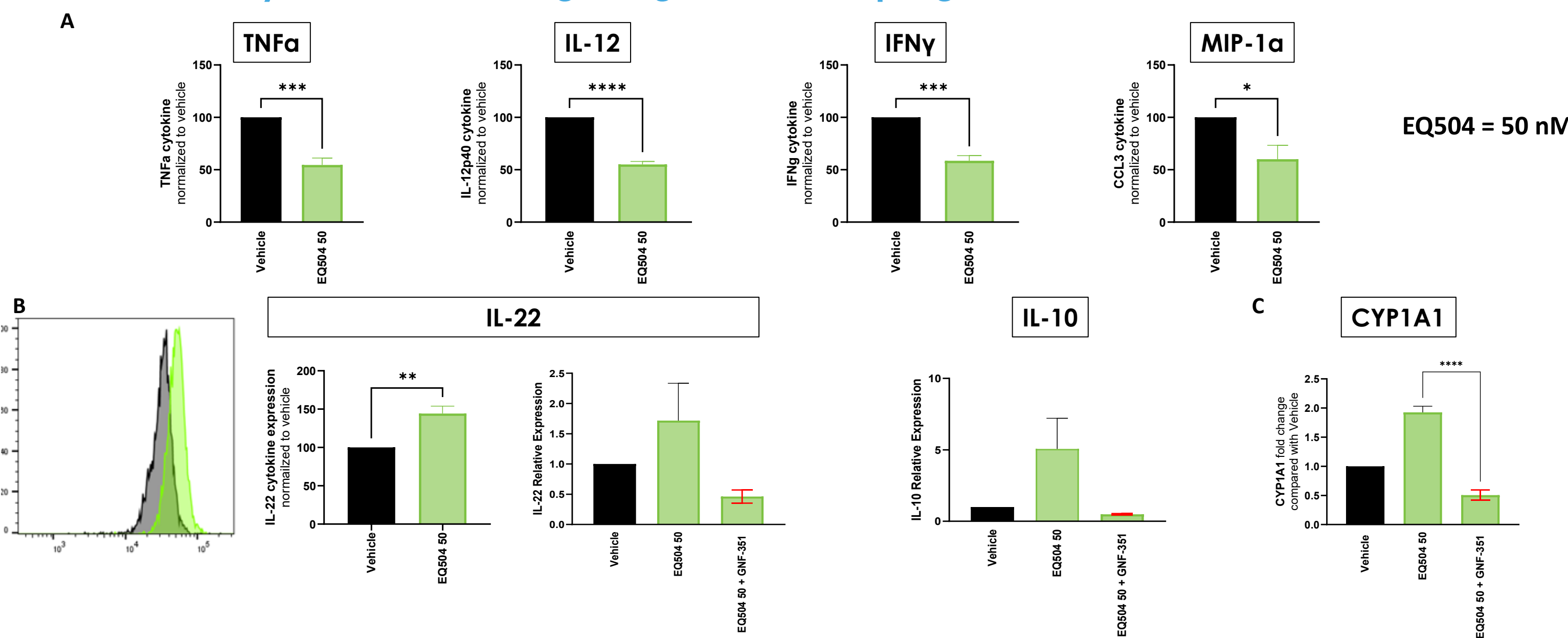
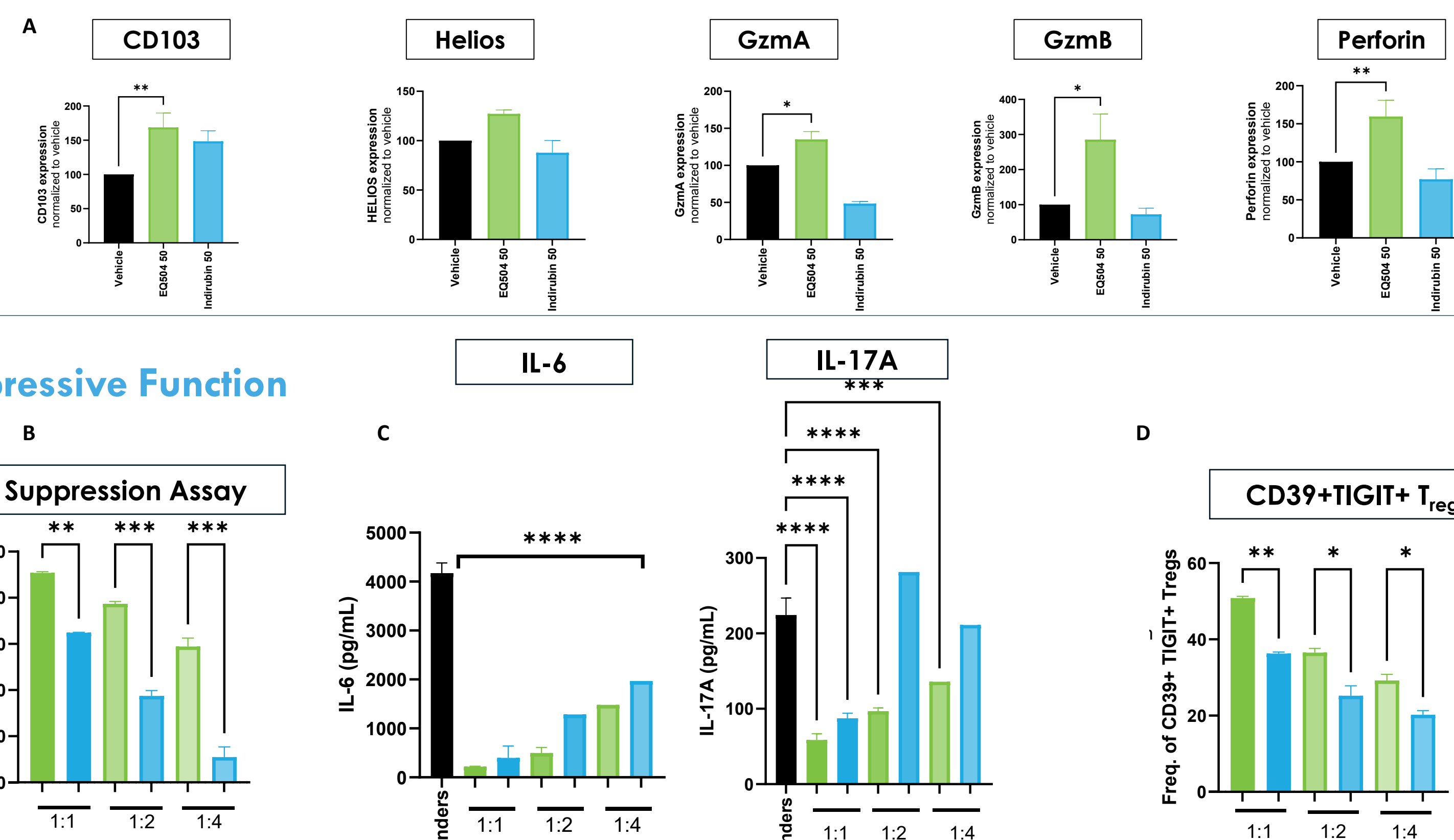


Figure 4. Isolated monocytes polarized towards an M2 phenotype with EQ504 for a total of 8 days. (A) EQ504 decreased several pro-inflammatory cytokines. (B) EQ504 increases IL-22 cytokine release confirmed by FACS and gene expression. Macrophage-derived IL-10 promotes intestinal mucosal wound healing⁶. IL-10 and IL-22 is AhR-dependent. (C) AhR engagement confirmed through CYP1A1 gene expression. Induction of CYP1A1 in M2 macrophages by EQ504 is AhR-dependent. *****p*<0.00001, ****p*<0.0001, ***p*<0.001, **p*<0.005 by Welch's *t*-test. Test article plus AhR antagonist GNF-351 shown in red.

EQ504 Programs Gut-Homing Regulatory T Cells with Enhanced Suppressive Function



IL-10, IL-22 and Target Engagement by CYP1A1 are AhR-dependent

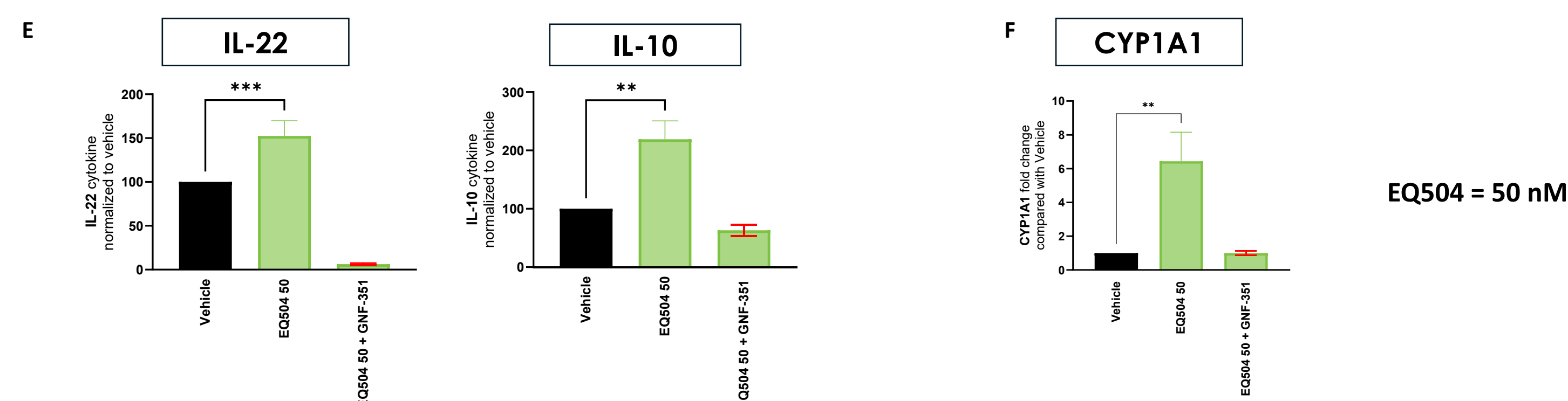


Figure 5. Gut homing: Naïve T cells treated with a standard T_{reg} cell differentiation cocktail and anti-CD3/CD28 stimulation for a total of 7 days with EQ504 or Indirubin. (A) Cells were assessed for markers associated with colonic tissue resident T_{reg} cells such as CD103, Helios, Granzyme A and B as well as Perforin^{7,8}. This data supports that T_{reg} cells differentiated with EQ504 express greater CD103 with suppressive activity which is required for T_{reg} cell localization^{7,8}. **Suppression Assay:** (B) Tresponders of the same donor were labeled with Cell Trace Violet. iT_{reg}s differentiated for 7 days were co-cultured with Tresponders at 1:1, 1:2, and 1:4 Tregs:T_{reg} ratio. Suppression assay was stimulated with CD3/CD28 for 4 days. Suppression of T_{reg}s differentiated with EQ504 have greater suppressive ability over Indirubin. Suppressive activity can also be observed by inhibition of (C) IL-6 and IL-17A. (D) When measuring the iT_{reg}s during suppression they continued to express higher levels of CD39+TIGIT+. **Gene expression and Target Engagement:** We observed an increase in (E) IL-10 and IL-22 during differentiation and suppression. The expression of CD39, TIGIT plus the release of IL-10 and IL-22 could be contributing to the suppressive activity. (F) Induction of CYP1A1 in iT_{reg}s by EQ504 is AhR-dependent. Test article plus AhR antagonist GNF-351 shown in red. *****p*<0.00001, ****p*<0.0001, ***p*<0.001, **p*<0.005 by Ordinary one-way ANOVA or Welch's *t*-test.

Conclusions

- EQ504 driven AhR modulation establishes immune tolerance by orchestrating both adaptive and innate immune responses.
- The simultaneous induction of T_{reg} and M2 associated functions, together with suppression of T_h17 and M1 driven inflammation, highlights the therapeutic potential of AhR targeting in immune-mediated diseases.
- These findings demonstrate EQ504 as a potent AhR modulator with a unique immunoregulatory profile, promoting immune tolerance and tissue repair in cells highly relevant to maintaining intestinal homeostasis and the treatment of IBD.

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Disclosures

This study was funded by Equillum, Inc. All authors are currently employees of Equillum. Jeanette Ampudia is a stockholder of Equillum. Stephen Connelly is currently an employee, stockholder, President and officer of Equillum. To request permission or to ask any questions about the poster, please contact IR@equillum.bio.com.