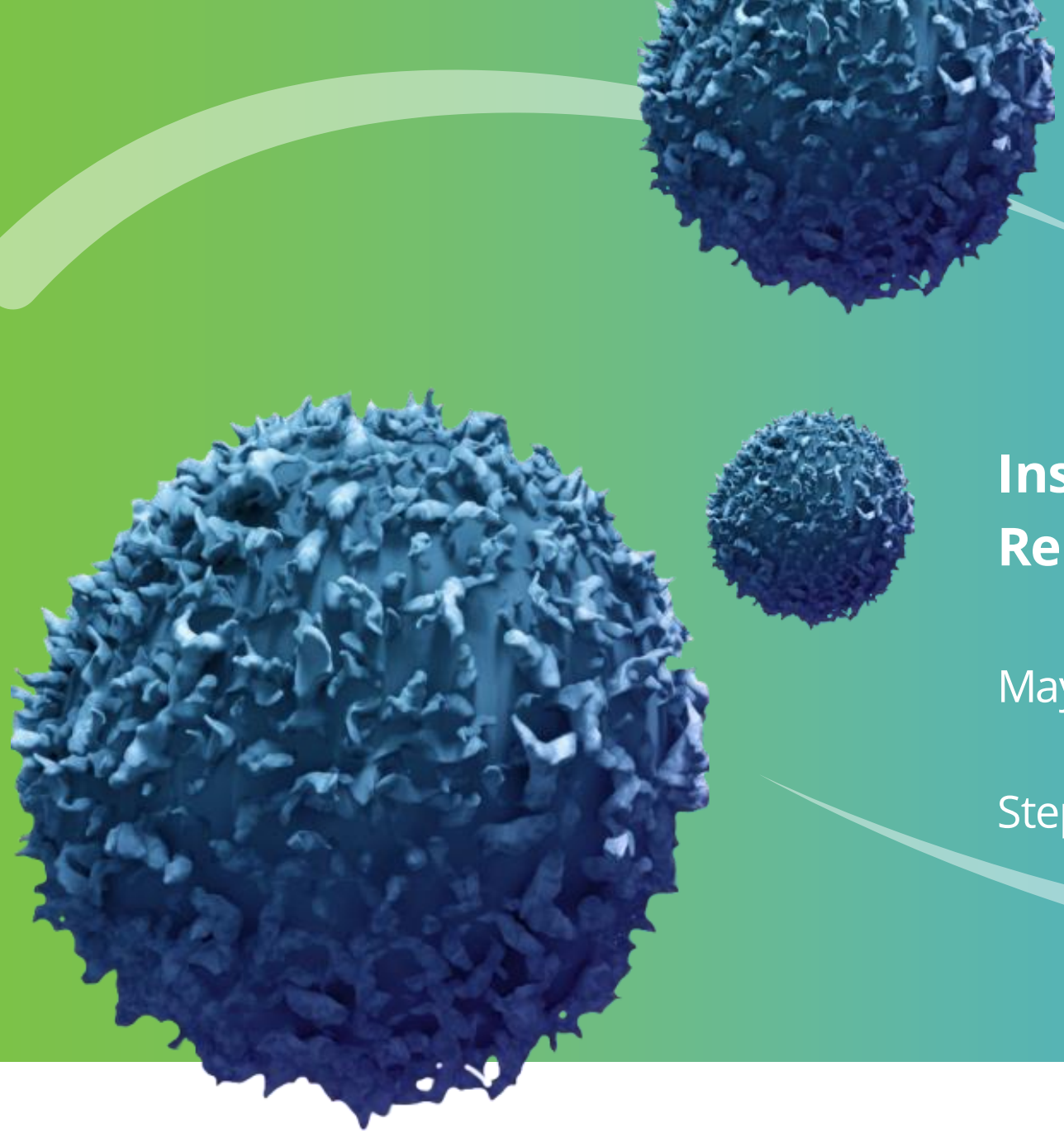
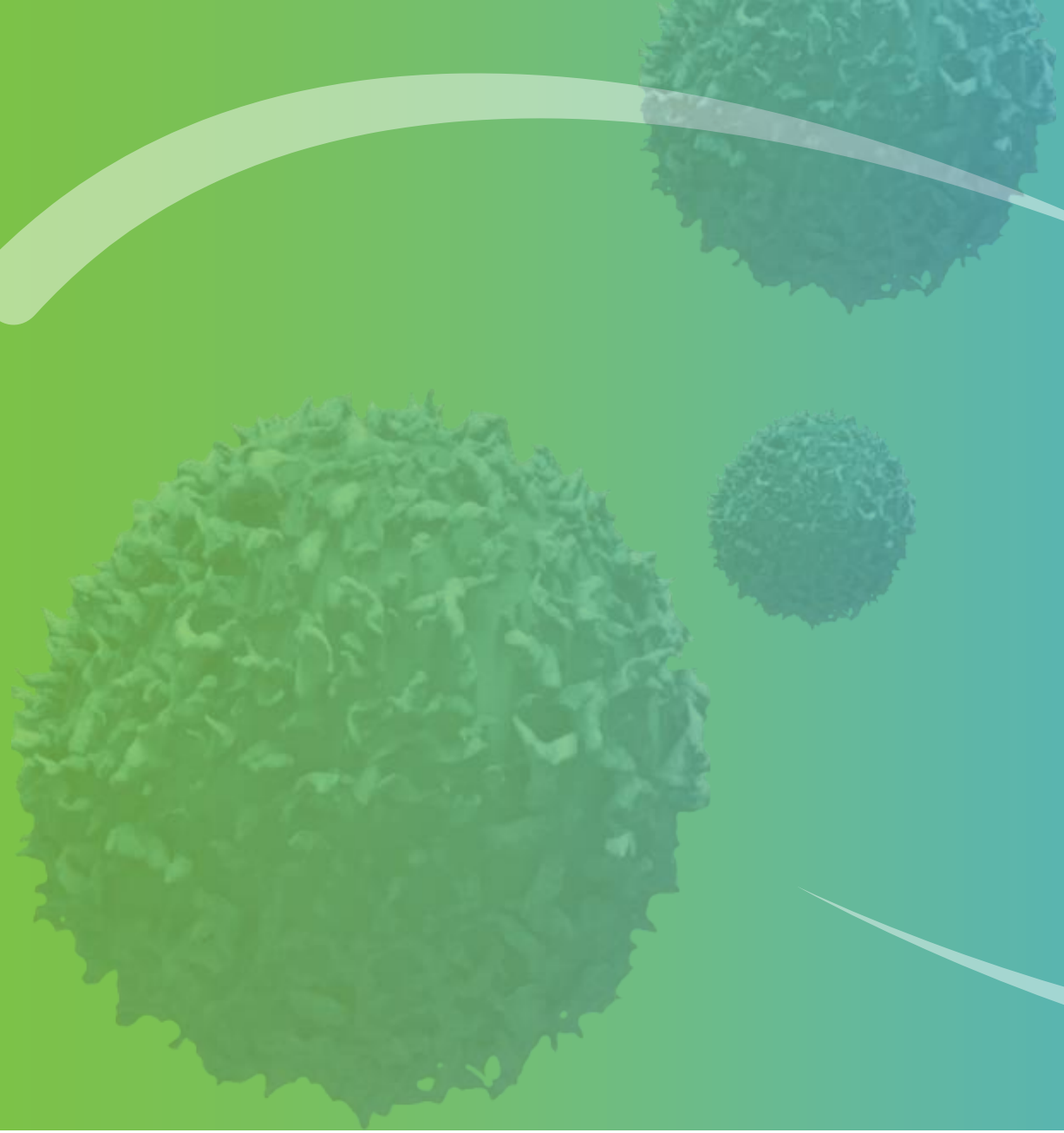


Insights into the Aryl Hydrocarbon Receptor and miR-124 Axis

May 27th 2026

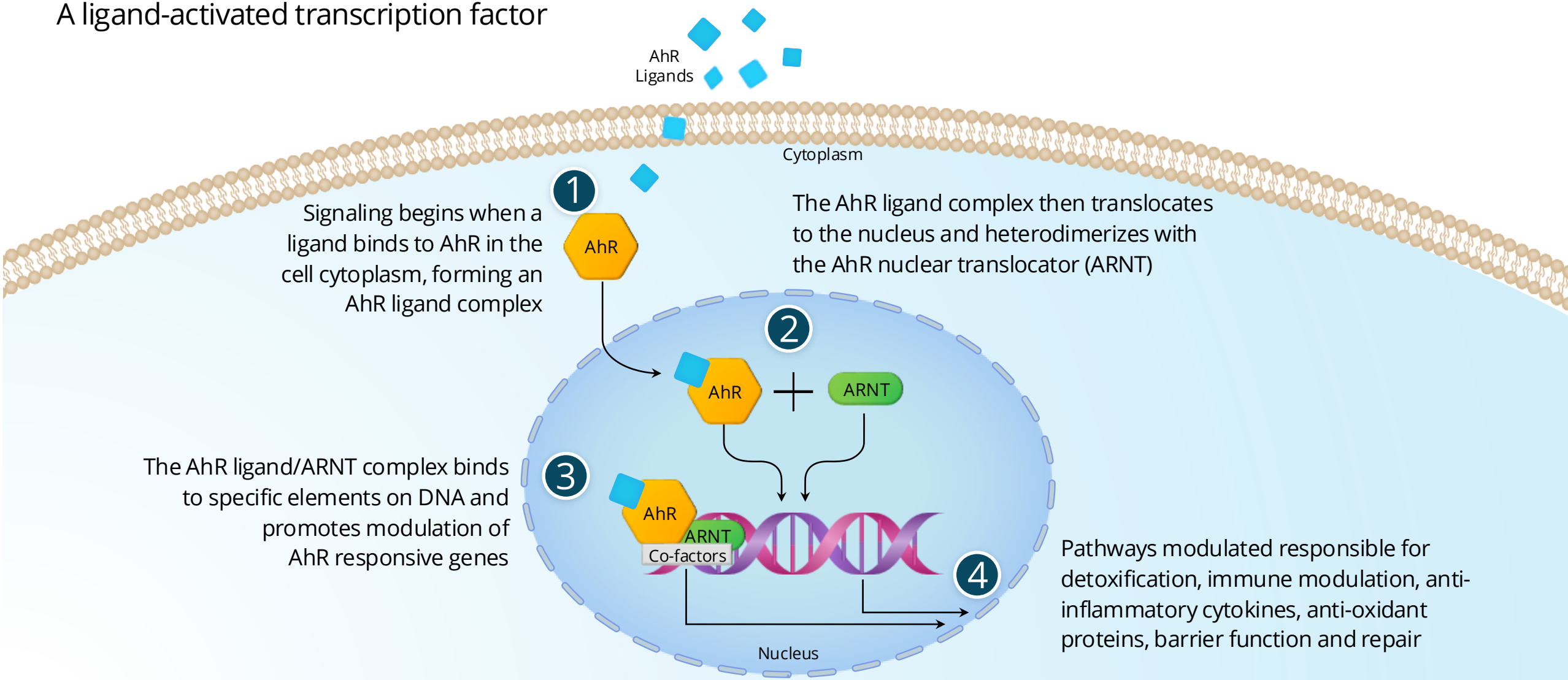
Stephen Connelly Ph.D., Chief Scientific Officer



The Aryl Hydrocarbon Receptor (AhR) as a Signaling Hub

The Aryl Hydrocarbon Receptor (AhR)

A ligand-activated transcription factor



Signaling begins when a ligand binds to AhR in the cell cytoplasm, forming an AhR ligand complex

The AhR ligand complex then translocates to the nucleus and heterodimerizes with the AhR nuclear translocator (ARNT)

The AhR ligand/ARNT complex binds to specific elements on DNA and promotes modulation of AhR responsive genes

Pathways modulated responsible for detoxification, immune modulation, anti-inflammatory cytokines, anti-oxidant proteins, barrier function and repair

equillium

Esser & Rannug., Pharmacol Rev., 2015; Rothhammer & Quintana, Nat. Rev. Immunol., 2019; Polonio *et al.*, Nat. Rev. Drug Discov., 2025; Coumoul *et al.*, Signal Transduct. Target Ther.., 2026

AhR as a Signaling Hub

Top 4 known genomic & non-genomic pathways induced by AhR by functional area

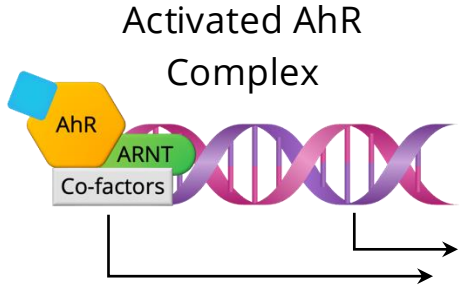
Xenobiotic Response

Genes induced:

CYP1A1/A2
CYP1B1
NQO1
UGT1A1,3,4,6,9

Immune Regulation

Co-factors:	Genes modulated:
STAT1	IL-1β
STAT3	IL-6
NF-κβ	**IL-10**
RORγt	**IL-22**



Barrier Function & Repair

Genes modulated:	Co-factors:
IL-22Rα	STAT3
REG3α/γ	β-catenin / Wnt
CLDN1 & 2	HIF-1α
MUC2	AP-1 (JUN/FOS)

Regulatory Micro-RNA (miR)

miRs Induced:

miR-132 / 212
miR-142
miR-150-5p
miR-335

Esser & Rannug., Pharmacol Rev., 2015; Rothhammer & Quintana, Nat. Rev. Immunol., 2019; Polonio *et al.*, Nat. Rev. Drug Discov., 2025; Coumoul *et al.*, Signal Transduct. Target Ther.. 2026; Nakahama *et al.*, Proc. Natl. Acad. Sci. U S A, 2013; Chinen *et al.*, International Immunology. 2013; Disner *et al.*, Front Mol Biosci., 2021; Zhang *et al.*, Mol. Cancer. Ther., 2011

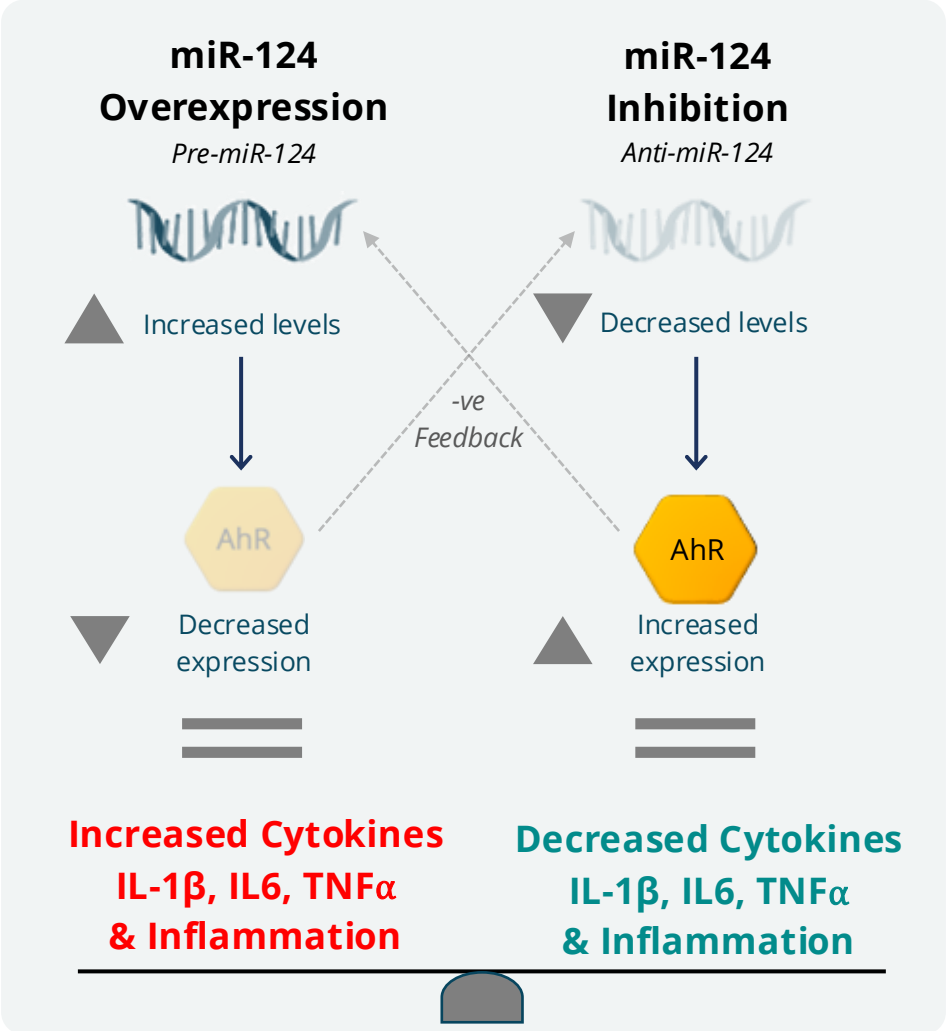
The AhR/miR-124 Axis

What is Known of the Relationship Between miR-124 and AhR

Original Article

MicroRNA-124 Promotes Intestinal Inflammation by Targeting Aryl Hydrocarbon Receptor in Crohn's Disease

- Zhao *et al.* reports an inverse correlation between miR-124 and AhR protein levels in tissues from active CD patients
- Overexpression of miR-124 decreased AhR expression and had a pro-inflammatory effect, while inhibition of miR-124 increased AhR expression and had an anti-inflammatory effect in cell and mouse models of inflammation
- This data suggests a negative, and potentially regulatory relationship between miR-124 and AhR expression



Zhao *et al.*, Journal of Crohn's and Colitis, 2016; Zhao *et al.*, Inflammation, 2020

The Obefazimod (ABX464) Paradox

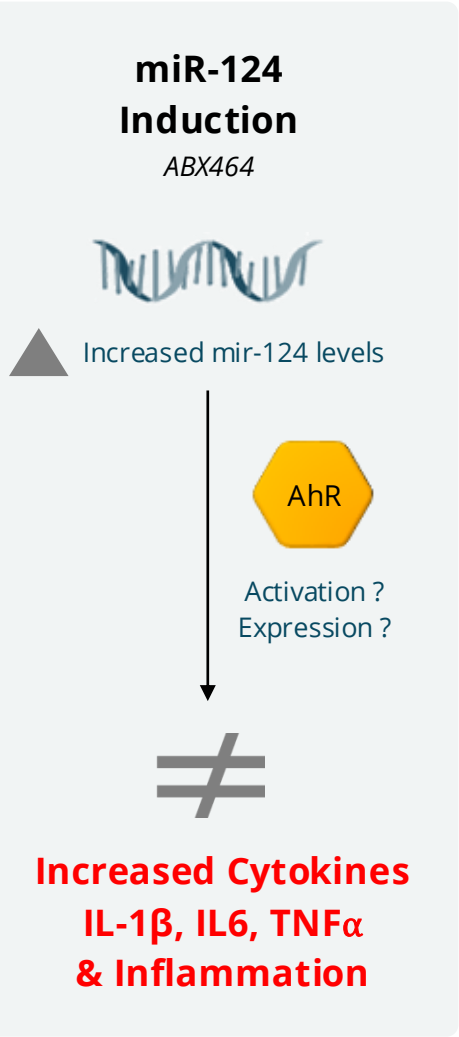
- The Zhao *et al*. data is at odds with the pre-clinical and clinical data with ABX464, where induction of miR-124 reduces inflammation and pro-inflammatory cytokines
- This model can be reconciled if ABX464 is an AhR modulator inducing miR-124

Key Questions

01 Is ABX464 itself an AhR modulator?

02 Does AhR modulation induce miR-124?

03 Functional consequences of AhR-blockade on induction of key downstream pathways?



Approach Taken

1. **In silico modelling:** Used computational docking to assess ligand binding potential to PAS-B domain in AhR

2. **AhR modulation by ABX464:** Used validated AhR-luciferase assay, AhR modulators and an AhR antagonist to compare and confirm activation of AhR through PAS-B binding domain mechanism

3. **AhR modulation and miR-124 induction:** Replicated published protocols to assess potential AhR-driven miR-124 induction in immune cells and changes in pro-inflammatory cytokine production

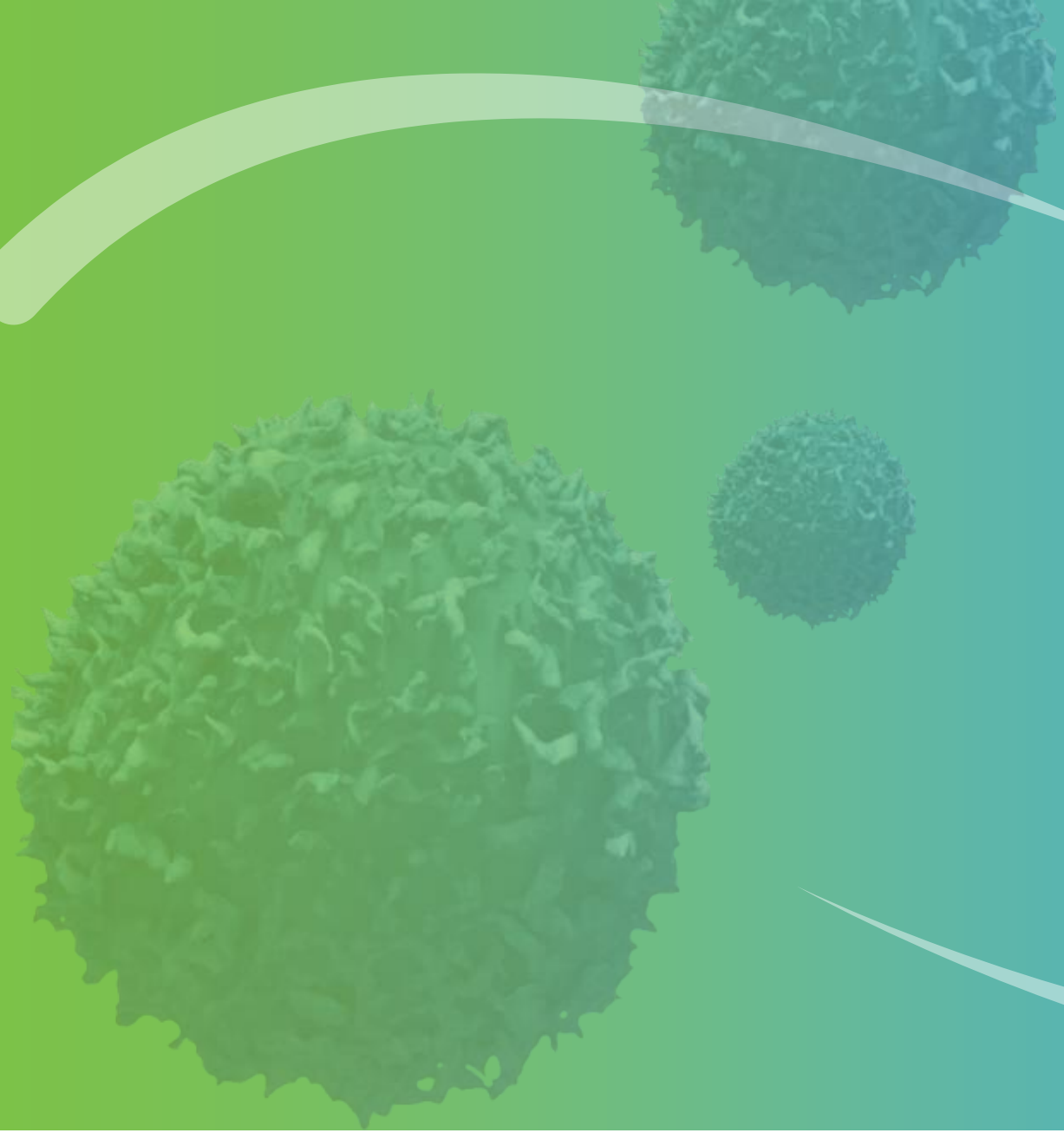

ARTICLE

ABX464 (Obefazimod) Upregulates miR-124 to Reduce Proinflammatory Markers in Inflammatory Bowel Diseases

Cécile Apolit, PhD[1,*], Noëlie Campos, PhD[1,*], Audrey Vautrin, PhD[1], Christina Begon-Pescia, PhD[2], Laure Lapasset, PhD[1], Didier Scherrer, PhD[1], Paul Gineste, PharmD[3], Hartmut Ehrlich, MD[3], Aude Garcel, PharmD, PhD[1], Julien Santo, PhD[1] and Jamal Tazi, PhD[1,3]

4. **Functional consequences of AhR-blockade on induction of key downstream pathways:** Used well-characterized models in regulatory macrophages (M2) and T cells (T_{reg}), as well as intestinal epithelial cell lines to assess the effects on AhR activation or antagonism on CYP1A1, miR-124, IL-10, IL-22 and CLDN2

Apolit *et al.*, Clinical and Translational Gastroenterology, 2022



In Silico Modeling of ABX464 and ABX464-N-Glu in AhR

AhR PAS-B Ligand Binding Domain

The large hydrophobic binding cleft can accommodate a diverse range of ligands

Endogenous Ligands
Synthesized *in situ* in tissues

Indoles and tryptophan derivatives

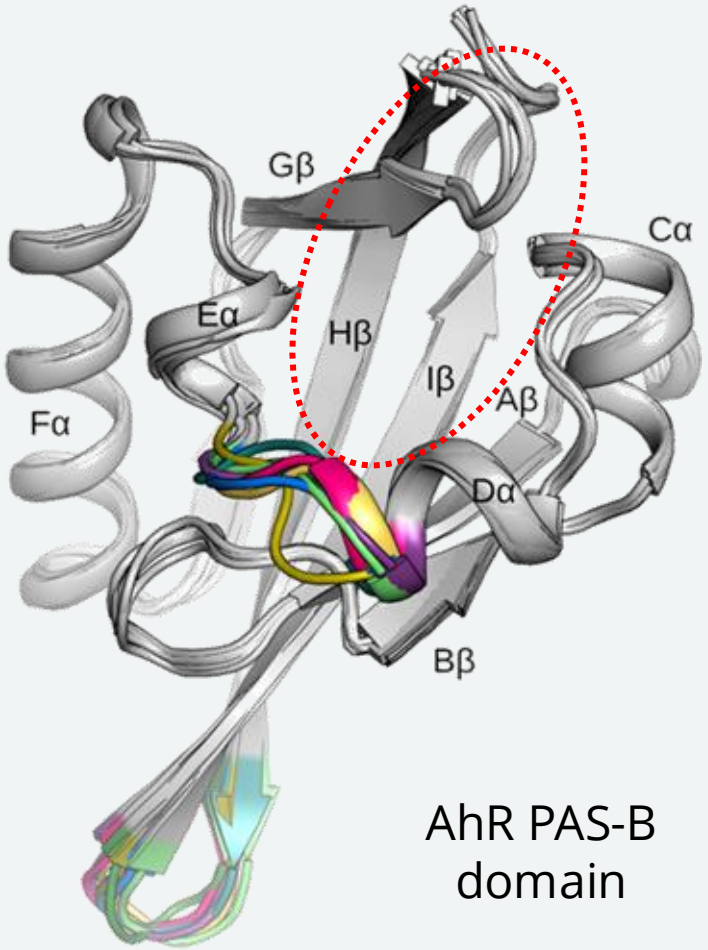
Natural Ligands
Diet or synthesized in our gut

Indoles and tryptophan derivatives
Flavonoids and polyphenols

Xenobiotics
Pollutants & pharmaceuticals

Halogenated aromatic hydrocarbons
Polycyclic aromatic hydrocarbons
13-FDA approved drugs

"In a 2025 computational drug discovery study targeting the AhR PAS-B domain, using similarity-based strategies to screen PubChem, ***77,359 potential AhR-binding molecules were identified"***



AhR PAS-B domain

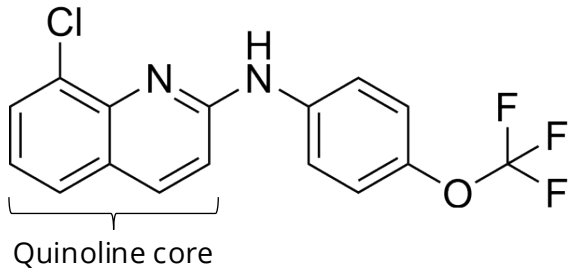
Safe, S., & Shrestha, R., Medicinal Research Reviews, 2018; Lin *et al.*, Eur. J. Med. Chem., 2022; Santini *et al.*, Sci Reports, 2025; Coumoul *et al.*, Signal Transduct Target Ther., 2026

Pharmacophore Analyses of ABX464

ABX464 shares structural similarity to other validated AhR modulators

ABIVAX

ABX464 / Obefazimod

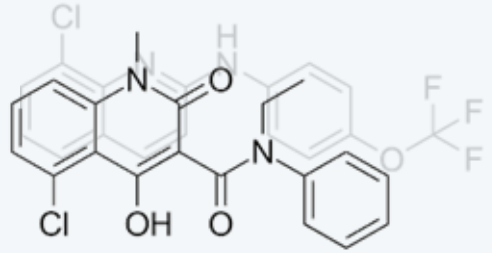


AhR Modulators

- ✓ Conformationally constrained
- ✓ Typically planar core
- ✓ Extended Aromatic heteroaromatic
- ✓ Moderate to high lipophilicity

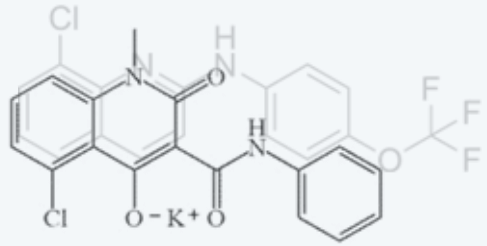
teva Active Biotech

Laquinimod



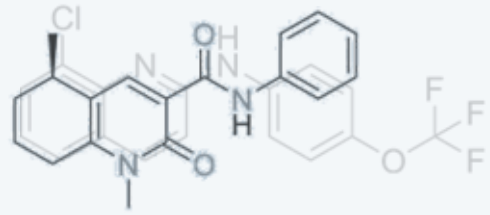
AQILION

AQ-312



Lilly

#68



Kaye *et al.*, PNAS, 2016; Lin *et al.*, Eur. J. Med. Chem., 2022; Sabinis. ACS Med Chem Lett., 2023; Russell *et al.*, DDW Abstract, 2024

Methodology Used for Ligand Docking

Chemical Computing Group

MOE
MOLECULAR OPERATING ENVIRONMENT

1

Identify structural templates
Assemble key structural templates and identify most appropriate one(s) to generate apo receptor

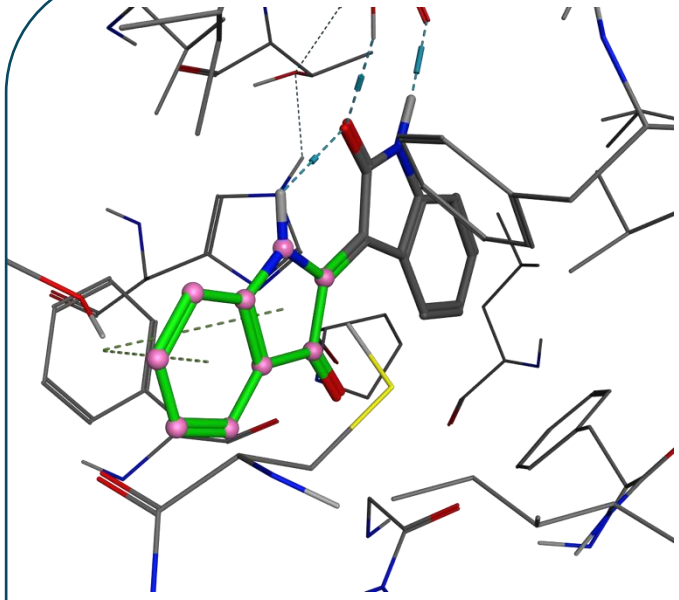
2

Induced-fit docking protocol
Enable receptor flexibility (PAS-B pocket adaptation) to capture ligand-dependent conformational adjustments

3

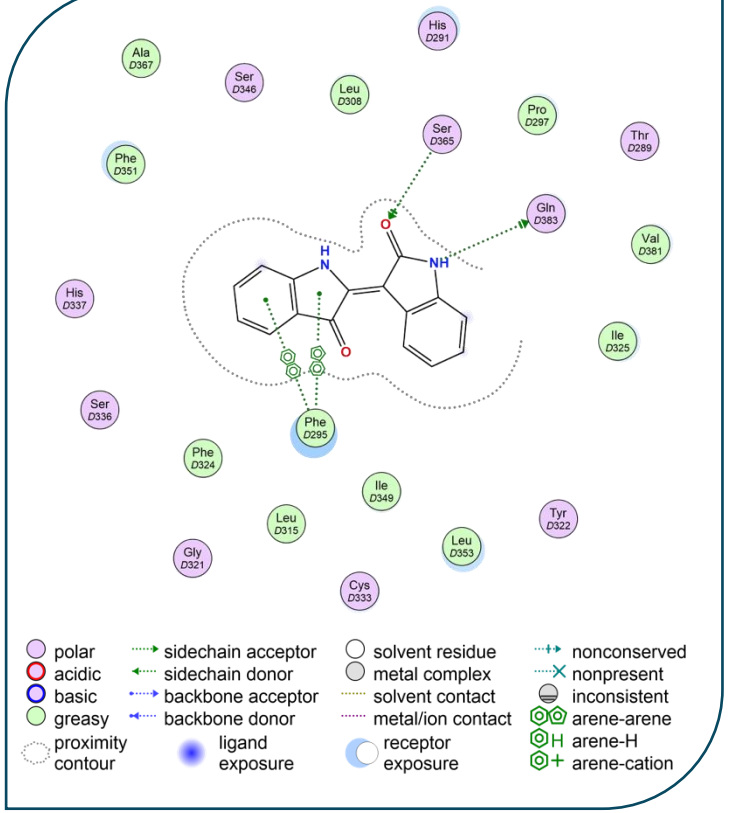
Prioritize best-fit pose
Rank by S- score and filtered by structural plausibility - aromatic orientation, pocket complementarity and steric clashes

3D Experimentally Solved Structure (7ZUB)



Indirubin anchors in AhR PAS-B through a conserved indole π–π stack that constrains ligand pose

2D Projection Ligand Interaction Map (7ZUB)

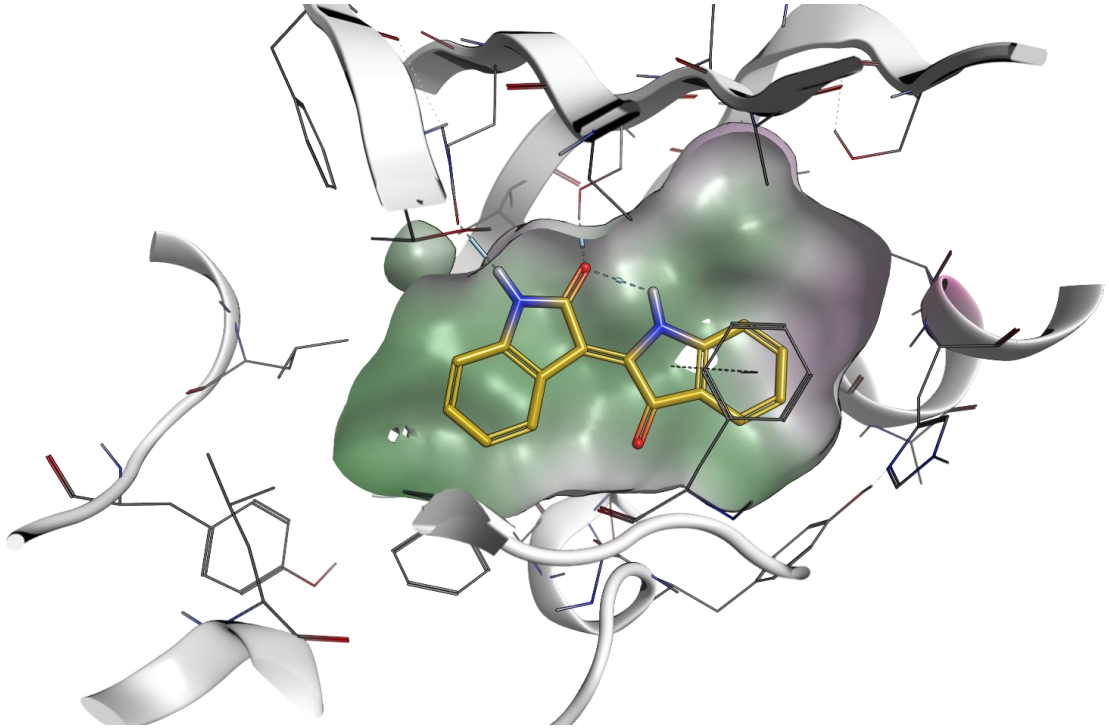


PDB 7ZUB from Gruszczyk *et al.*, Nat. Commun., 2022
Figures generated using MOE v2024.0601. Docking: Triangle Matcher placement, London dG scoring, induced-fit refinement, and GBVI/WSA dG rescoring within the AhR PAS-B pocket.

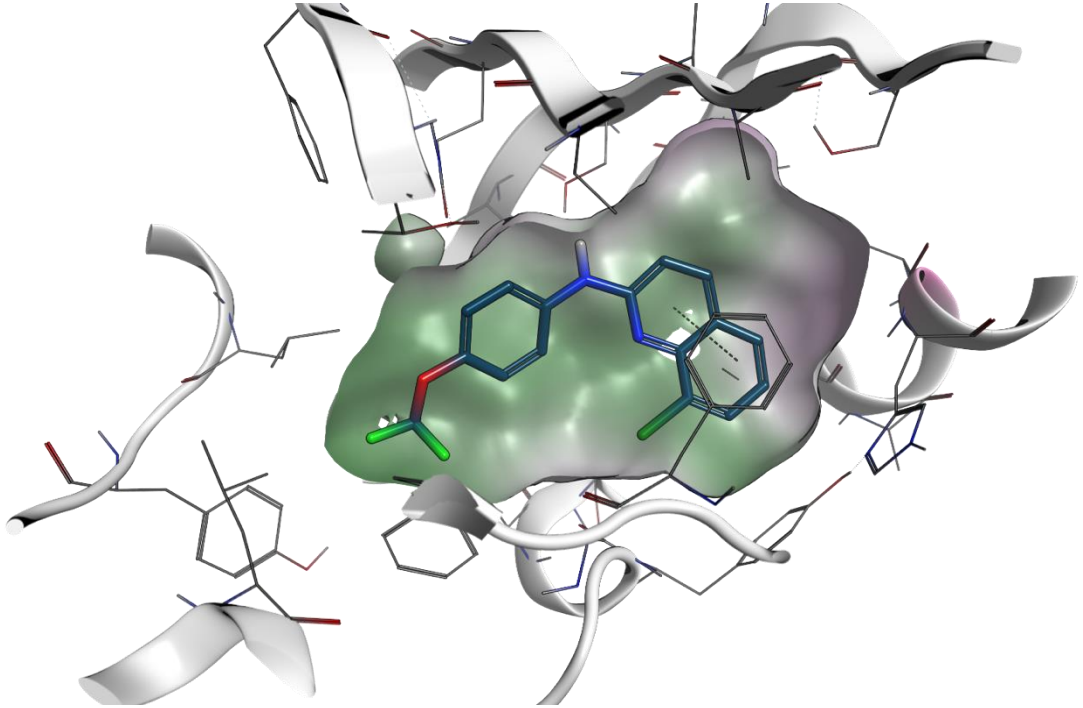
Ligand Binding Maps

ABX464 docks into the lipophilic PAS-B pocket leveraging conserved π-π stacking with key residue Phe295

Indirubin (7ZUB)



ABX464 (model)

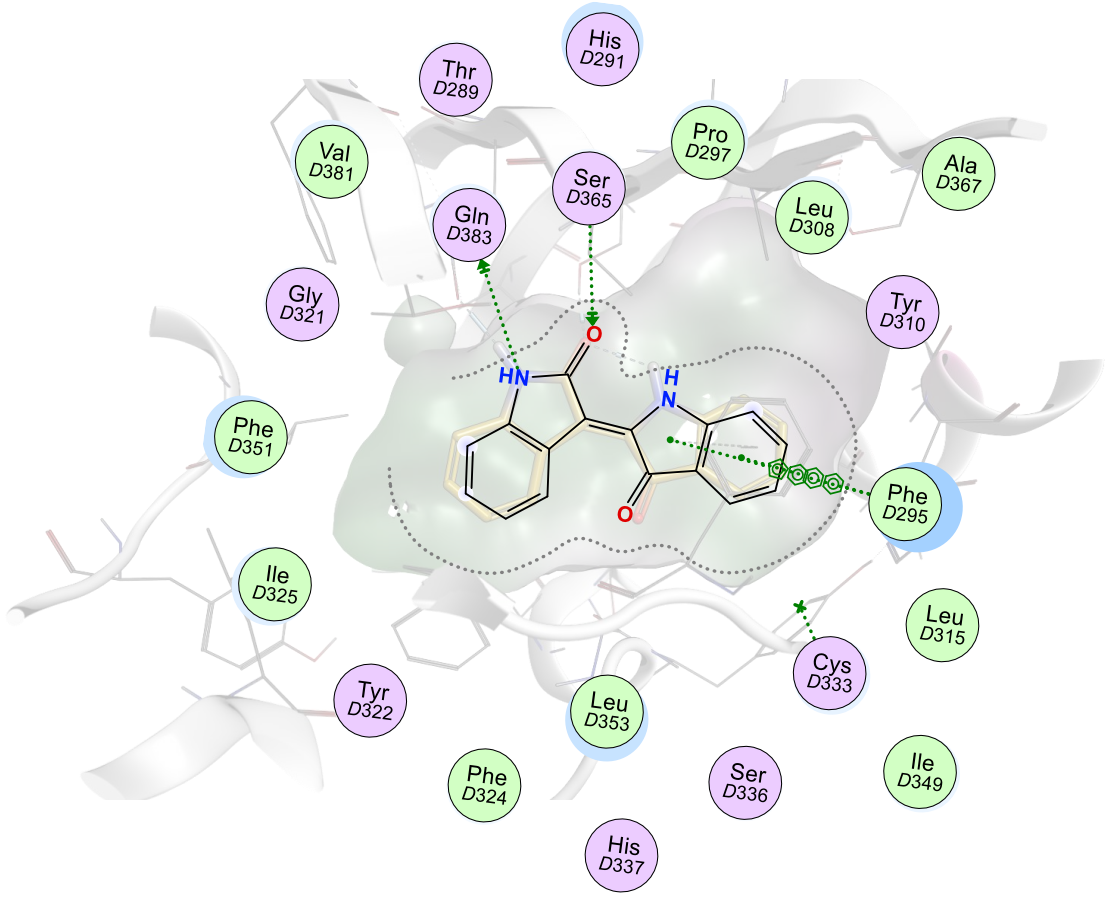


PDB 7ZUB from Gruszczyk *et al.*, Nat. Commun., 2022
Figures generated using MOE (MOE 2024.0601)

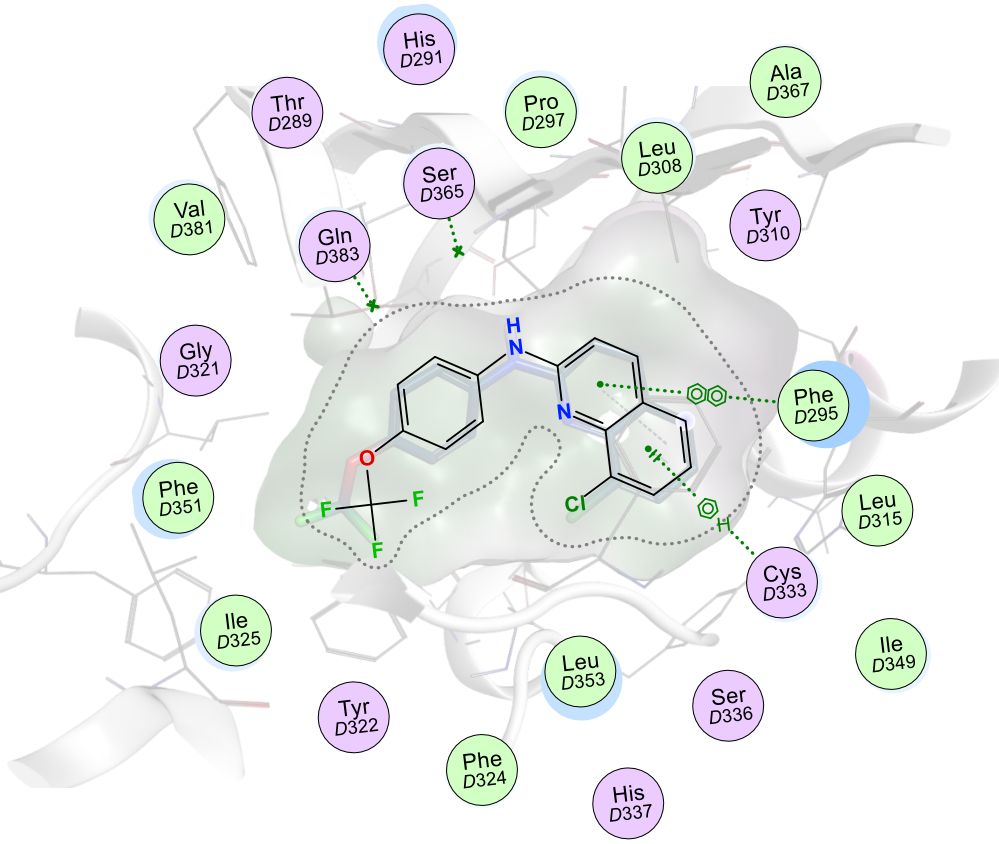
Ligand Binding Maps

ABX464 docks into the lipophilic PAS-B pocket leveraging conserved π-π stacking with key residue Phe295

Indirubin (7ZUB)



ABX464 (model)

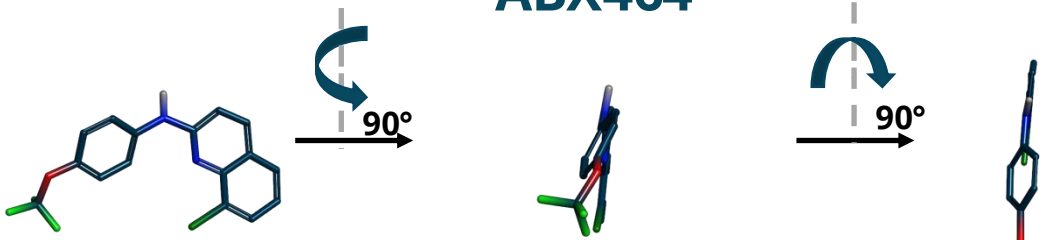


PDB 7ZUB from Gruszczyk *et al.*, Nat. Commun., 2022
Figures generated using MOE (MOE 2024.0601)

Effects of Glucuronidation of ABX464 on Potential PAS-B Binding

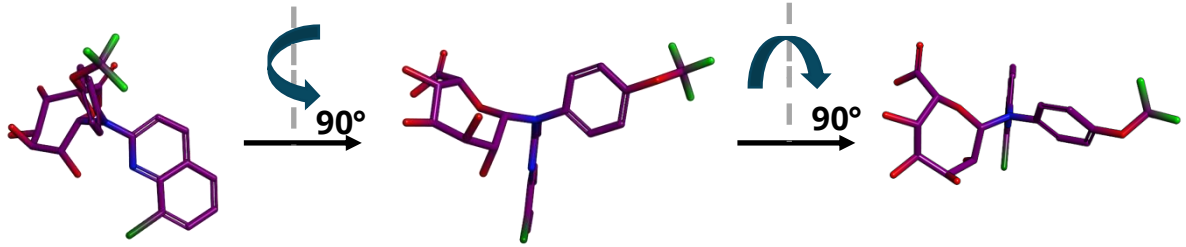
Glucuronidation introduces polar steric bulk, disrupts planarity and hinders binding in the PAS-B pocket

ABX464



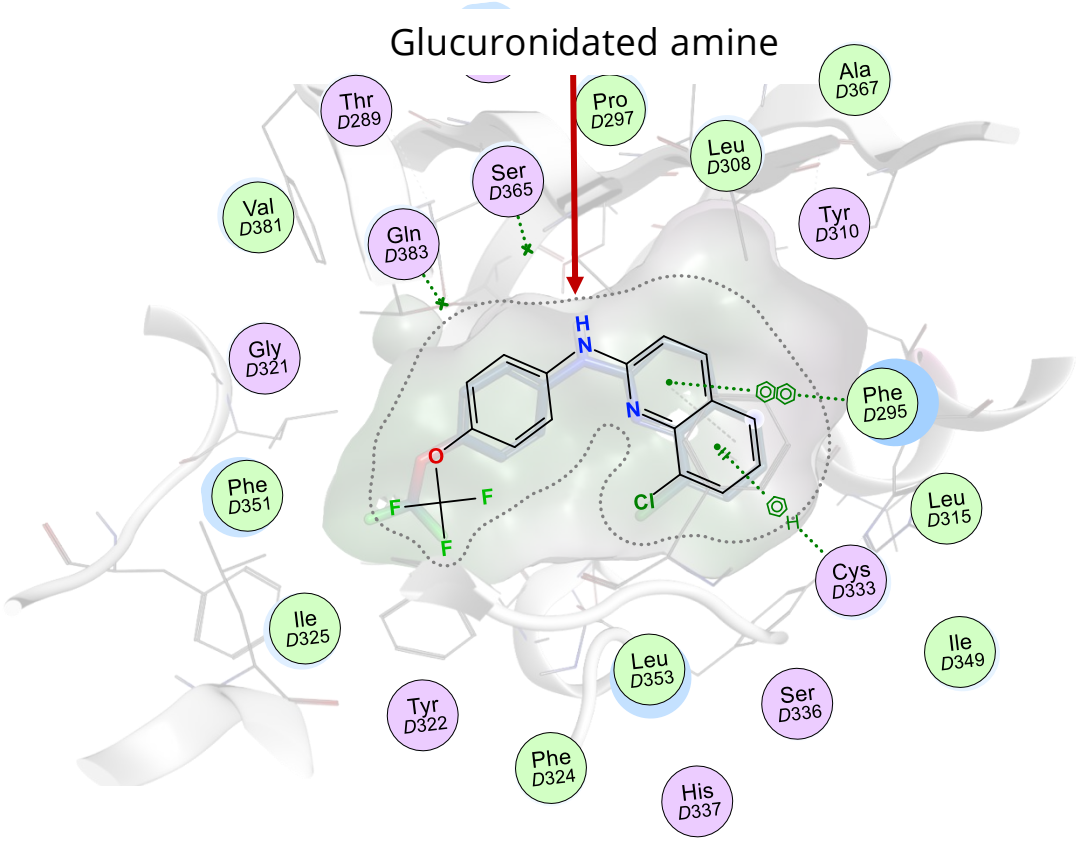
Likely Active

ABX464-N-Glu



Likely Inactive

ABX464 (model)



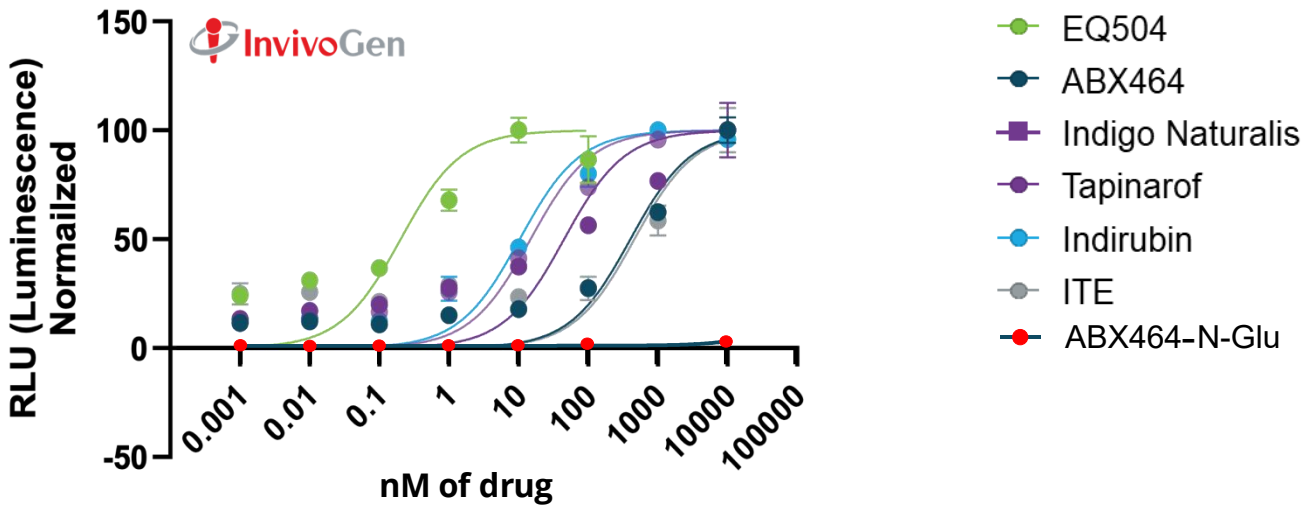
Figures generated using MOE (MOE 2024.0601)

Activation of AhR by ABX464

Comparing Potencies of AhR Modulators

ABX464 is a moderately potent AhR modulator, but ABX464-N-Glu does not bind or modulate AhR

AhR-luciferase Assay



- The AhR-luciferase assay provides a highly sensitive, quantitative and physiologically relevant readout directly linking ligand binding to transcriptional output

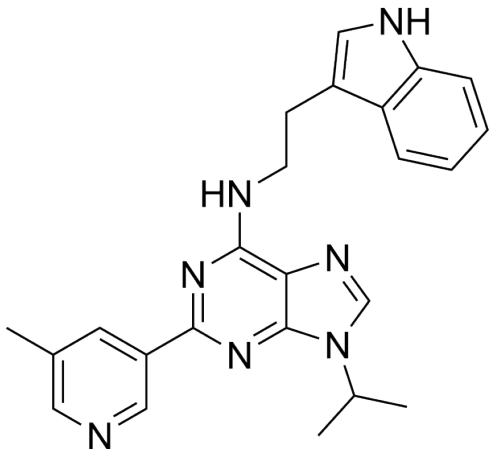
Compound Potency EC_{50}

Compound	EC_{50} (nM)
EQ504	0.203
Indirubin	10.39
Indigo naturalis	14.89
Tapinarof	45.21
ABX464	404.2
ITE	461.8
ABX464-N-Glu	Not detectable

HepG2-Lucia™ AhR cells and manufacturer provided protocols were obtained from InvivoGen (Cat. #hpgl-ahr, #rep-qlc4lg1). Cells alone and media alone were run as background controls. Data were fitted to a four-parameter concentration–response curve (non-linear regression model with variable slope, smallest and largest values fixed to 0 and 100). ABX464: MedChem Cat. #HY-100870, *Indigo naturalis*: Saiki *et al* BMJ Open Gastro., 2021, (indigo (7.2%) and indirubin (0.44%)), Tapinarof: MedChem Cat. #HY-109044, Indirubin: MedChem Cat. #HY-N0117, ITE: MedChem Cat. #HY-19317

AhR Antagonist GNF-351 Inhibits AhR Activation

GNF-351 inhibits AhR activation by both EQ504 and ABX464 confirming binding to PAS-B domain

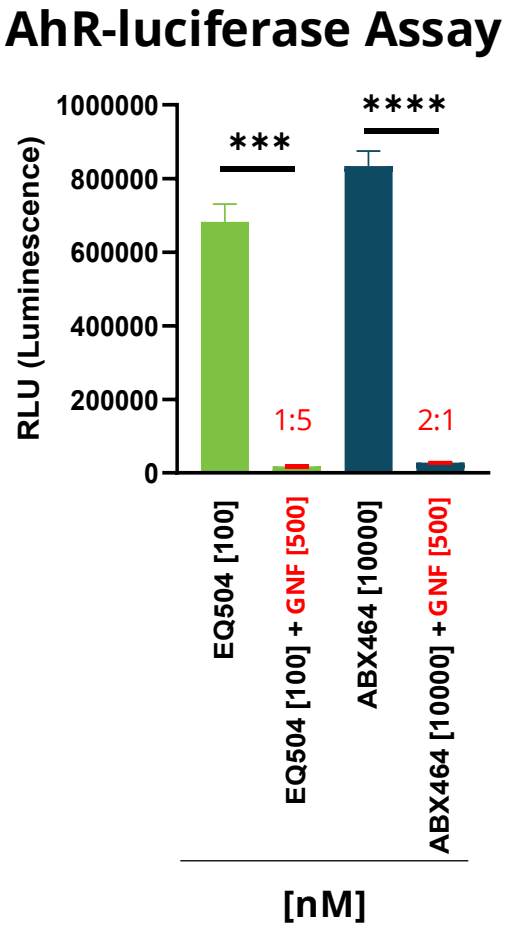


GNF-351 (GNF)

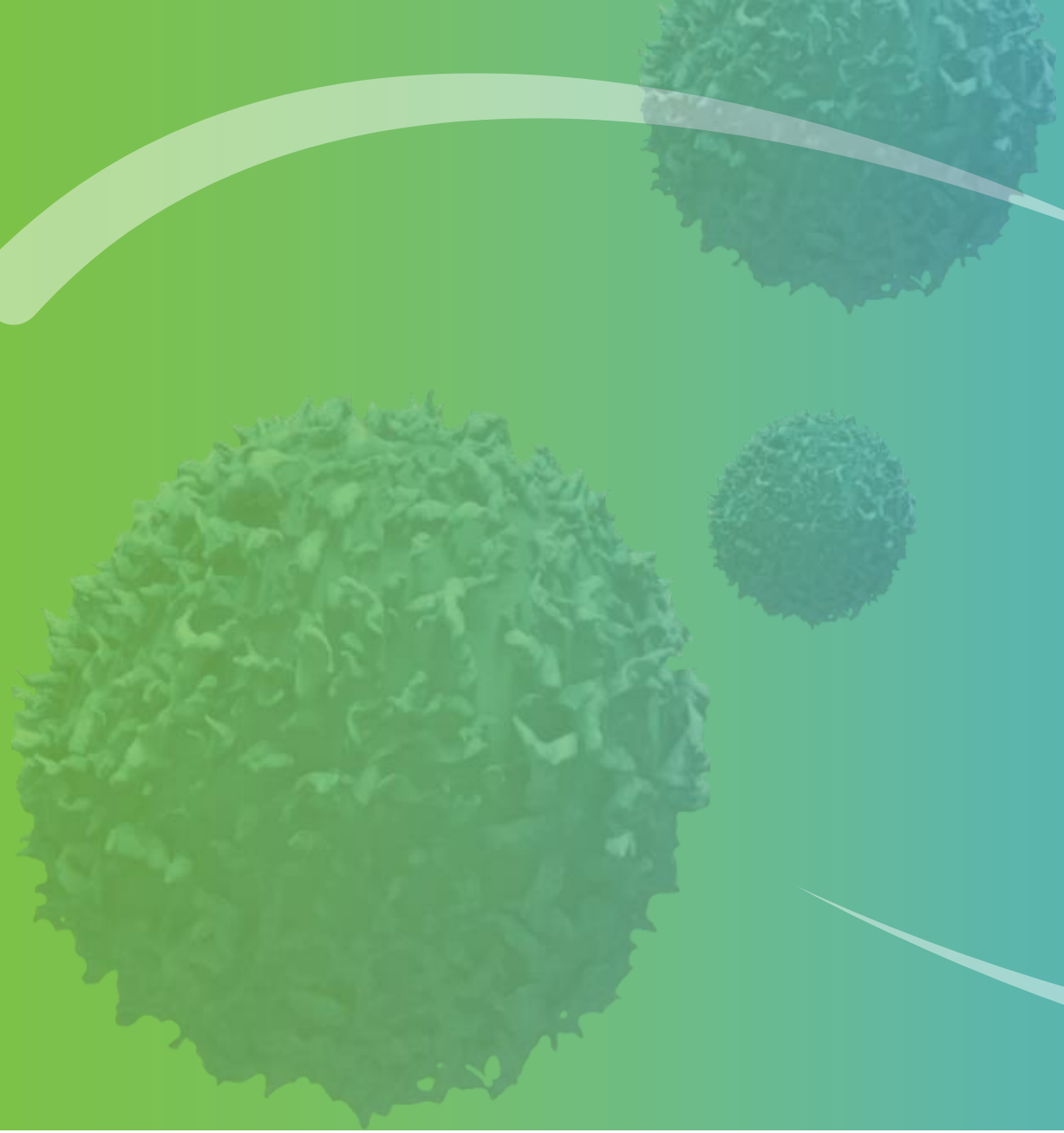
CAS No. : 1227634-69-6

IC_{50} of 62 nM

- GNF is a high-affinity antagonist of the AhR that blocks the PAS-B ligand-binding pocket, enabling confirmation that agonist activity is direct, on-target
- Has been widely used as a research tool to specifically block AhR transcriptional responses *in vitro* to confirm AhR-specific pathway attribution in multiple cell types



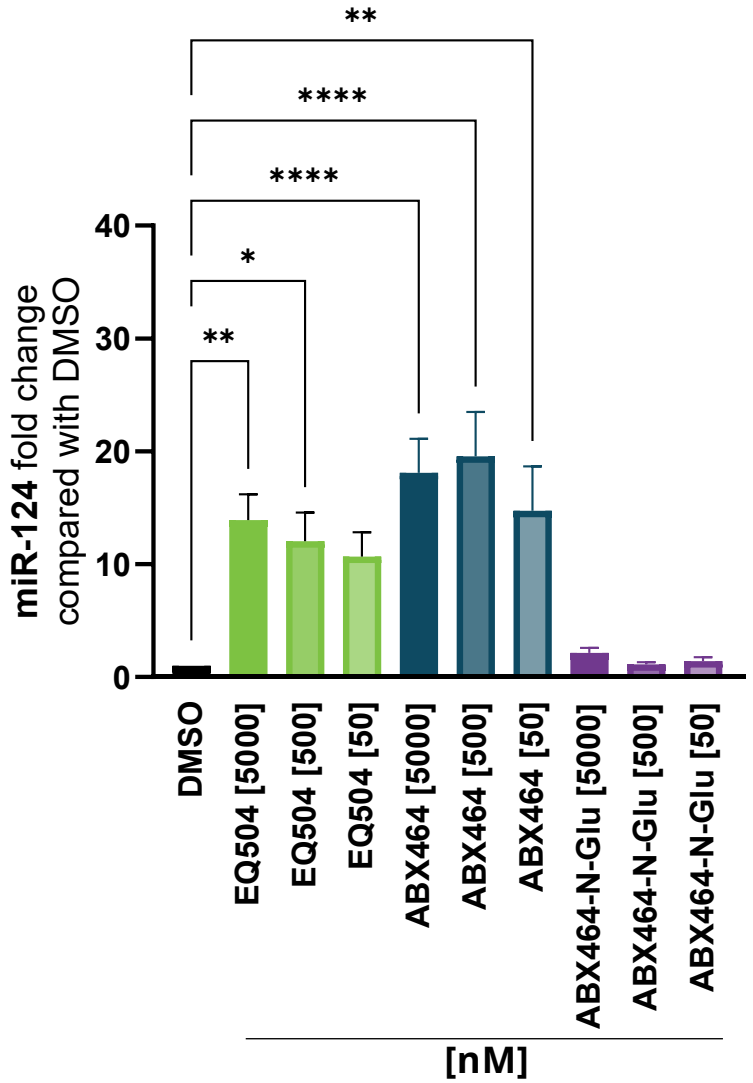
HepG2-Lucia™ AhR cells and manufacturer provided protocols were obtained from InvivoGen (Cat. #hpgl-ahr, #rep-qlc4lg1). Cells alone and media alone were run as background controls. AhR Luciferase cells were blocked with GNF-351 for one hour prior to the addition of EQ504 or ABX464 *****p<0.00001, ***p<0.0001 ,**p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA.* Test article to AhR antagonist GNF ratio shown in red. GNF-351: MedChem Cat. #HY-102023

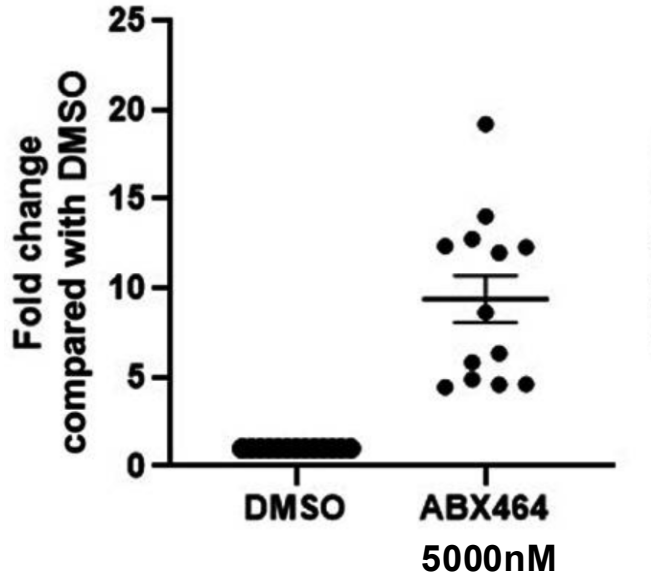


Induction of miR-124 by AhR Activation in Immune Cells

Induction of miR-124 in PBMCs

EQ504 & ABX464, but not ABX464-N-Glu, significantly induce miR-124 expression in PBMCs



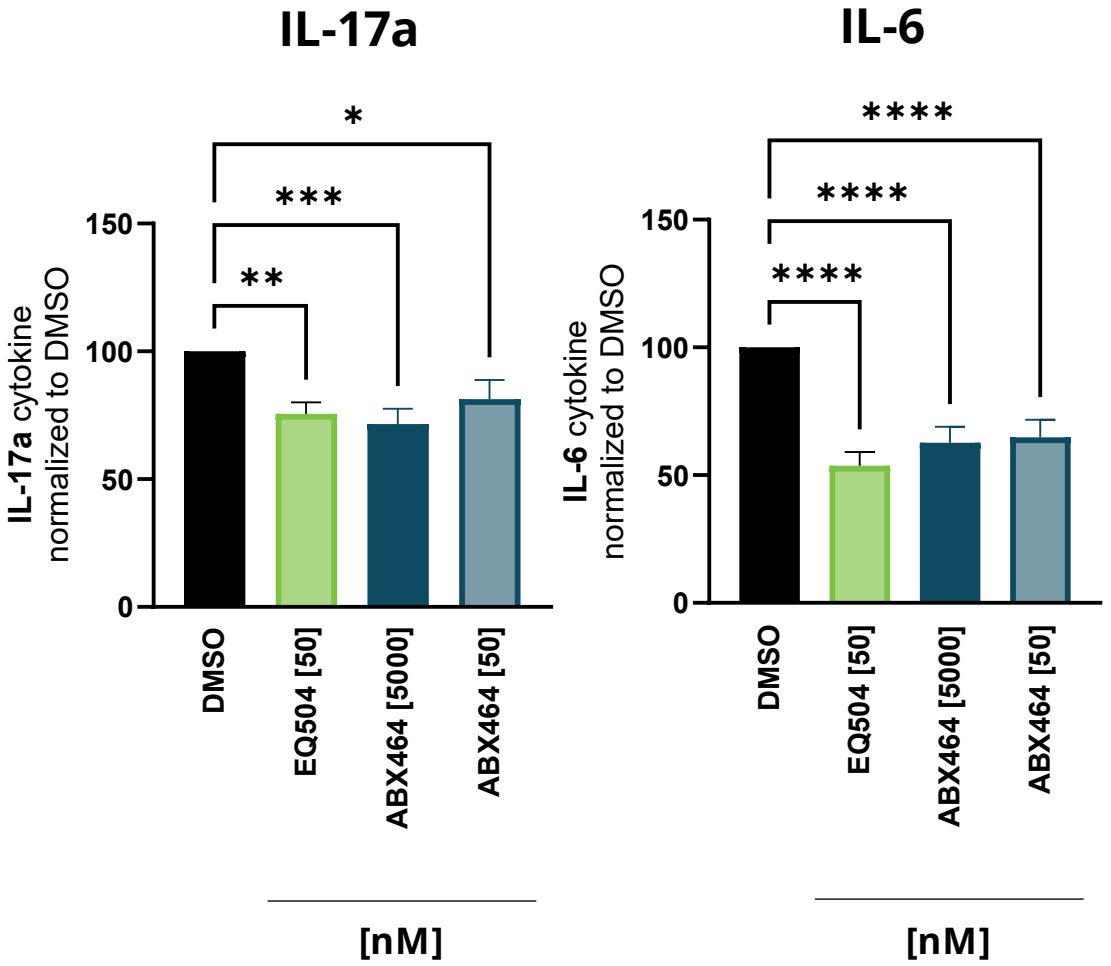


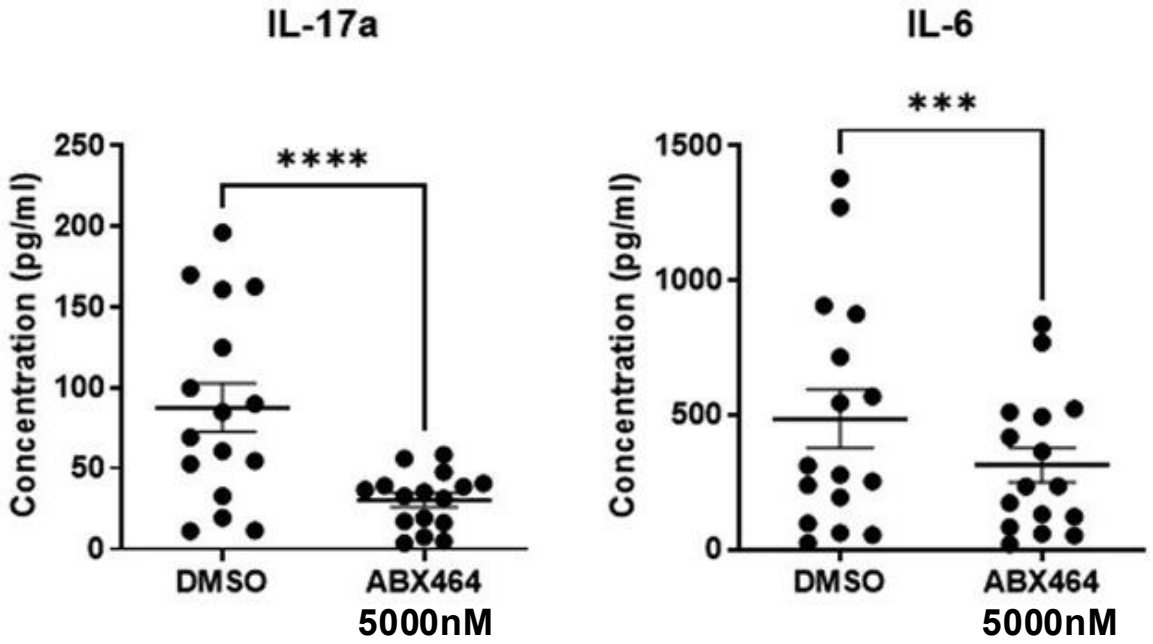
Abivax data, Figure 1a from Apolit *et al.*, 2022

Total PBMCs treated with TransAct (CD3/CD28) stimulation for 48hrs, washed and then treated with EQ504 or ABX464 for 6 days per Apolit *et al.*, Clinical and Translational Gastroenterology, 2022. Total of 8 donors were completed. *****p<0.00001, ***p<0.0001 ,**p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA.*

Pro-Inflammatory Cytokine Profiling in PBMCs

EQ504 & ABX464 significantly inhibit key pro-inflammatory cytokines release in PBMCs



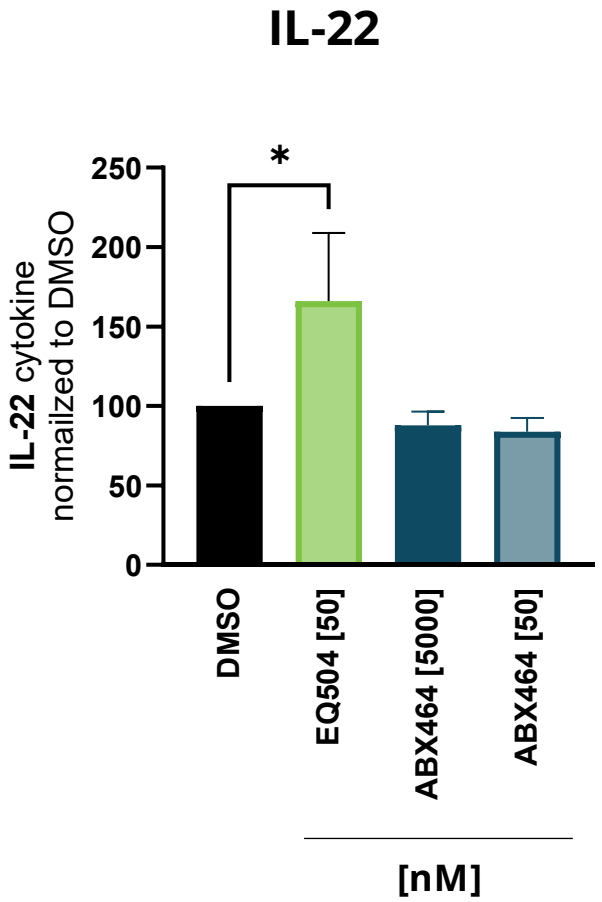


Abivax data, Figure 2a from Apolit *et al.*, 2022

Total PBMCs treated with TransAct (CD3/CD28) stimulation for 48hrs, washed and then treated with EQ504 or ABX464 for 6 days Apolit *et al.*, Clinical and Translational Gastroenterology, 2022. Total of 8 donors were completed. *****p<0.00001, ***p<0.0001 ,**p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA.*

Anti-inflammatory Cytokine Profiling in PBMCs

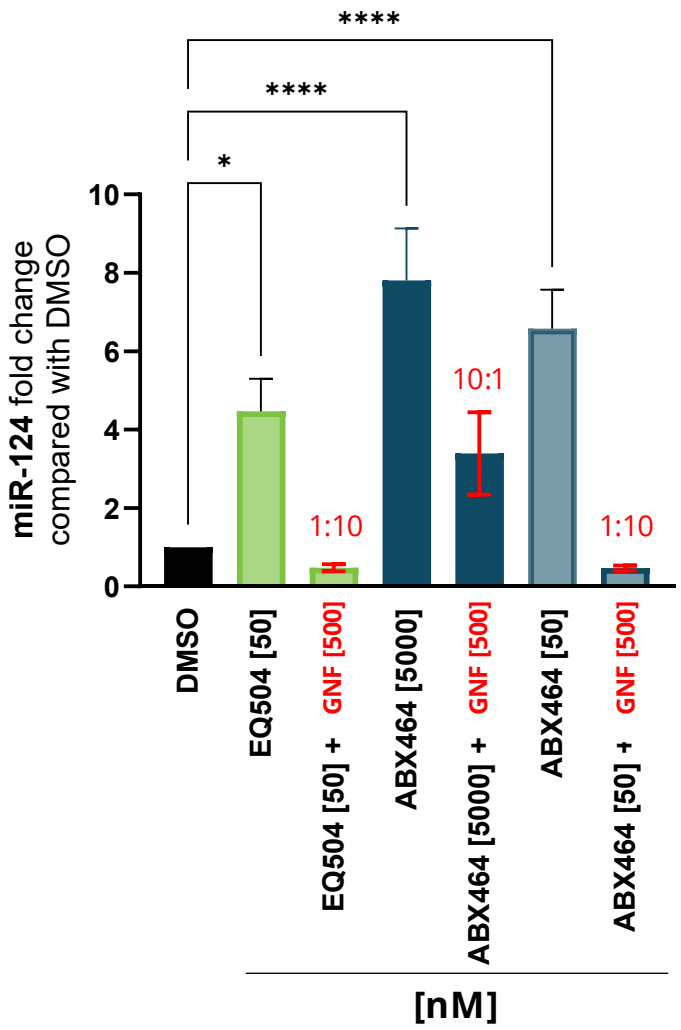
EQ504, but not ABX464, significantly induces IL-22 release in PBMCs

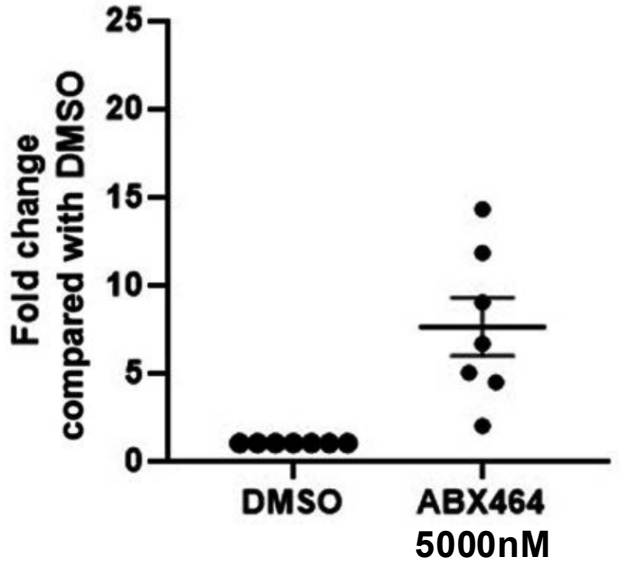


Total PBMCs treated with TransAct (CD3/CD28) stimulation for 48hrs, washed and then treated with EQ504 or ABX464 for 6 days (per Apolit et al., 2022) Total of 8 donors were completed. *****p<0.00001, ***p<0.0001 , **p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA.*

Effects of AhR Modulation and miR-124 in CD4+ T Cells

Induction of miR-124 in isolated CD4⁺ T cells by both EQ504 & ABX464 is AhR-dependent



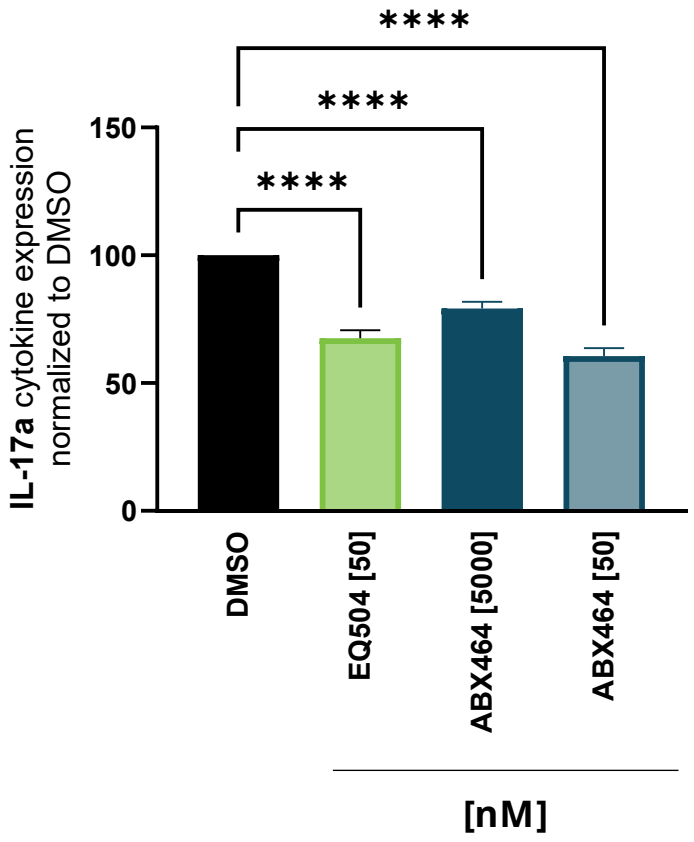


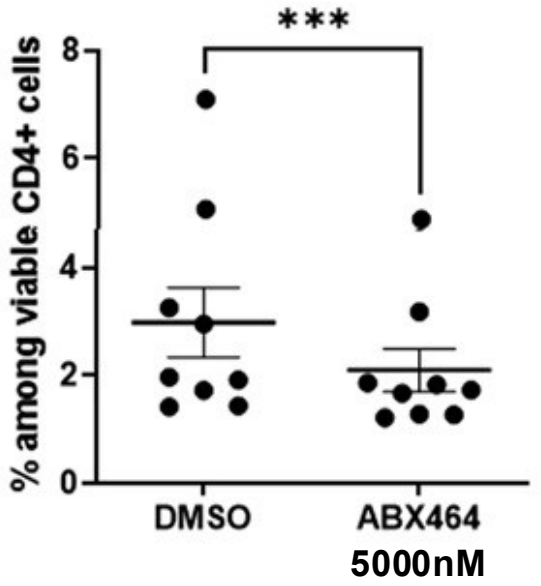
Abivax data, Figure 1b from Apolit *et al.*, 2022

Isolated CD4 T cells treated with TransAct (CD3/CD28) stimulation for 48hrs, washed and blocked with GNF-351 (500 nM) for one hour, prior to EQ504 or ABX464 treatment for 6 days Apolit *et al.*, Clinical and Translational Gastroenterology, 2022. A total of 8 donors have been completed. *****p<0.00001, ***p<0.0001 , **p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA*. Test article to AhR antagonist GNF ratio shown in red.

Effects on IL-17a Expressing Cells in Isolated CD4+ T Cells

EQ504 & ABX464 significantly reduced number of IL17a+ expressing cells in isolated CD4+ T cells



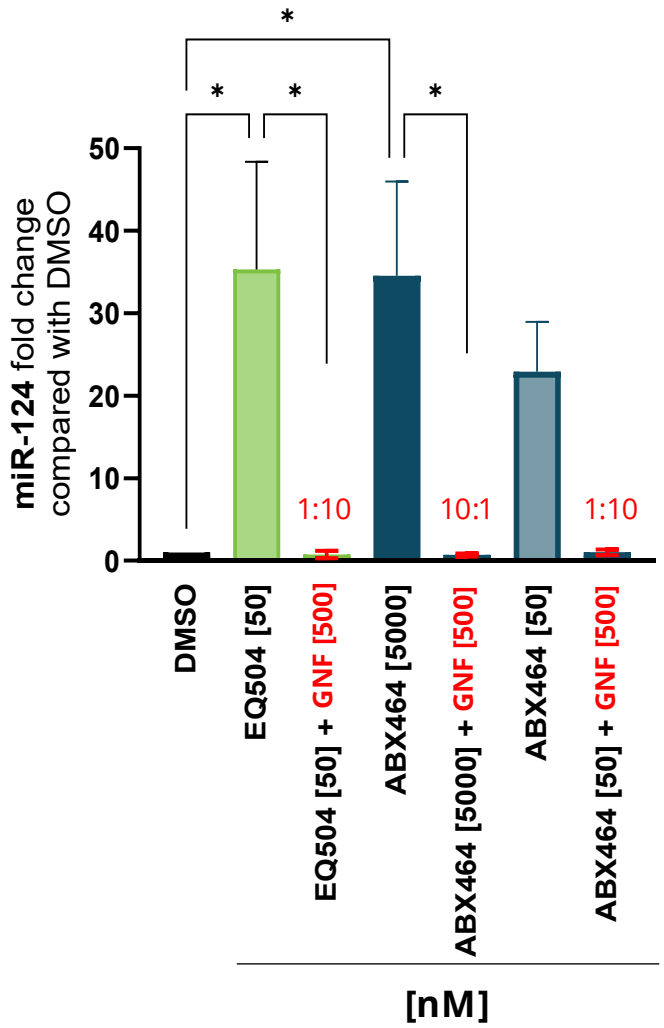


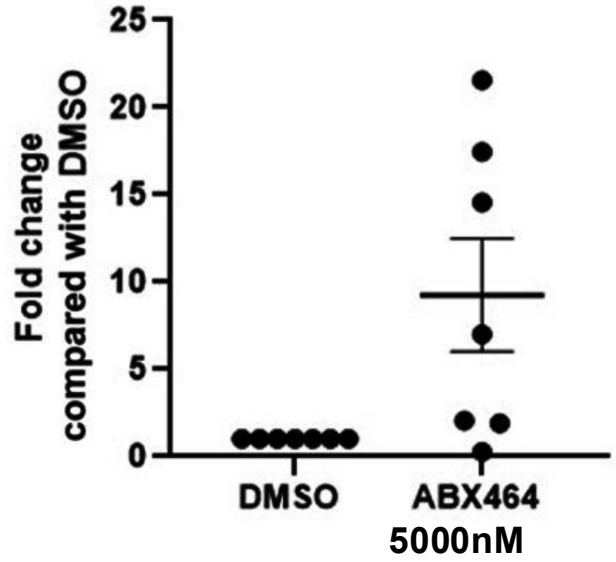
Abivax data, Figure 3c from Apolit *et al.*, 2022

Isolated CD4 T cells treated with TransAct (CD3/CD28) stimulation for 48hrs, washed and then treated with EQ504 or ABX464 for 6 days (per Apolit et al., 2022). A total of 8 donors have been completed. *****p<0.00001, ***p<0.0001 ,**p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA.*

Effects of AhR Modulation and miR-124 in M1 Macrophages

Induction of miR-124 in M1 macrophage cells by both EQ504 & ABX464 is AhR-dependent



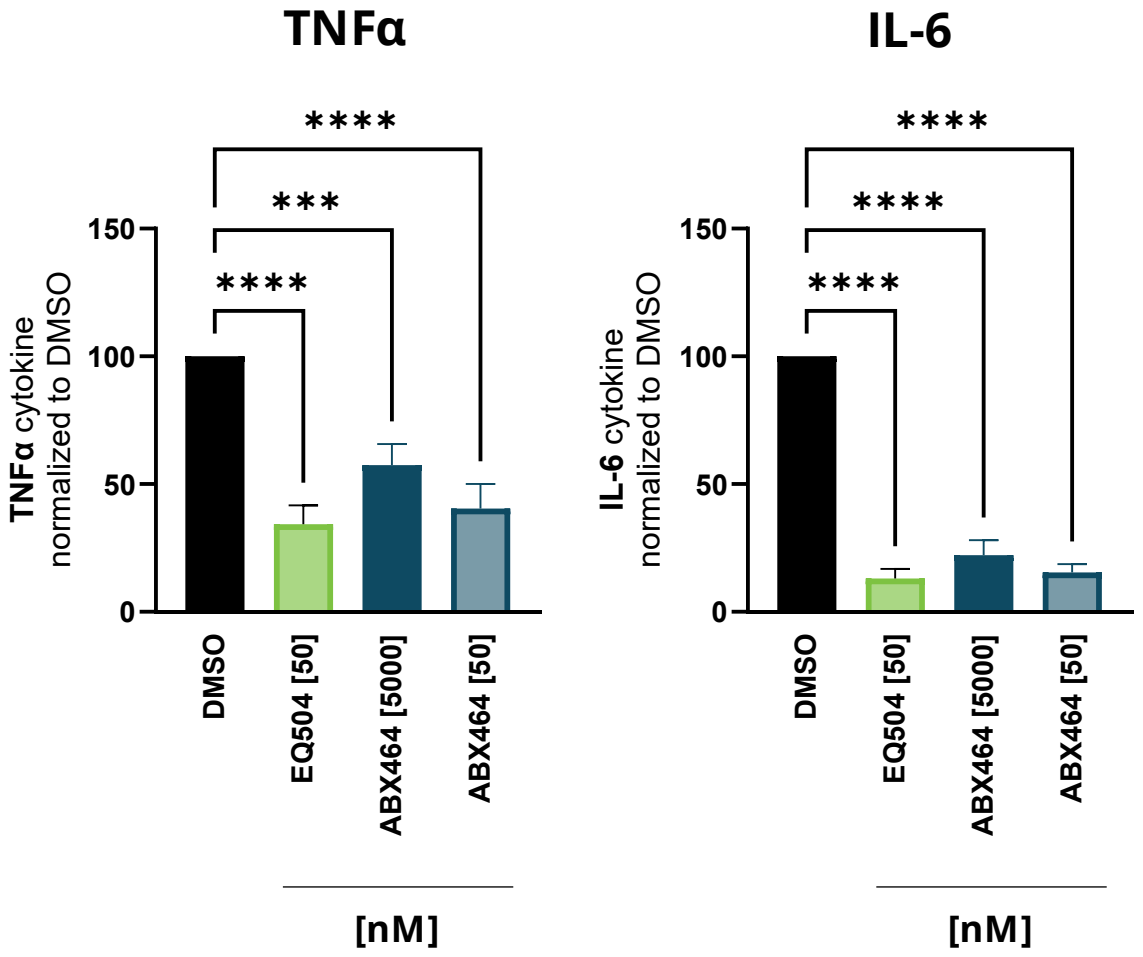


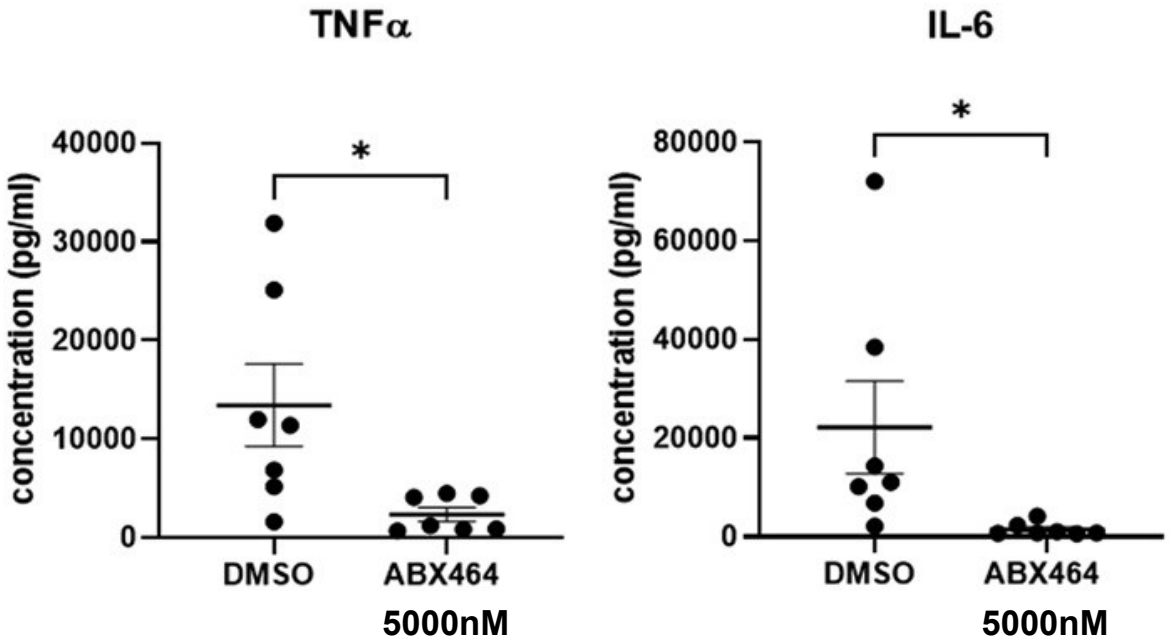
Abivax data, Figure 1c from Apolit *et al.*, 2022

Monocytes were isolated and then treated with GM-CSF for 2 days. Cells were washed and then blocked with GNF-351 (500 nM) for one hour, prior to EQ504 or ABX464 treatment. Cells were under EQ504 or ABX464 treatment for 6 days. On day 7 cells were treated with LPS and IFNγ. Takedown of assay was on day 8 Apolit *et al.*, Clinical and Translational Gastroenterology, 2022. Total of 6 donors have been completed. ****$p<0.00001$, ***$p<0.0001$, **$p<0.001$, *$p<0.005$ *vs DMSO by Ordinary one-way ANOVA*. Test article to AhR antagonist GNF ratio shown in red.

Pro-Inflammatory Cytokine Profiling in M1 Macrophages

EQ504 & ABX464 significantly inhibit key pro-inflammatory cytokines release in M1 cells





Abivax data, Figure 3d from Apolit *et al.*, 2022

Monocytes were isolated and then treated with GM-CSF for 2 days. Cells were under EQ504 or ABX464 treatment for 6 days. On day 7 cells were treated with LPS and IFNγ. Takedown of assay was on day 8 Apolit *et al.*, Clinical and Translational Gastroenterology, 2022. Total of 6 donors have been completed. *****p<0.00001, ***p<0.0001 ,**p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA.*

Pro-Inflammatory Cytokine Profiling in M1 Macrophages

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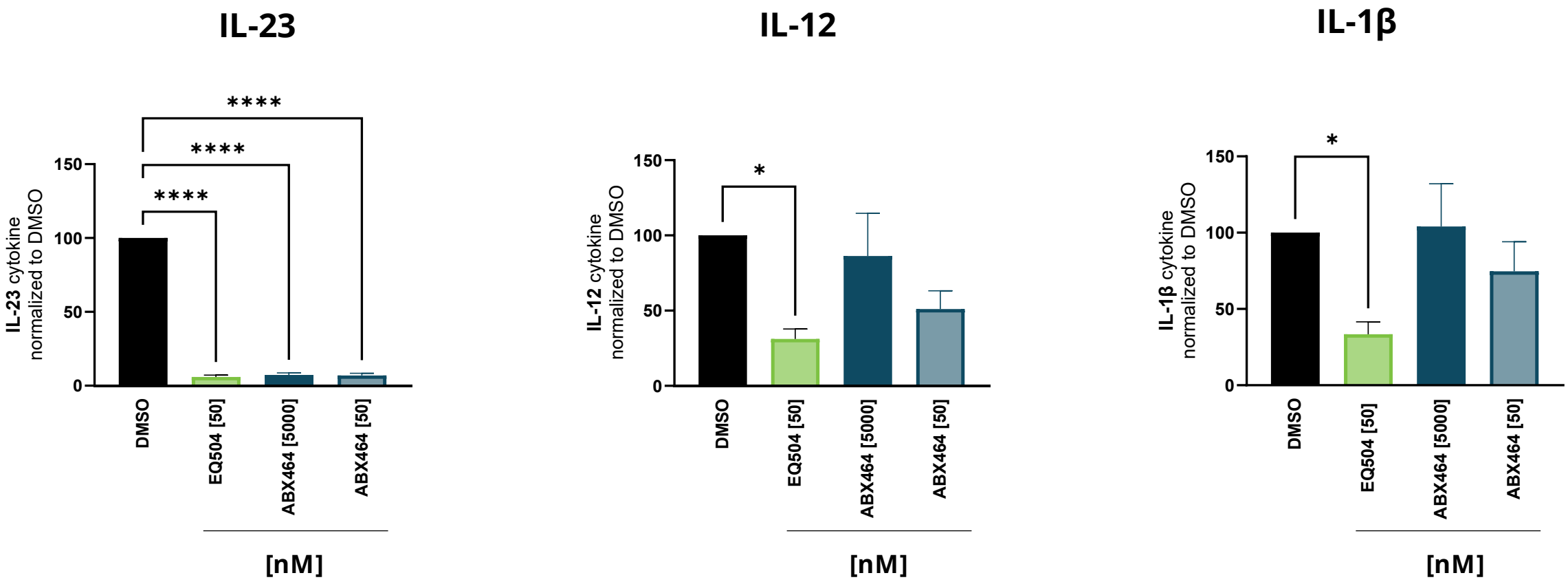

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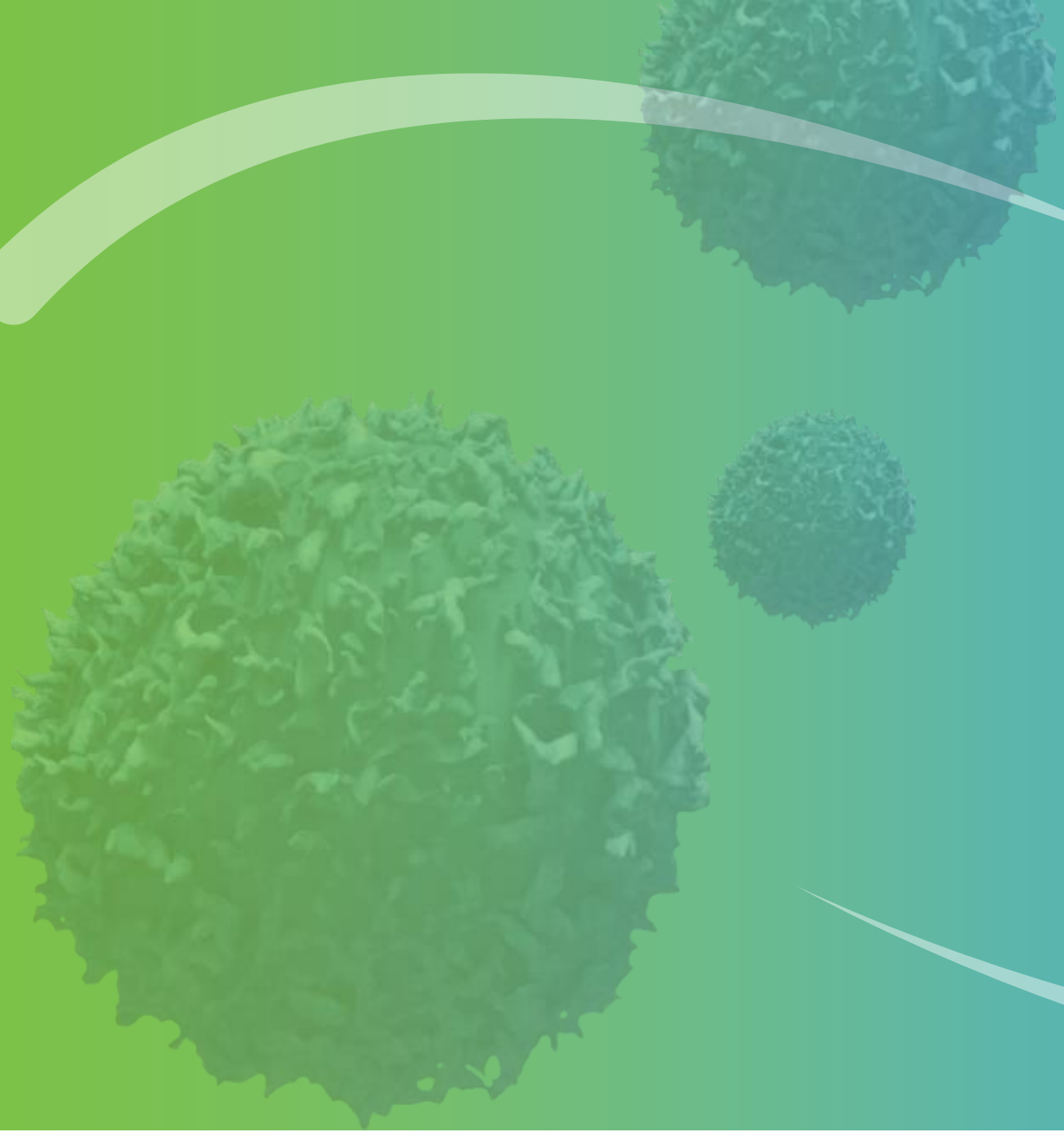
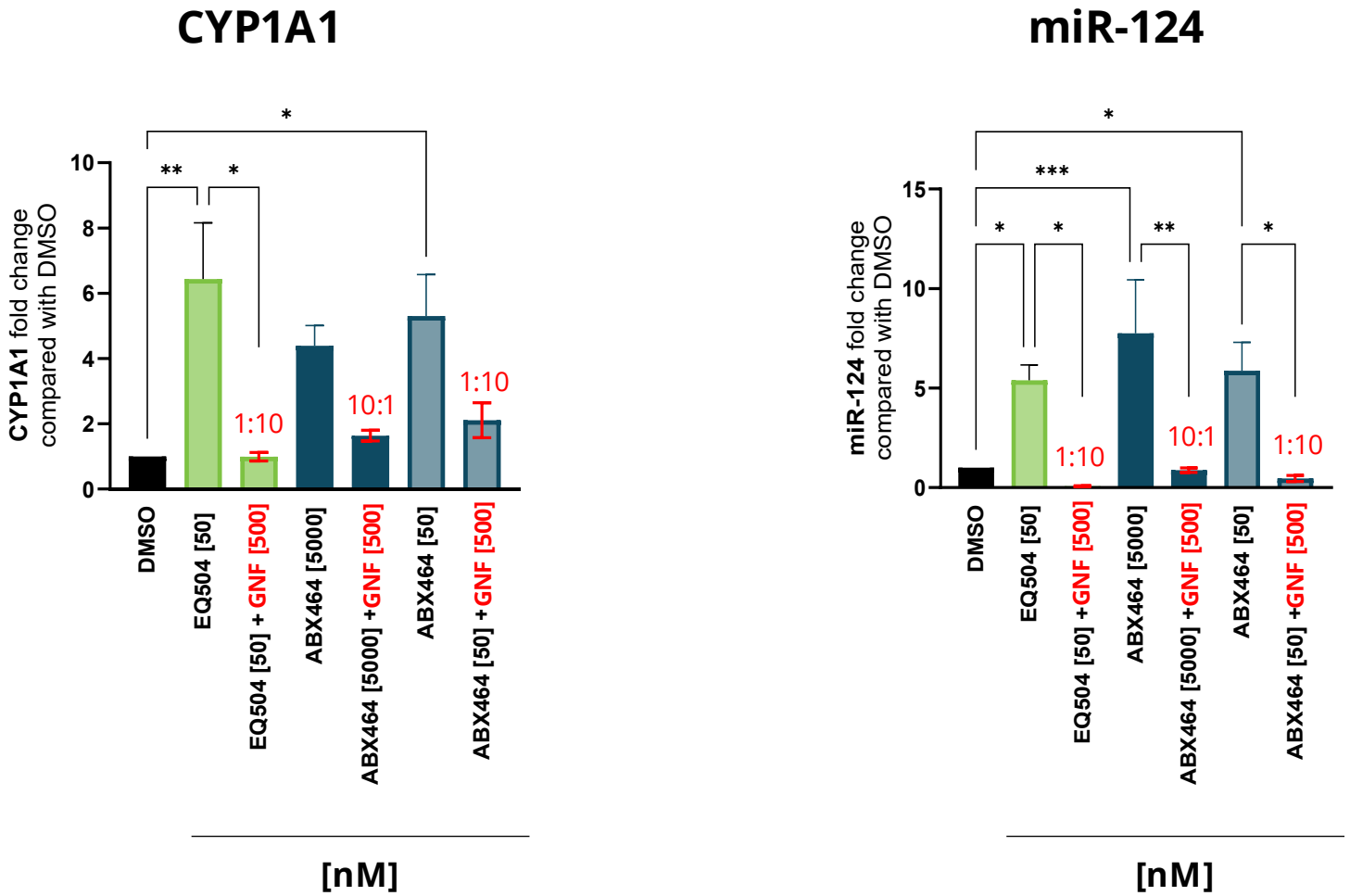


Functional Consequence of AhR-antagonism on CYP1A1, miR-124, IL-10 and IL-22

- ✓ Regulatory T Cells (T_{reg})
- ✓ Regulatory Macrophages (M2)

Effects of AhR Modulation on CYP1A1 and miR-124 in Regulatory T cells

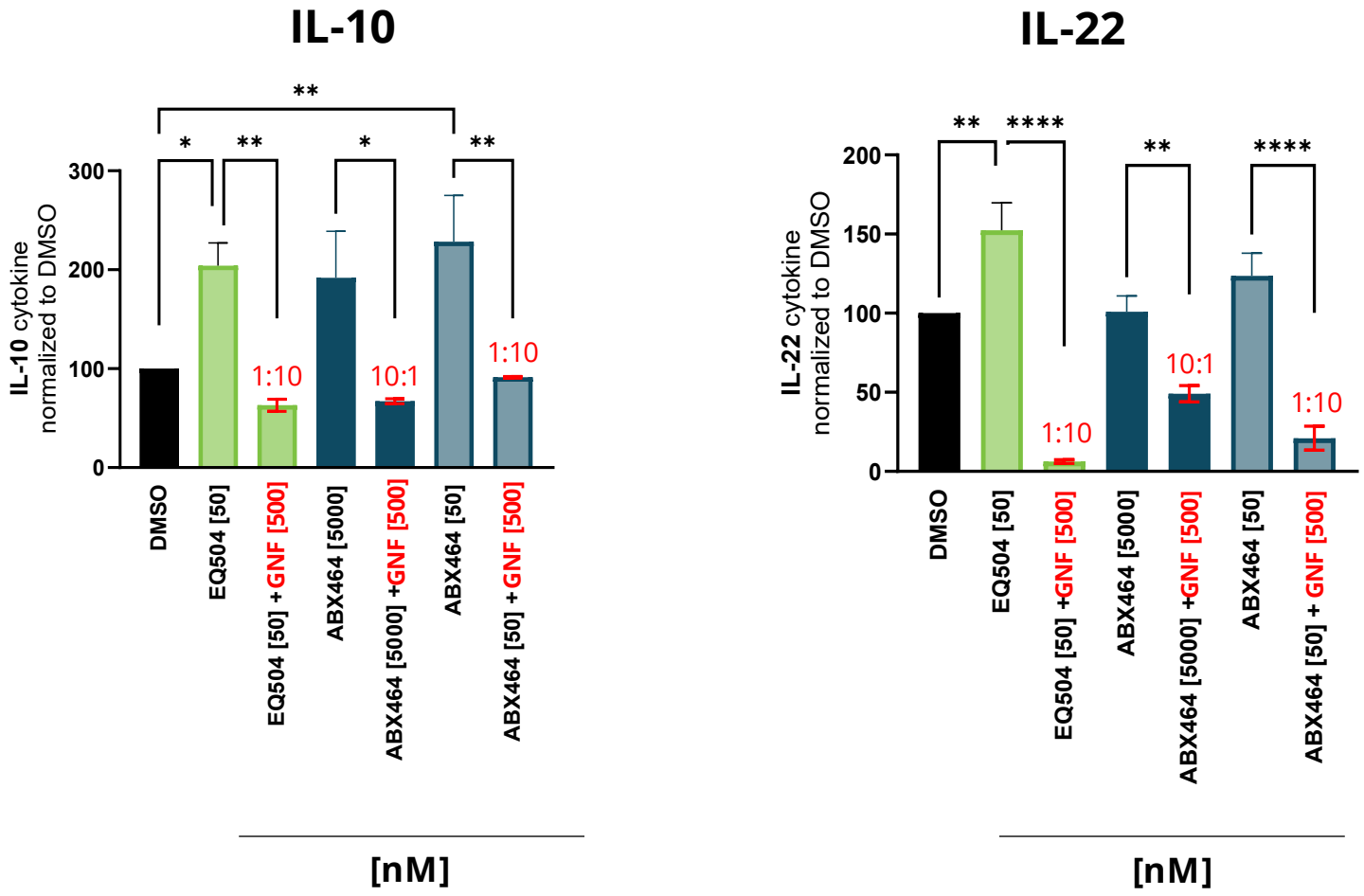
Induction of CYP1A1 and miR-124 in regulatory T cells by both EQ504 & ABX464 is AhR-dependent



Naïve T cells were enriched and treated with a standard T_{reg} cocktail with low CD3/CD28 stimulation for a total of 7 days. Cells were blocked with GNF-351 (500 nM) for one hour, prior to EQ504 or ABX464 treatment. Total of 4 donors have been completed. *****p<0.00001, ***p<0.0001 ,**p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA*. Test article to AhR antagonist GNF ratio shown in red.

AhR Antagonism Inhibits IL-10 & IL-22 Expression in Regulatory T Cells

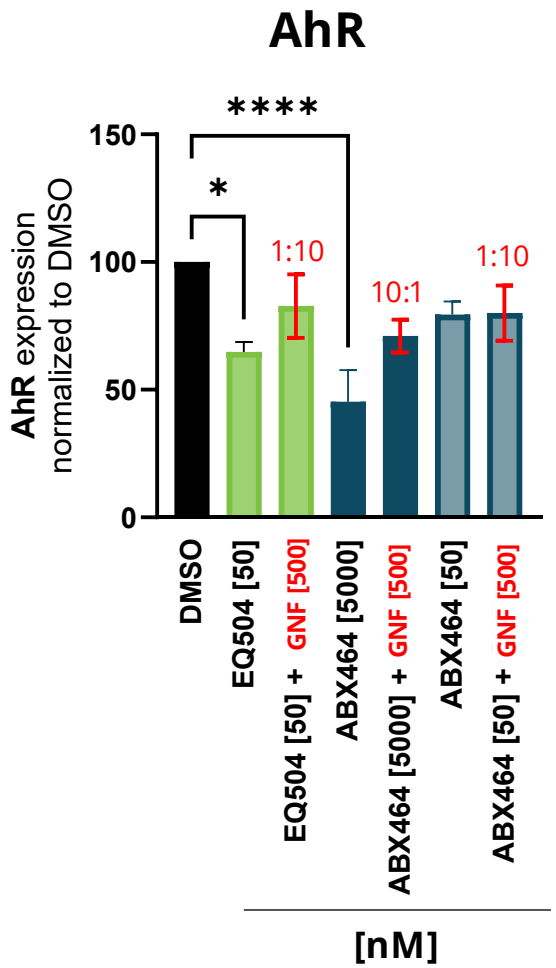
Induction of IL-10 and IL-22 in regulatory T cells by both EQ504 & ABX464 is AhR-dependent



Naïve T cells were enriched and treated with a standard T_{reg} cocktail with low CD3/CD28 stimulation for a total of 7 days. Cells were blocked with GNF-351 (500 nM) for one hour, prior to EQ504 or ABX464 treatment. Total of 4 donors have been completed. *****p<0.00001, ***p<0.0001 ,**p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA*. Test article to AhR antagonist GNF ratio shown in red.

AhR Antagonism Inhibits Decrease of AhR in Regulatory T Cells

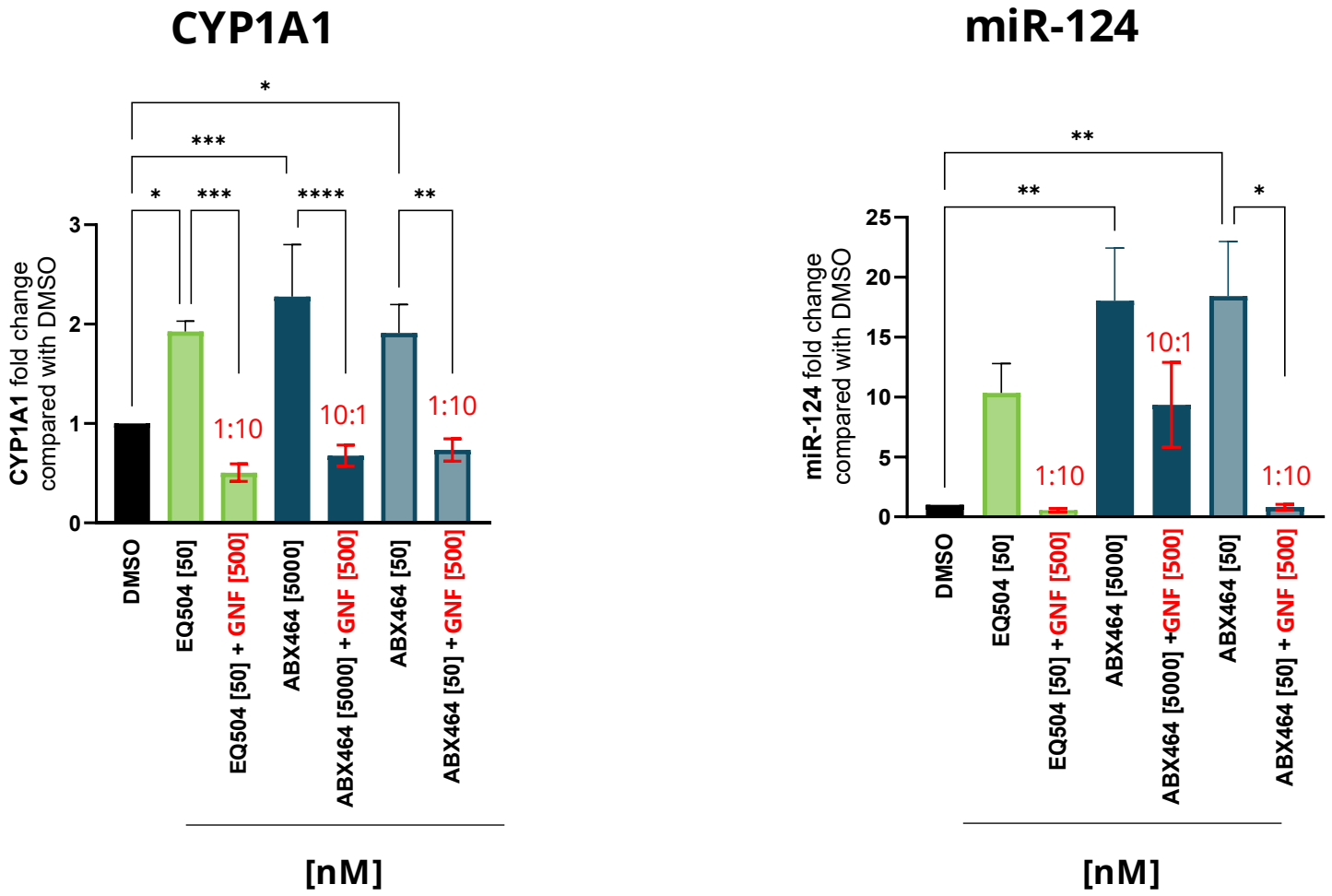
EQ504 & ABX464 decrease AhR expression in regulatory T cells



Naïve T cells were enriched and treated with a standard T_{reg} cocktail with low CD3/CD28 stimulation for a total of 7 days. Cells were blocked with GNF-351 (500 nM) for one hour, prior to EQ504 or ABX464 treatment. Total of 4 donors have been completed. ****$p<0.00001$, **$p<0.001$, *$p<0.005$ *vs DMSO by Ordinary one-way ANOVA*. Test article to AhR antagonist GNF ratio shown in red.

Effects of AhR Modulation on CYP1A1 and miR-124 in M2 Macrophages

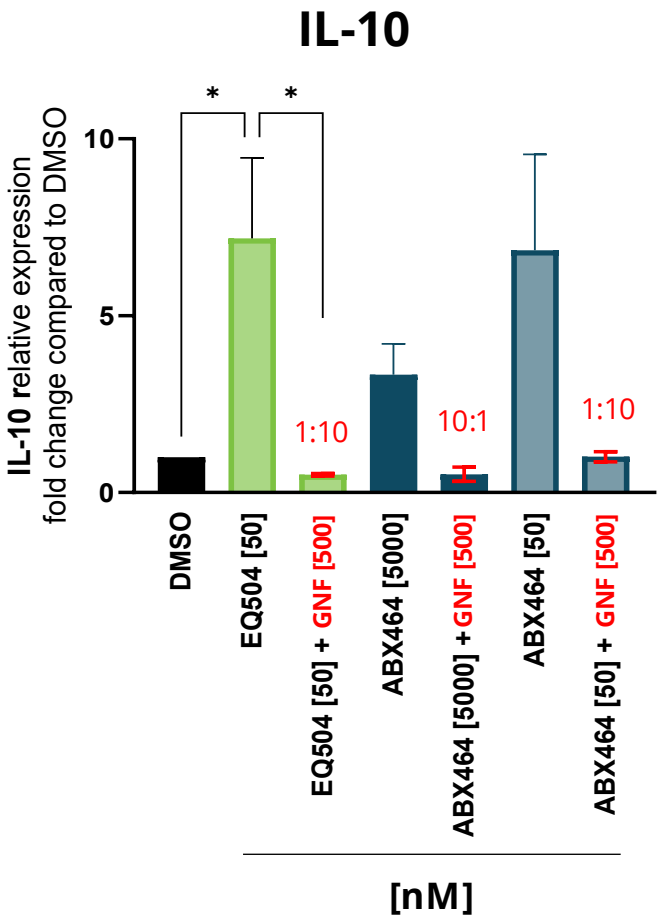
Induction of CYP1A1 and miR-124 in M2 macrophages by both EQ504 & ABX464 is AhR-dependent

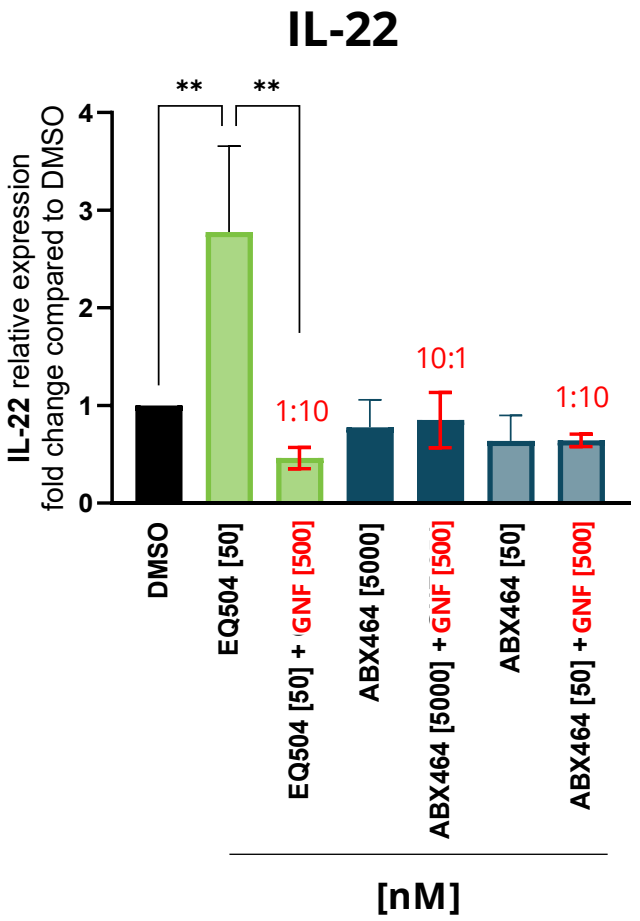


Monocytes were isolated and then treated with M-CSF for 2 days. Cells were then blocked with GNF-351 (500 nM) for one hour, prior to EQ504 or ABX464 treatment. Cells were under EQ504 or ABX464 treatment for 6 days. On day 7 cells were treated with IL-6, IL-13 and IL-4. Takedown of assay was on day 8. Total of 6 donors have been completed. *****p<0.00001, ***p<0.0001 , **p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA.* Test article to AhR antagonist GNF ratio shown in red.

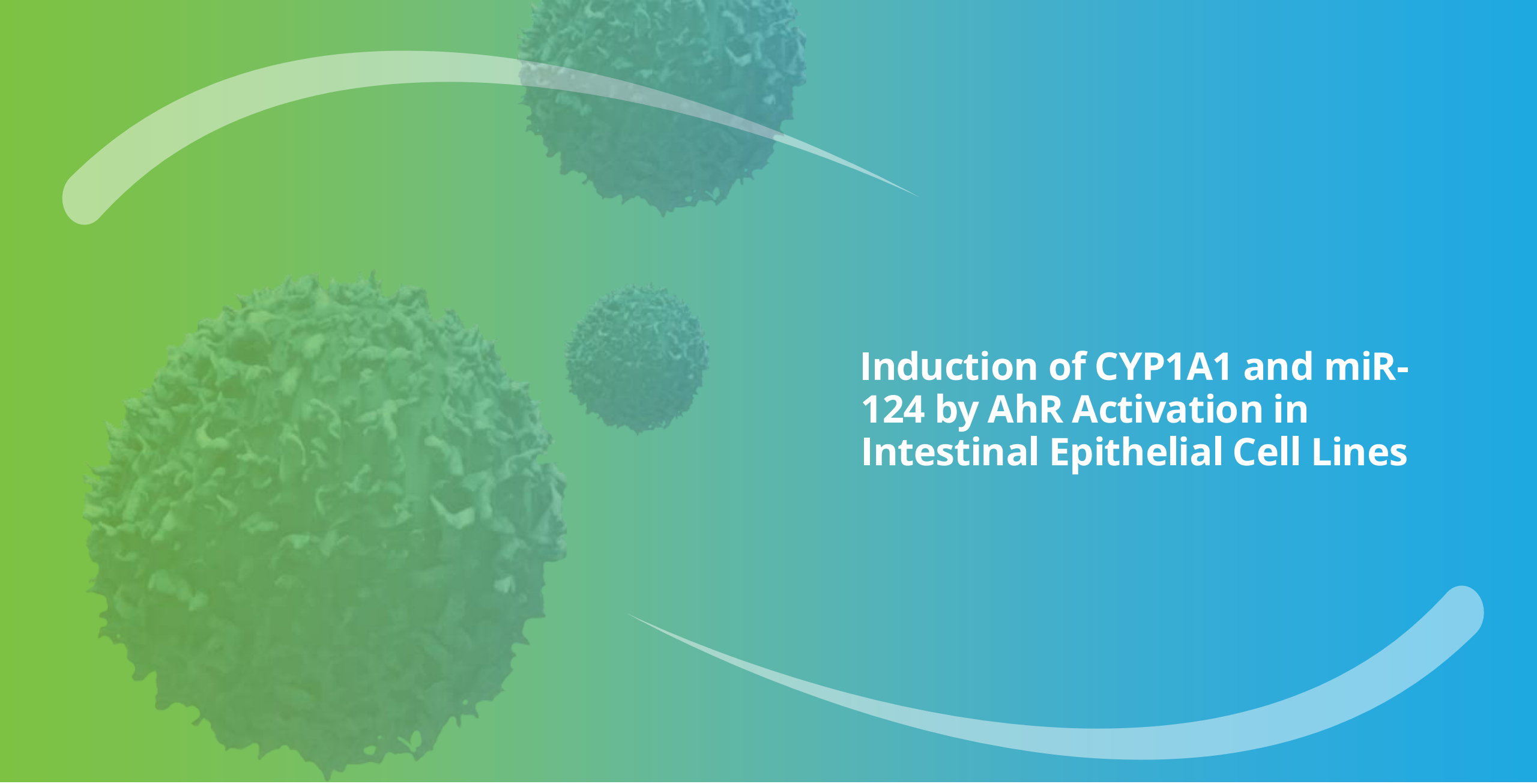
Effects of AhR Modulation on IL-10 and IL-22 in M2 Macrophages

Induction of IL-10 and IL-22 in M2 macrophages by both EQ504 & ABX464 is AhR-dependent





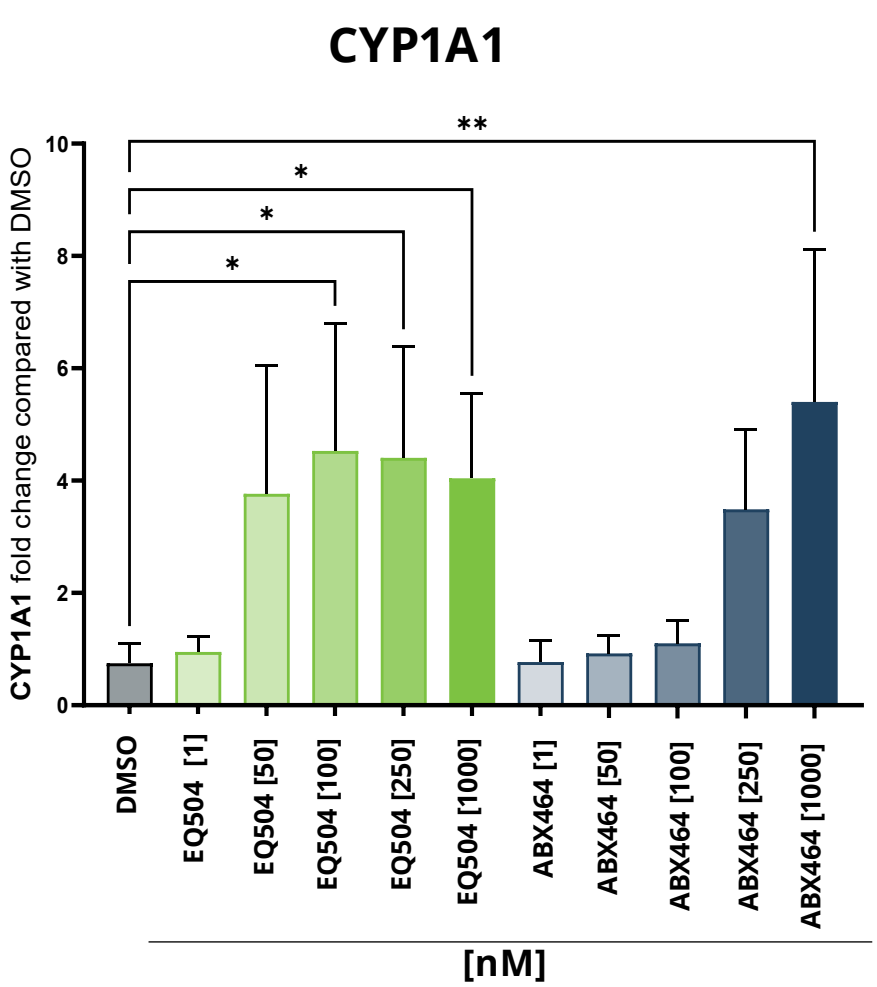
Monocytes were isolated and then treated with M-CSF for 2 days. Cells were then blocked with GNF-351 (500 nM) for one hour, prior to EQ504 or ABX464 treatment. Cells were under EQ504 or ABX464 treatment for 6 days. On day 7 cells were treated with IL-6, IL-13 and IL-4. Takedown of assay was on day 8. Total of 6 donors have been completed. *****p<0.00001, ***p<0.0001, **p<0.001, *p<0.005 vs DMSO by Ordinary one-way ANOVA*. Test article to AhR antagonist GNF ratio shown in red.

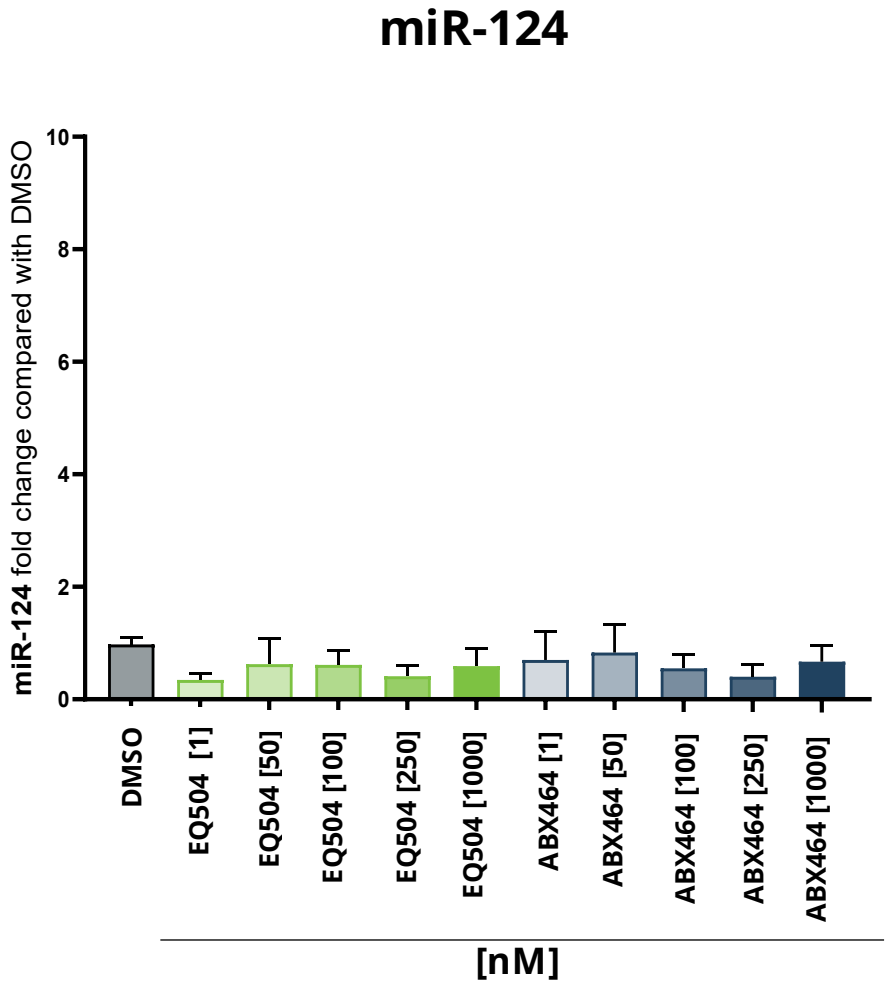


Induction of CYP1A1 and miR-124 by AhR Activation in Intestinal Epithelial Cell Lines

Induction of CYP1A1 and miR-124 in Intestinal Epithelial Cell Line

EQ504 & ABX464, significantly induce CYP1A1, but not miR-124

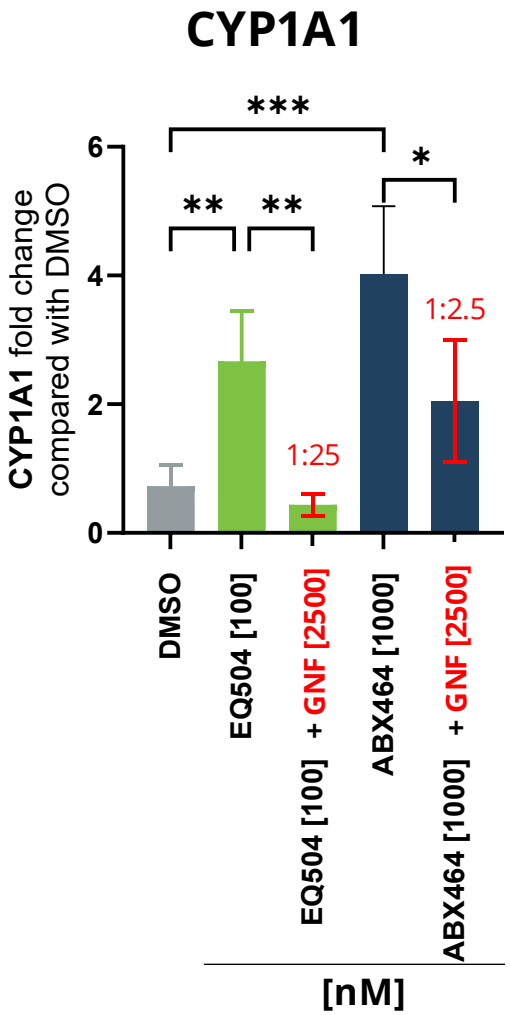




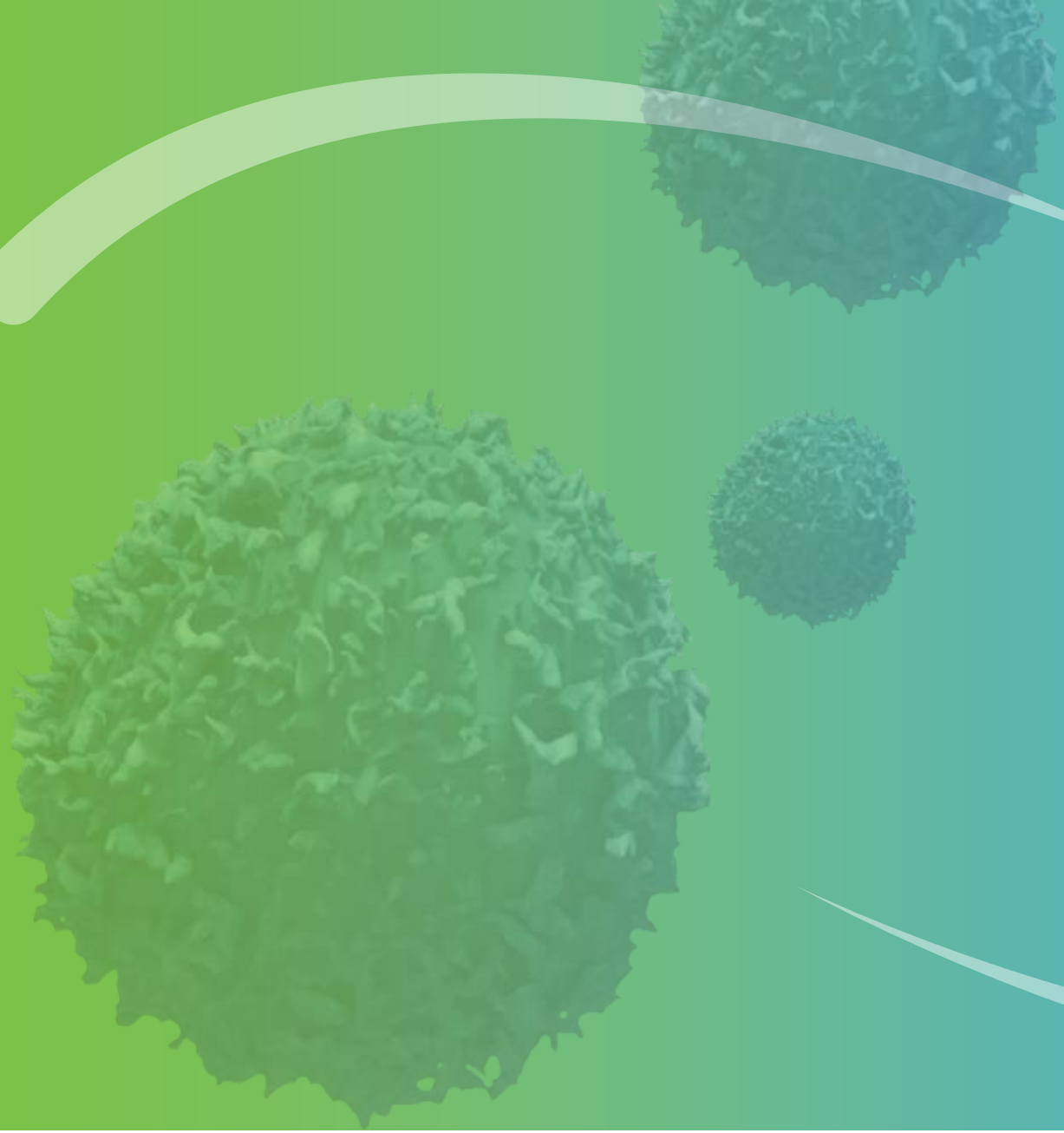
Confluent Caco2 Epithelial Cells were treated with EQ504 or ABX464 for 18h, then CYP1A1 and miR-124 expression were analyzed by RT-qPCR. n = 3-6. Data shown are representative of two independent experiments. . *****p<0.00001, ***p<0.0001 ,**p<0.001, *p<0.005 versus DMSO by one-way ANOVA.*

Effects of AhR Modulation and CYP1A1 in Epithelial Cells

Induction of CYP1A1 in epithelial cells by both EQ504 & ABX464 is AhR-dependent



CYP1A1 gene expression analysis in confluent Caco2 cells treated with EQ405 or ABX464 for 18h and pre-treated for 1h with vehicle or 2.5µM GNF-351. n = 3-6. Data shown are from one representative experiment out of at least two independent experiments. . *****p<0.00001, ***p<0.0001 ,**p<0.001, *p<0.005 analyzed by one-way ANOVA.* Test article to AhR antagonist GNF ratio shown in red.

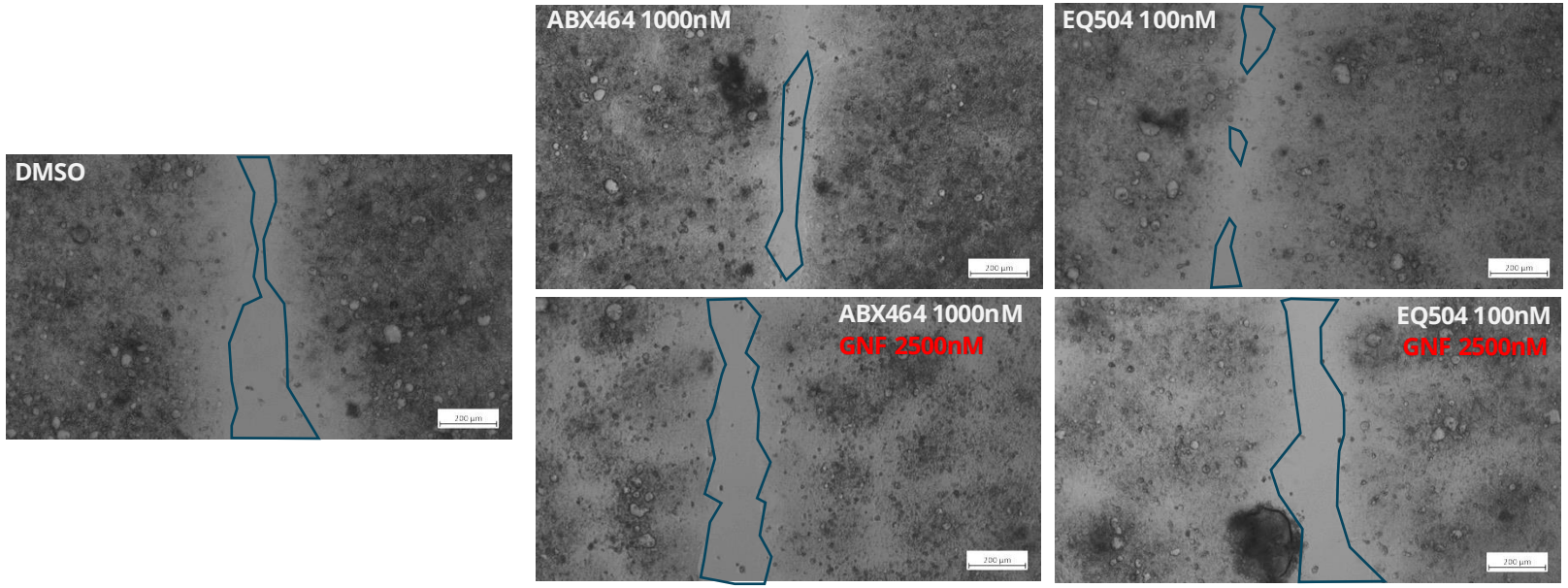


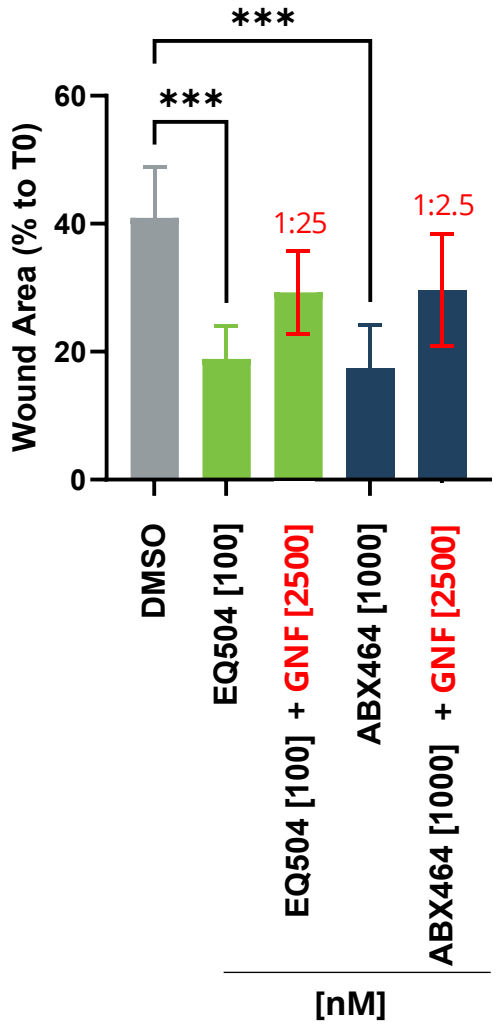
Functional Consequence of AhR-antagonism on CYP1A1, miR-124 and CLDN2

- ✓ Wound Healing in T84
- ✓ TEER Assay in Caco2

Effects of EQ504 and ABX464 on Intestinal Cell Wound Healing

EQ504 & ABX464, significantly improve wound healing in T84 intestinal epithelial cells in AhR-dependent manner

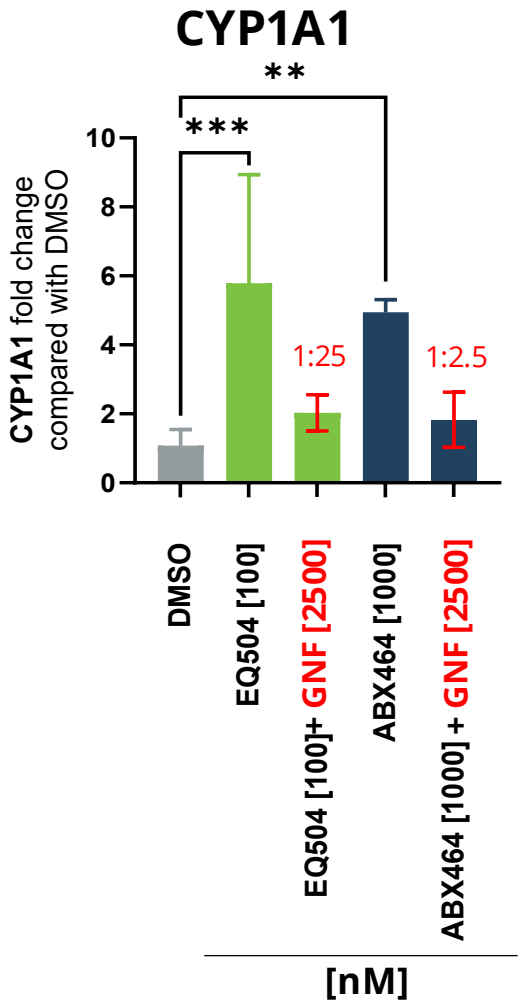




T84 cells in a standard scratch assay, see Lanis *et al.*, Mucosal Immunol., 2017. Scratch area was measured at day 0 (T0) and day 3 using the wound healing size tool master macro on ImageJ and expressed as % scratch area vs day 0. n =3-6. Data shown are from one representative experiment of at least two independent experiments. . *****p<0.00001, ***p<0.0001 , **p<0.001, *p<0.005 vs DMSO by one-way ANOVA.* Test article to AhR antagonist GNF ratio shown in red.

Effects of EQ504 and ABX464 on CYP1A1 Induction in Intestinal Cells

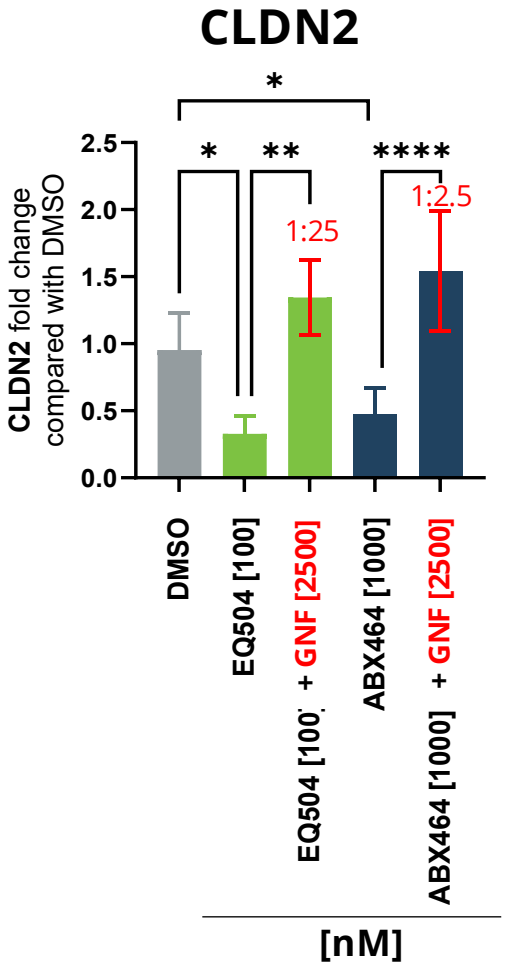
Induction of CYP1A1 in T84 epithelial cells by both EQ504 and ABX464 is AhR-dependent



CYP1A1 expression, analyzed by Real Time qPCR, from T84 cells in a standard scratch assay after 4 days of treatment with DMSO, EQ504 100nM or ABX464 1000nM ± GNF-351 2500nM. n=3-12 replicates per group. Data shown are from one representative experiment out of at least two independent experiments. . *****p<0.00001, ***p<0.0001 ,**p<0.001, *p<0.005 vs DMSO by one-way ANOVA.* Test article to AhR antagonist GNF ratio shown in red.

Effects of EQ504 and ABX464 on Tight Junction Protein CLDN2

Reduction of CLDN2 in T84 intestinal epithelial cells by both EQ504 & ABX464 is AhR-dependent

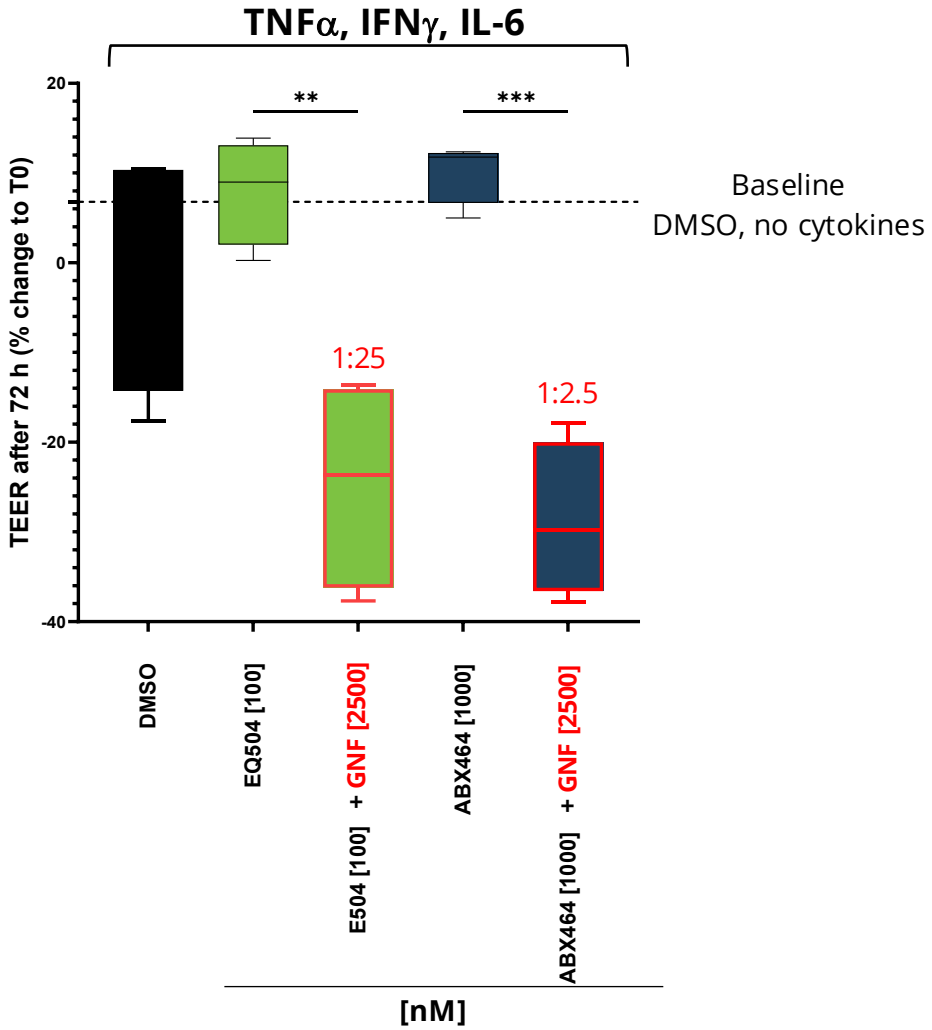


- Elevated CLDN2 is associated with impaired epithelial barrier function (“leaky gut”)
- CLDN2 is increased in ulcerative colitis and DSS colitis
- AhR activation has been associated with reduced CLDN2 expression in intestinal models
- Decreased CLDN2 may reflect restoration of epithelial barrier function following AhR agonist treatment

Claudin 2 expression, analyzed by Real Time qPCR, from T84 cells in a standard scratch assay after 4 days of treatment with DMSO, EQ504 100nM or ABX464 1000nM ± GNF-351 2500nM. n=3-12 replicates per group. Data shown are from one representative experiment of at least two independent experiments. . *****p<0.00001*, ****p<0.0001* , ***p<0.001*, **p<0.005 vs DMSO by one-way ANOVA*. Test article to AhR antagonist GNF ratio shown in red. “Zeissig *et al.*, Gut 2007. Luettig *et al.*, Tissue Barriers 2015. Cuzic *et al.*, Front. Pharmacol., 2021. Yuanhang Ma et al., Chem Biol Interact. 2018. Da Rocha et al., Int J Mol Sci. 2024.”

Effects of EQ504 and ABX464 on Barrier Integrity in a TEER assay

EQ504 & ABX464 significantly protect the epithelial barrier integrity in Caco2 in AhR-dependent manner

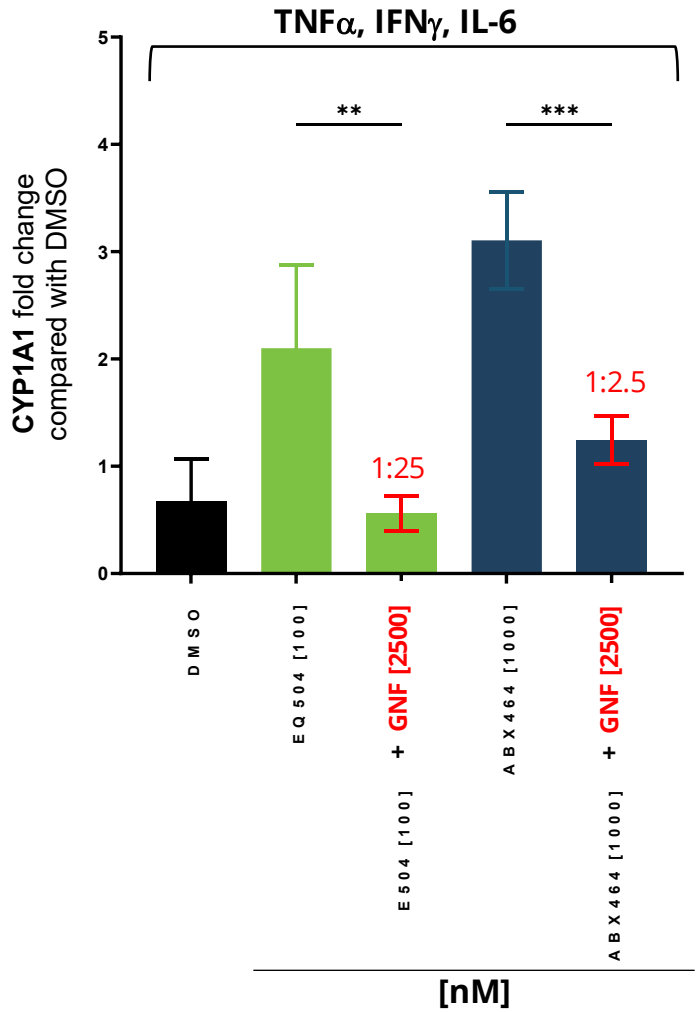


- TEER (transepithelial electrical resistance) is a commonly used assay to assess epithelial barrier integrity in vitro
- The assay measures electrical resistance across a polarized epithelial cell monolayer, with lower resistance indicating increased barrier permeability
- Changes in TEER can be used to evaluate barrier disruption and recovery following inflammatory stimulation or compound treatment

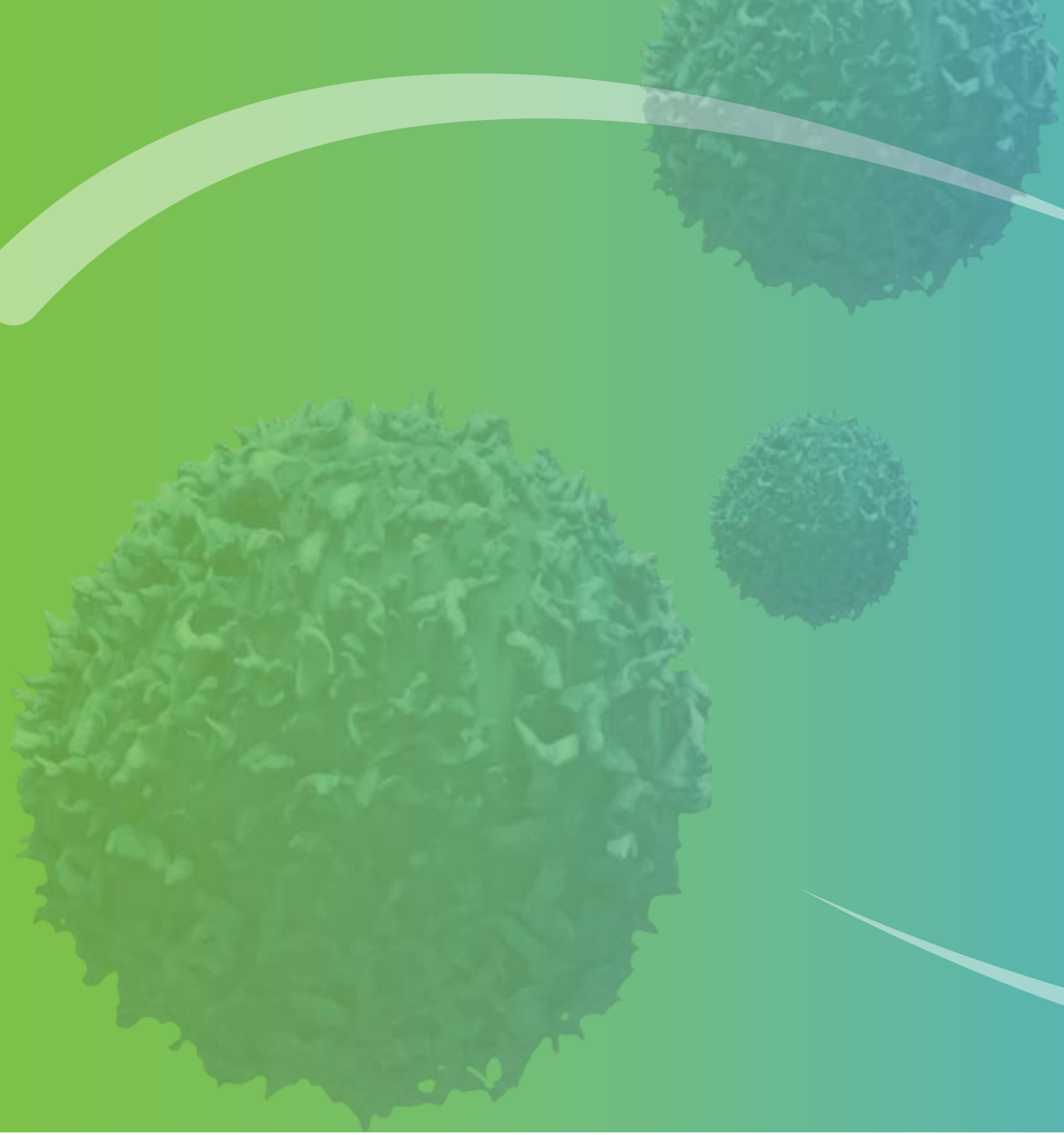
Caco2 polarized monolayer was used in a standard Transepithelial Electrical Resistance (TEER) assay, see Yu et al., Int J Biol Sci 2018 . TNFα 20 ng/ml, IFNγ 10 ng/ml and IL-6 10 ng/ml were used to disrupt the integrity of the barrier. Dashed line represents DMSO without cytokines treatment. n =4 . Data shown are from one representative experiment out of at least two independent experiments. *****p<0.00001, ***p<0.0001 , **p<0.001, *p<0.005 vs non-GNF treatment by one-way ANOVA.* Test article to AhR antagonist GNF ratio shown in red.

Effects of EQ504 and ABX464 on CYP1A1 Induction in Intestinal Cells

Induction of CYP1A1 in Caco2 epithelial cells by both EQ504 & ABX464 is AhR-dependent



CYP1A1 expression analyzed in Caco2 from a Transepithelial Electrical Resistance (TEER) assay. TNFα 20 ng/ml, IFNγ 10 ng/ml and IL-6 10 ng/ml were used to disrupt the integrity of the barrier. CYP1A1 expression at 72h was analyzed by Real Time qPCR. n=4, Data shown are from one representative experiment out of at least two independent experiments. *****p<0.00001, ***p<0.0001 , **p<0.001, *p<0.005 vs non-GNF treatment by one-way ANOVA.* Test article to AhR antagonist GNF ratio shown in red.



Summary and Conclusions

Summary and Conclusions

01 Is ABX464 itself an AhR Modulator?

- ABX464 is a moderately potent AhR modulator (ca. 400nM) that binds to the canonical PAS-B domain of AhR, but ABX464-N-Gluc does not bind nor modulate AhR

02 Does AhR Modulation Induce miR-124?

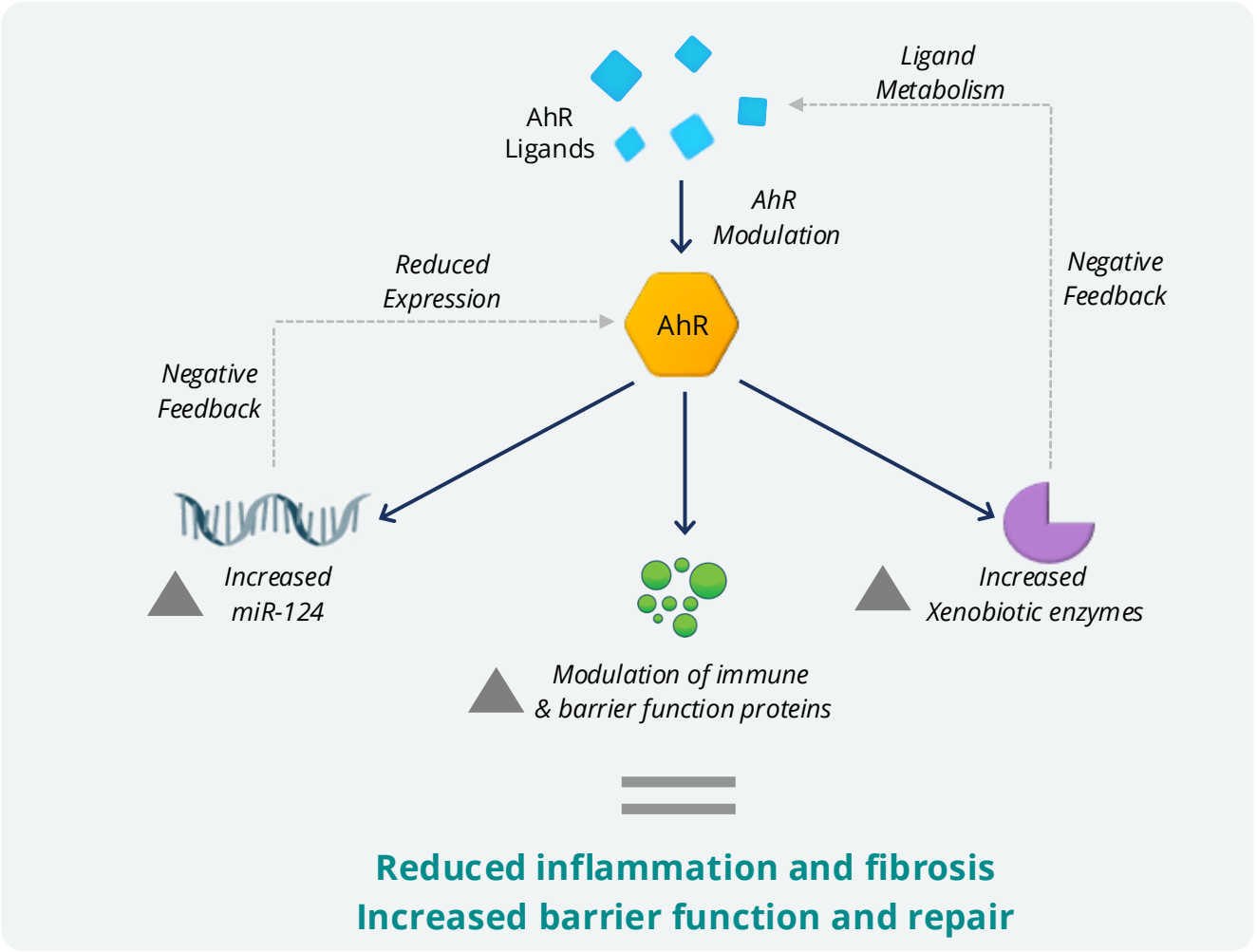
- AhR modulation by EQ504 and ABX464, but not ABX464-N-Glu, induces miR-124 and decreases pro-inflammatory cytokines in multiple immune cells
- AhR antagonism inhibits induction of miR-124, indicating miR-124 is downstream of AhR activation
- No miR-124 induction in epithelial cell lines suggesting miR-124 is contextually important for immune cells

03 Functional consequences of AhR-blockade on induction of key downstream pathways?

- AhR antagonism inhibits induction of CYP1A1, miR-124, IL-10, IL-22 in regulatory macrophages and T cells
- AhR antagonism inhibits induction of CYP1A1 and CLDN2 in epithelial cell lines, worsening healing
- AhR antagonism inhibits induction of CYP1A1 in epithelial cell lines worsening barrier integrity

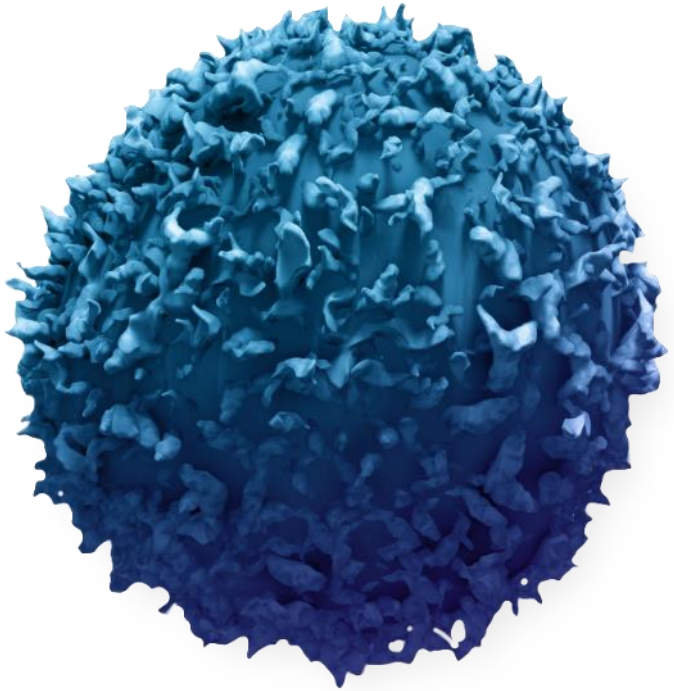
The AhR/miR-124 Axis

Toward a unifying mechanistic framework linking ligand-dependent AhR signaling to miR-124



Zhao *et al.*, Journal of Crohn's and Colitis, 2016; Zhao *et al.*, Inflammation, 2020; Coumoul *et al.*, Signal Transduct Target Ther., 2026

Thank You



Key Acknowledgements

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