

**The role of the AhR signaling pathway in host
responses to *Salmonella* Typhimurium infection and
in tuft cell-mediated intestinal immunity**

Inaugural-Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Michelle Mayer

aus Köln

Düsseldorf, Januar 2025

aus dem Life & Medical Sciences (LIMES) Institut
Abteilung Immunologie und Umwelt
der Rheinischen Friedrich-Wilhelms-Universität Bonn

Gedruckt mit der Genehmigung der
der Mathematisch-Naturwissenschaftlichen Fakultät
der Heinrich-Heine-Universität Düsseldorf

Berichterstatter:

1. Prof. Dr. rer. nat. Irmgard Förster
Abteilung Immunologie und Umwelt
Rheinische Friedrich-Wilhelms-Universität Bonn
2. Prof. Dr. rer. nat. Johannes H. Hegemann
Funktionelle Genomforschung der Mikroorganismen
Heinrich-Heine-Universität Düsseldorf

Tag der mündlichen Prüfung: 23.05.2025

Eidesstattliche Versicherung

Ich versichere an Eides statt, dass die Dissertation selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist.

Ich habe keine früheren Promotionsversuche unternommen und habe diese Dissertation in vorgelegter oder ähnlicher Form in keiner anderen Institution eingereicht.

Teile dieser Arbeit wurden in ähnlicher Form bereits veröffentlicht und sind entsprechend kenntlich gemacht:

Mayer M*, Cengiz-Dartenne SC*, Thiem M, Hatzfeld P, Semeniuk A, Wang N, Strugnell RA, Förster I, Weighardt H. Dysregulation of stress erythropoiesis and enhanced susceptibility to *Salmonella Typhimurium* infection in aryl hydrocarbon receptor-deficient mice. *The Journal of Infectious Diseases*. 2024 Jun 6;jiae304. doi: 10.1093/infdis/jiae304. Epub ahead of print. PMID: 38842164.

**contributed equally to the study*

Düsseldorf, den _____

Michelle Mayer

Table of contents

List of abbreviations	IV
List of figures	IX
List of tables	XI
Summary	XII
1. Introduction	1
1.1. The immune system	1
1.1.1. The innate immune system	2
1.1.2. The adaptive immune system	3
1.2. The Aryl hydrocarbon Receptor	4
1.2.1. The AhR pathway	5
1.2.2. The diversity of AhR ligands	7
1.2.3. AhR and AhRR expression in different cell types	8
1.2.4. Functions of AhR in immune responses to infections	9
1.3. <i>Salmonella</i> infection in the mouse model	10
1.4. Immune regulation in the intestine	10
1.4.1. Components of the small intestinal barrier	11
1.4.2. The small intestinal tuft cell-ILC2 circuit	13
1.4.3. The role of AhR signaling in gut immunity	16
2. Aims of the thesis	18
3. Materials and Methods	19
3.1. Materials	19
3.1.1. Equipment	19
3.1.2. Consumables	21
3.1.3. Chemicals reagents, enzymes and recombinant proteins	22
3.1.4. Solutions and buffers	25
3.1.5. Kits and assays	28
3.1.6. Antibodies	28
3.1.7. Bacterial strains	30
3.1.8. Mouse lines	31

3.1.9. Software	31
3.2. Methods	32
3.2.1. Mouse housing conditions	32
3.2.2. <i>Salmonella</i> infection model	32
3.2.3. Blood/serum analysis	32
3.2.4. Preparation of single-cell suspensions from bone marrow and spleen	33
3.2.5. Succinate treatment	33
3.2.6. Preparation of single-cell suspensions from small intestine	33
3.2.7. Restimulation of LP cells	34
3.2.8. Isolation of small intestinal crypts	34
3.2.9. Small intestinal organoid culture	35
3.2.10. Cryopreservation of small intestinal organoids	36
3.2.11. Co-culture of crypts and LP cells	36
3.2.12. Dissociation of small intestinal crypts and organoids	37
3.2.13. Flow cytometry	37
3.2.14. Histology	38
3.2.15. Statistical analysis	39
4. Results	41
4.1. Dysregulation of stress erythropoiesis and enhanced susceptibility to <i>Salmonella</i> Typhimurium infection in AhR-deficient mice	41
4.1.1. AhR ^{-/-} mice display increased susceptibility to TAS2010 infection	41
4.1.2. TAS2010 infection leads to increased splenomegaly and altered splenic architecture in AhR ^{-/-} mice	42
4.1.3. AhR ^{-/-} mice show macrocytic anemia and reduced bone marrow erythropoiesis upon infection	45
4.1.4. Reduced bone marrow erythropoiesis in AhR ^{-/-} mice correlates with an increase in stress erythropoiesis upon TAS2010 infection	47
4.2. Modulation of AhR signaling influences the tuft cell-mediated immunity in the mouse small intestine	50
4.2.1. AhR activation restricts the IL-13-driven tuft cell expansion and induces <i>AhRR</i> expression by IECs in mouse intestinal organoids	51
4.2.2. Modulation of AhR signaling has no effect on <i>Atoh1</i> expression by tuft cells in organoids	57

4.2.3. Direct co-culture of intestinal crypts with LP cells suppresses <i>AhRR</i> expression by IECs upon AhR activation	59
4.2.4. Genetic modulation of AhR signaling in mice alters the tuft cell frequency in the small intestinal epithelium	61
4.2.5. Loss of AhR in mice impairs the succinate-induced activation of the intestinal tuft cell-ILC2 immune circuit	68
5. Discussion	72
5.1. AhR deficiency in mice results in unrestrained EME and elevated susceptibility to STM infection	72
5.2. Modulation of AhR signaling affects tuft cell-mediated immunity in the mouse small intestine	75
5.2.1. Influence of AhR signaling on tuft cell differentiation under homeostatic conditions	75
5.2.2. Role of AhR signaling in tuft cell-ILC2 circuit activation	79
5.2.3. Differences in <i>AhRR</i> expression by IECs <i>in vitro</i> compared to <i>in vivo</i>	81
5.2.4. Use of small intestinal organoids as <i>in vitro</i> model	83
6. References	85
7. Appendix	102
8. Curriculum Vitae	106
9. List of publications	108
10. Acknowledgements	109

List of abbreviations

	3MC	3-Methylcholanthrene
A	Ach	Acetylcholine
	ADP	Adenosine diphosphate
	AhR	Aryl hydrocarbon receptor
	AhRR	Aryl hydrocarbon receptor repressor
	AIP	Aryl hydrocarbon receptor-interacting protein
	ALOX5	Arachidonate 5-lipoxygenase
	AMP	Antimicrobial peptide
	APC	Antigen presenting cell
	ARNT	Aryl hydrocarbon receptor nuclear translocator
	ATOH1	Atonal basic helix-loop-helix transcription factor 1
B	BaP	Benzo(a)pyrene
	BasoE	Basophilic erythroblast
	BCR	B cell receptor
	BFU-E	Burst-forming unit-erythroid
	bHLH	Basic helix-loop-helix
	BMP	Bone morphogenetic protein
	BSA	Bovine serum albumin
C	°C	Degree Celsius
	Ca/Ca²⁺	Calcium
	CFU	Colony-forming unit
	CFU-E	Colony-forming unit-erythroid
	ChAT	Choline acetyltransferase
	ChrgA	Chromogranin A
	cm	Centimeter
	COVID-19	Coronavirus disease 2019
	COX	Cyclooxygenase
	CRTH2	Chemoattractant receptor-homologous molecule expressed on T _H 2 cells
	CYP	Cytochrome P450
	CysLT	Cysteinyl leukotrienes
D	DAMP	Damage-associated molecular pattern

	DAPCO	Triethylenediamine
	DAPI	4-6-Diamidino-2-phenylindole
	DC	Dendritic cell
	DCLK1	Doublecortin-like kinase 1
	DIM	3,3'-Diindolylmethane
	DLL	Notch delta-like ligand
	DMEM	Dulbecco's Modified Eagle Medium
	DMH1	Dorsomorphin homolog 1
	DMSO	Dimethylsulfoxide
	DNA	Deoxyribonucleic acid
	DPBS (PBS)	Dulbecco's phosphate-buffered saline
	DSS	Dextran sodium sulfate
	DTT	Dithiothreitol
E	EDTA	Ethylene-diaminetetraacetic acid
	EEC	Enteroendocrine cells
	EGF	Epidermal growth factor
	EGFP	Enhanced green fluorescent protein
	EME	Extramedullary erythropoiesis
	EPO	Erythropoietin
	eYFP	Enhanced yellow fluorescent protein
F	FACS	Fluorescence-activated cell sorting
	FCS	Fetal calf serum
	FICZ	6-Formylindolo[3,2-b]carbazole
	FoxP3	Forkhead box P3
G	g	Gram/G-Force
	GALT	Gut-associated lymphoid tissue
	GATA-3	GATA-binding protein 3
	GFR	Growth Factor Reduced
	GNAT3	α -Gustducin
H	h	Hour
	HBSS	Hank's balanced salt solution
	HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
	HES1	Hairy and enhancer of split 1
	HCl	Hydrogen chloride

	HIF	Hypoxia-inducible factor
	HIV	Human immunodeficiency virus
	HSC	Hematopoietic stem cells
	HSP90	Heat shock protein 90 kDa
I	I3C	Indole-3-carbinol
	ICZ	Indolo-[3,2-b]carbazole
	IDO1	Indoleamine 2,3-dioxygenase 1
	IEC	Intestinal epithelial cell
	IEL	Intraepithelial lymphocyte
	IFN	Interferon
	Ig	Immunoglobulin
	IL	Interleukin
	IL-4Rα	Interleukin-4 receptor- α
	ILC	Innate lymphoid cell
	ILF	Isolated lymphoid follicle
	ISC	Intestinal stem cell
K	KLRG1	Killer cell lectin-like receptor G1
L	LB	Luria-Bertani
	LGR5	Leucine-rich repeat-containing G-protein-coupled receptor 5
	LP	Lamina propria
	LP-CM	Conditioned medium from lamina propria cells
	LTi cell	Lymphoid tissue inducer cell
	LysP	Lysozyme-P
M	mAChR	Muscarinic acetylcholine receptor
	M cell	Microfold cell
	MCHC	Mean corpuscular hemoglobin concentration
	MCV	Mean corpuscular volume
	Mg	Magnesium
	MGV	Mean grey value
	MHC	Major histocompatibility complex
	min	Minute
	μg	Microgram
	μl	Microliter

	ml	Milliliter
	mLN	Mesenteric lymph node
	μm	Micrometer
	μM	Micromolar
	mM	Millimolar
	MMMΦ	Marginal metallophilic macrophage
	mRBC	Mature red blood cell
	MUC2	Mucin 2
	MZMΦ	Marginal zone macrophage
N	Na⁺	Sodium
	NaCl	Sodium chloride
	NFAT	Nuclear factor of activated T cells
	NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
	NK cell	Natural killer cell
	nm	Nanometer
	NOD	Nucleotide-binding oligomerization domain
O	OD	Optical density
	O-GlcNAc	O-linked-N-acetylglucosaminylation
	OrthoE	Orthochromatic erythroblasts
P	PAMP	Pathogen-associated molecular pattern
	PAS	PER-ARNT-SIM
	PFA	Paraformaldehyde
	PGD₂	Prostaglandin D ₂
	PLCβ2	Phospholipase Cβ2
	PLP	Periodate-Lysine-Paraformaldehyde
	PolyE	Polychromatic erythroblast
	POU2F3	POU class 2 homeobox 3
	PP	Peyer's patch
	ProE	Proerythroblast
	PRR	Pattern recognition receptor
R	RBC	Red blood cell
	Retic	Reticulocyte
	RIG-I	Retinoic acid-inducible gene-I

	RNA	Ribonucleic acid
	RORγt	Retinoic acid-related orphan receptor gamma T
	rpm	Revolutions per minute
	RPMI	Roswell Park Memorial Institute
S	SCF	Stem cell factor
	SD	Standard deviation
	SDS	Sodium dodecyl sulfate
	sec	Second
	SEM	Standard error of the mean
	SEP	Stress erythroid progenitor
	SOX	SRY (sex-determining region Y)-box transcription factor
	ST2	Suppression of tumorigenicity 2
	STAT	Signal transducers and activators of transcription
	STM	<i>Salmonella enterica</i> serovar Typhimurium
	SUCNR1	Succinate receptor 1
T	TA zone	Transit-amplifying cell zone
	T-bet	T-box-containing protein expressed in T cells
	TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
	TCR	T cell receptor
	TDO	Tryptophan 2,3-dioxygenase
	TGF	Transforming growth factor
	T_h cell	T helper cell
	TiPARP	TCDD-inducible poly-ADP-ribose polymerase
	TLR	Toll-like receptors
	TNF	Tumor necrosis factor
	TPO	Thrombopoietin
	T_r1 cell	Type 1 regulatory T cell
	T_{reg} cell	Regulatory T cell
	TRPM5	Transient receptor potential cation channel subfamily M member 5
W	wk	Week
	WT	Wildtype
X	XRE	Xenobiotic response element

List of figures

Figure 1.1 Cellular and humoral components of the innate and adaptive immune system _____	4
Figure 1.2 Scheme of AhR functional domains _____	5
Figure 1.3 The canonical AhR signaling pathway _____	6
Figure 1.4 Structure of the small intestinal mucosa _____	13
Figure 1.5 The small intestinal tuft cell-ILC2 circuit _____	15
Figure 4.1 AhR deficiency in mice results in higher susceptibility to TAS2010 infection _____	42
Figure 4.2 AhR ^{-/-} mice display increased splenomegaly and remodeling of splenic immune cell compartments upon infection _____	44
Figure 4.3 TAS2010 infection results in macrocytic anemia and reduced bone marrow erythropoiesis in AhR ^{-/-} mice _____	46
Figure 4.4 Splenomegaly in AhR ^{-/-} mice is mainly caused by enhanced stress erythropoiesis _____	48
Figure 4.5 Expansion of splenic stress erythroid progenitors and elevated EPO and IL-6 serum levels in AhR ^{-/-} mice upon TAS2010 infection _____	49
Figure 4.6 Differentiation of mouse small intestinal organoids derived from adult somatic intestinal stem cells _____	51
Figure 4.7 AhR activation appears to limit IL-13-mediated tuft cell expansion and induces <i>AhRR</i> -EGFP expression by IECs in intestinal organoids _____	52
Figure 4.8 Tuft cell gating strategy of <i>AhRR</i> reporter intestinal organoids _____	53
Figure 4.9 Tuft cell expansion upon IL-13 treatment is restricted by AhR activation in intestinal organoids _____	54
Figure 4.10 3MC and FICZ stimulation of intestinal organoids induce <i>AhRR</i> -EGFP expression by IECs as well as tuft cells _____	55
Figure 4.11 Paneth cell and EEC gating strategy of intestinal organoids _____	56
Figure 4.12 Frequencies of Paneth cells and EECs are not altered upon IL-13 stimulation in intestinal organoids _____	57

Figure 4.13 Tuft cell gating strategy of <i>Atoh1</i> reporter intestinal organoids	58
Figure 4.14 <i>Atoh1</i> -tdTomato expression by tuft cells is not influenced by AhR signaling	59
Figure 4.15 3MC-induced <i>AhRR</i> -EGFP expression by IECs is inhibited by direct co-culture of intestinal crypts with LP cells	60
Figure 4.16 DCLK1 marks tuft cells in the small intestine of WT, <i>AhR</i> ^{-/-} , <i>AhRR</i> ^{E/E} and TripleCyp ^{-/-} <i>AhRR</i> ^{E/E} mice	62
Figure 4.17 Intestinal tuft cell numbers are altered by genetic modulation of AhR signaling	63
Figure 4.18 Gating strategy for <i>ex vivo</i> analysis of small intestinal tuft cells	64
Figure 4.19 Gating strategy for <i>ex vivo</i> analysis of small intestinal ILC2s	64
Figure 4.20 Genetic ablation of AhR enhances whereas loss of CYP1 enzymes decreases the frequency of intestinal tuft cells	65
Figure 4.21 Genetic modulation of AhR signaling does not significantly affect the frequency and proliferation of intestinal ILC2s	66
Figure 4.22 <i>Ex vivo</i> isolated IECs including tuft cells barely express <i>AhRR</i> -EGFP	67
Figure 4.23 Tuft cell expansion upon succinate treatment is limited in <i>AhR</i> ^{-/-} mice	69
Figure 4.24 <i>AhR</i> ^{-/-} mice show impaired succinate-induced tuft cell expansion	70
Figure 4.25 Reduced ILC2 proliferation in <i>AhR</i> ^{-/-} mice upon succinate treatment	71
Appendix Figure 7.1 Characterization of splenic immune cell subsets	102
Appendix Figure 7.2 Analysis of erythroid cells in blood and bone marrow	103
Appendix Figure 7.3 Analysis of erythroid cells in spleen and niche factors in serum regulating erythropoiesis	104

List of tables

Table 3.1 | Equipment _____ 19

Table 3.2 | Consumables _____ 21

Table 3.3 | Chemical reagents, enzymes and recombinant proteins _____ 22

Table 3.4 | Solutions and buffers _____ 25

Table 3.5 | Kits and assays _____ 28

Table 3.6 | Flow cytometry antibodies and streptavidins _____ 28

Table 3.7 | Histology antibodies and streptavidins _____ 30

Table 3.8 | *Salmonella* Typhimurium strains _____ 30

Table 3.9 | Mouse lines _____ 31

Table 3.10 | Software _____ 31

Appendix Table 7.1 | Contributions of Michelle Mayer to the publication of *Mayer et al.*,
2024 _____ 105

Summary

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that integrates environmental, dietary, microbial, and metabolic cues to control various biological processes. AhR activation induces the expression of its repressor (AhRR) and the xenobiotic-metabolizing cytochrome P450 (CYP) enzymes, which regulate AhR activity by metabolizing AhR ligands. The influence of the AhR on the outcome of infectious diseases strongly depends on the type of pathogen and the local context. In this thesis, it was studied how modulation of AhR signaling impacts host responses to *Salmonella Typhimurium* (STM) infection and the tuft cell-mediated intestinal immunity.

Infection of mice with the attenuated STM strain TAS2010 leads to a chronic systemic disease that resembles human typhoid fever. AhR-deficient (AhR^{-/-}) mice were highly susceptible to TAS2010 infection compared to wildtype mice as indicated by increased bacterial burden and mortality. Furthermore, STM infection led to macrocytic anemia in AhR^{-/-} mice and induced elevated extramedullary erythropoiesis in the spleen. This resulted in enhanced splenomegaly in AhR^{-/-} mice, which was mainly caused by a vast expansion of immature erythroid cells and accompanied by a destruction of the splenic microarchitecture. Elevated serum levels of erythropoietin upon infection as well as increased numbers of splenic stress erythroid progenitors already in steady state probably drive this effect, which might be causative for the splenic remodeling, thereby disturbing an effective host defense of AhR^{-/-} mice against STM infection.

The small intestinal epithelium contains a rare taste-chemosensory cell type, named tuft cell. By acting as luminal sentinels, tuft cells are crucial to induce type 2 immunity to helminths. Upon helminth infection, tuft cell-derived interleukin (IL)-25 activates type 2 innate lymphoid cells (ILC2s) to secrete IL-13, which drives tuft cell hyperplasia. In mouse epithelial-only intestinal organoids used as *in vitro* model, AhR activation reduced IL-13-mediated tuft cell expansion. In line, enhanced AhR activity in the absence of the AhR-dependent CYP enzymes *in vivo* also decreased tuft cell numbers, whereas global AhR deficiency enhanced the frequency of intestinal tuft cells in steady state. In contrast to the *in vitro* results, activation of the whole tuft cell-ILC2 circuit by succinate treatment was significantly restrained in AhR^{-/-} mice. These results demonstrate that an inclusion of immune cells, especially ILC2s, in organoid systems is essential to better model the tuft cell-mediated intestinal immunity.

1. Introduction

Infectious diseases still represent one of the leading causes of deaths worldwide as demonstrated by the recent COVID-19 (coronavirus disease 2019) pandemic. According to the World Health Organization, COVID-19 was globally responsible for 8.8 million deaths in 2021. Additionally, other respiratory infections, diarrheal diseases, HIV (human immunodeficiency virus) infection, tuberculosis, and malaria cause serious health problems and collectively claim millions of lives each year. Hereby, the disease severity depends on the virulence and dose of a pathogen but also particularly on the host immune system. Thus, it is essential to further understand immune mechanisms in response to infections to improve the clinical outcome of various infectious diseases.

1.1. The immune system

The immune system is a complex and interactive network of specialized tissues, cells and molecules that protects the body from infections, toxins, and harmful cell changes. It mediates defense against a wide variety of pathogens, including bacteria, viruses, fungi and parasites. In order to induce an effective protection, the immune system first needs to recognize pathogens, leading to the activation of immune effector functions to limit and finally eliminate infections. Immunodeficiencies can result in the development of chronic infections or even a complete failure of immune defense mechanisms. In contrast, an overactivation of the immune system may cause allergies or autoimmune diseases that damage the host's own tissues. To adjust the balance between fight and tolerance functions, regulatory mechanisms should prevent an overshooting of the immune system. Finally, the generation of an immunological memory allows a rapid and effective response to infections with previously encountered pathogens (Murphy & Weaver, 2017).

In mammalian species, the immune system is comprised of an innate and an adaptive part. While innate immune responses are rapidly induced against a broad spectrum of conserved pathogen patterns, the more specific recognition system of the adaptive immunity responds slower and develops immunological memory. However, a correct interplay of both systems is essential to mount an effective and long-lasting immunity.

1.1.1. The innate immune system

As the first line of defense, physical barriers, such as the epidermis of the skin or the epithelium of the respiratory and gastrointestinal tract, prevent the entry of pathogens into the body. The low pH of the skin and the stomach as well as the gastrointestinal mucus layer, which contains antimicrobial enzymes and peptides, additionally form chemical barriers to eliminate most pathogens. Moreover, commensal bacteria reside on the skin or in the gastrointestinal tract in symbiosis with the host to serve as biological barriers. Known as colonization resistance, intestinal microbiota can prevent the incursion of harmful microorganisms by the competition for nutrients, the production of antimicrobial compounds, immune regulation, and the modulation of virulence factors (Murphy & Weaver, 2017; Rangan & Hang, 2017).

If pathogens successfully overcome these first layers of host protection, they encounter defense mechanisms and patrolling cells of the innate immune system. Pathogen-associated molecular pattern (PAMPs) or damage-associated molecular patterns (DAMPs) are recognized by pattern recognition receptors (PRRs) expressed by innate immune cells and epithelial cells. PRRs include membrane-bound C-type lectin receptors and toll-like receptors (TLRs) and cytoplasmic TLRs, nucleotide-binding oligomerization domain (NOD)-like receptors, and retinoic acid-inducible gene-I (RIG-I)-like receptors. Innate immune cell types are myeloid cells, such as granulocytes (neutrophils, eosinophils, and basophils), macrophages, and dendritic cells (DCs), as well as innate lymphoid cells (ILCs) (Figure 1.1). ILCs comprise five different subsets, which are defined by their distinct cytokine and transcription factor profile: natural killer (NK) cells, lymphoid tissue inducer cells (LTi), group 1 ILCs (ILC1s), group 2 ILCs (ILC2s), and group 3 ILCs (ILC3s). Activated innate immune cells secrete antimicrobial peptides (AMPs) and cytokines that modulate inflammatory responses. Chemotactic cytokines, called chemokines, promote the recruitment of further innate effector cells. Macrophages, neutrophils, and DCs can eliminate pathogens by phagocytosis and degrade them within phagolysosomes. Pathogen-derived peptides formed during digestion are then presented on major histocompatibility complex (MHC) molecules to stimulate the adaptive immune system. Particularly, DCs are specialized antigen presenting cells (APCs), linking innate and adaptive immunity (Murphy & Weaver, 2017).

1.1.2. The adaptive immune system

Upon activation, antigen-loaded DCs migrate from peripheral tissue sites via lymphatic vessels to secondary lymphoid organs such as draining lymph nodes. Here, peptide/MHC complexes are recognized by thymus-derived lymphocytes (T cells) via their antigen-specific T cell receptor (TCR) on the cell surface. Whereas antigens presented on MHC class I (MHC I) are recognized by CD8⁺ T cells, CD4⁺ T cells are stimulated by peptides on MHC class II (MHCII) (Figure 1.1). Apart from the stimulation of the TCR, naive T cells require the binding of costimulatory molecules and cytokine signals to get activated. Cytotoxic CD8⁺ T cells are able to eliminate infected or cancerous target cells. In contrast, CD4⁺ T cells further differentiate into different T helper (T_h) cell subsets, which are characterized by the expression of key transcription factors and a specific cytokine profile. T_h1 cells, defined by T-bet expression and interferon (IFN)- γ and tumor necrosis factor (TNF)- β production, drive type I immunity against intracellular viral or bacterial pathogens. GATA-3⁺ T_h2 cells secrete interleukin (IL)-4, IL-5, and IL-13 and participate in type II immune responses against extracellular parasites or during allergies. T_h17 cells, which depend on ROR γ t and produce IL-17 and IL-22, mediate the defense against extracellular bacteria and fungi, especially at mucosal sites. Additionally, CD4⁺ T cells give rise to FoxP3⁺ regulatory T (T_{reg}) cells that secrete anti-inflammatory cytokines such as IL-10 and transforming growth factor (TGF)- β to suppress autoreactive and overshooting immune reactions, maintaining homeostasis and self-tolerance. In addition to conventional T cells expressing a TCR composed of an α - and a β -chain, unconventional TCR $\gamma\delta$ ⁺ T cells are not restricted to classical MHC antigen presentation and promote rapid innate-like immune responses at mucosal barriers (Figure 1.1). NK T cells, which share characteristics with innate NK cells and conventional T cells, express a semi-invariant TCR $\alpha\beta$, recognizing glycolipid antigens presented on the MHC I-like CD1d glycoprotein. Through rapid production of cytokines, NK T cells can shape innate and adaptive immune responses (Murphy & Weaver, 2017).

The activation of bone marrow-derived lymphocytes (B cells) is induced by the binding of the B cell receptor (BCR) to its cognate antigen. Followed by internalization and presentation of this antigen via MHCII, T_h cells specific for the same antigen interact with B cells to activate them via the release of cytokines. Thus, naive B cells start to proliferate and differentiate into plasma cells, potent in the production of pathogen-

specific antibodies, also called immunoglobulins (Ig) (Figure 1.1). As a part of humoral immunity, secreted antibodies can neutralize or opsonize pathogens to activate the complement system or facilitate phagocytosis. After a primary immune response, some T and B cells develop long-lasting immune memory, allowing the body to quickly and efficiently respond to previously encountered pathogens. However, the formation of immunological memory is not an exclusive feature of the adaptive immune system, as innate immune cells also show a memory response, called “trained immunity” to some pathogenic stimuli (Murphy & Weaver, 2017; Netea *et al*, 2020).

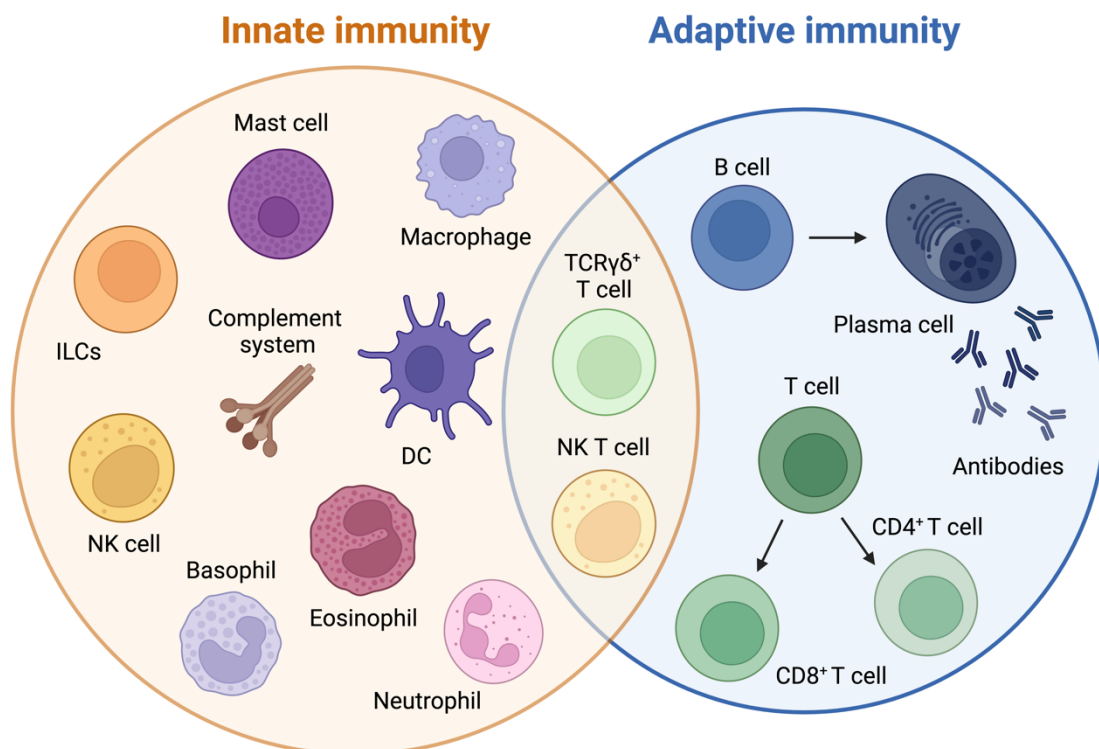


Figure 1.1 | Cellular and humoral components of the innate and adaptive immune system

Innate immunity is mediated by macrophages, mast cells, dendritic cells (DCs), granulocytes (basophils, eosinophils, and neutrophils), innate lymphoid cells (ILCs), natural killer (NK) cells, and the complement system. The adaptive immune system is comprised of B cells, differentiating into antibody-secreting plasma cells, and T cells, giving rise to CD8⁺ cytotoxic T cells and CD4⁺ T helper cells.

1.2. The Aryl hydrocarbon Receptor

The aryl hydrocarbon receptor (AhR) is an evolutionarily conserved ligand-activated transcription factor that acts as a sensor for environmental and endogenous polycyclic aromatic hydrocarbons to modulate the immune system in health and disease (Stockinger *et al*, 2014; Gutiérrez-Vázquez & Quintana, 2018). Originally, the AhR has been known for its response to small exogenous chemical substances (xenobiotics),

where it mediates the detoxification of these chemicals by the induction of xenobiotic metabolizing enzymes (Denison & Nagy, 2003; Barouki *et al*, 2012). The AhR belongs to the PER-ARNT-SIM (PAS) protein family of basic helix-loop-helix (bHLH) transcription factors (Gu *et al*, 2000). Further members of this protein family are the hypoxia-inducible factors (HIFs), which respond to cellular oxygen deprivation (Wang *et al*, 1995). All bHLH-PAS proteins share a similar structure of functional domains. They contain a bHLH motif, composed of a basic region responsible for DNA binding and a HLH dimerization domain, as well as PAS domains, which are involved in protein dimerization but are also essential for the sensing of diverse ligands. The AhR consists of the N-terminal bHLH motif, two PAS domains, termed PAS-A and PAS-B, and a glutamine (Q)-rich region (Figure 1.2). While the bHLH motif functions as the DNA-binding site, it also participates with both PAS domains in the heterodimerization of the AhR with the AhR nuclear translocator (ARNT) (Fukunaga *et al*, 1995). The PAS-B domain additionally serves as the ligand binding site. Moreover, the Q-rich domain at the C-terminus is critical for its transcriptional activation (Kumar *et al*, 2001). In its inactive state, the AhR is stabilized by a complex of chaperons, which bind to the AhR via regions of the bHLH and PAS-B domain (Kudo *et al*, 2018).

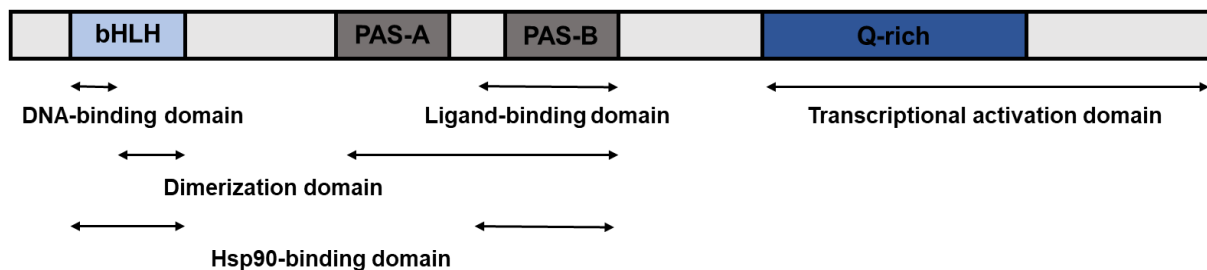


Figure 1.2 | Scheme of AhR functional domains

The AhR is a member of the PER-ARNT-SIM (PAS) protein family of basic helix-loop-helix (bHLH) transcription factors. The N-terminal bHLH domain mediates DNA binding and additionally facilitates together with the two PAS domains, PAS-A and PAS-B, the dimerization with interaction partners such as the AhR nuclear translocator (ARNT). The PAS-B domain also serves as the ligand binding site. Regions in the bHLH and PAS-B domains are involved in binding to the chaperon complex in the inactive state of the AhR. The C-terminal located glutamine (Q)-rich domain is essential for transactivation (adapted from Stockinger *et al*, 2014).

1.2.1. The AhR pathway

In the absence of ligands, the AhR is located in the cytoplasm as part of an inactive protein complex with chaperons, such as the heat shock protein 90 kDa (HSP90), the AhR-interacting protein (AIP, also known as XAP2), p23, and the protein kinase SRC

(Perdew, 1988; Carver & Bradfield, 1997; Meyer & Perdew, 1999; Kazlauskas et al, 1999; Dong et al, 2011) (Figure 1.3). Once a ligand, which enters the cytosol by diffusion across the plasma membrane, binds to the cytosolic AhR, a conformational change is induced. Because of the so exposed nuclear localization signal, the AhR translocates into the nucleus, where it dissociates from the chaperons and dimerizes with ARNT, also known as HIF-1 β (Ikuta et al, 1998; Probst et al, 1993). The formed AhR-ARNT complex binds to specific DNA consensus sequences, also known as xenobiotic response element (XRE), in the promoter region of AhR target genes to regulate their expression (Durrin et al, 1987).

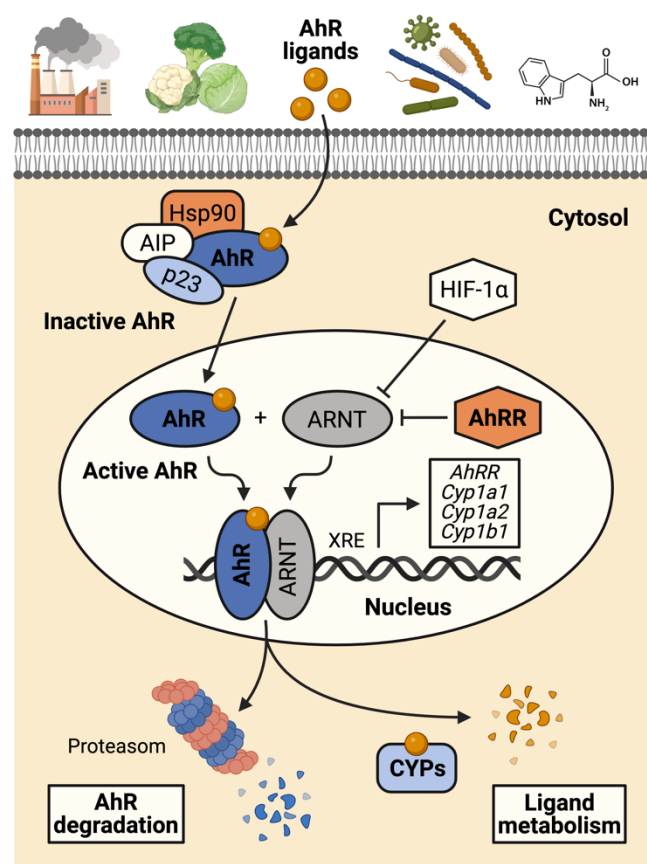


Figure 1.3 | The canonical AhR signaling pathway

The inactive AhR stabilized by chaperons is located in the cytoplasm, where environmental ligands enter via diffusion across the cell membrane. Upon ligand binding, the AhR translocates to the nucleus and dissociates from the chaperons to dimerize with the AhR nuclear translocator (ARNT). The AhR/ARNT complex binds to xenobiotic response elements (XRE) of its target genes, inducing their gene expression. AhR activation is regulated in a negative feedback loop by ligand-metabolizing cytochrome P450 (CYP) enzymes and the AhR repressor (AhRR), which competes with AhR for the binding with ARNT. The hypoxic inducible factor (HIF)-1 α can similarly interfere with the AhR pathway. Additionally, AhR activation is regulated by proteasome-mediated degradation of the AhR (adapted from Rothhammer & Quintana, 2019).

These target genes include genes encoding the xenobiotic-metabolizing cytochrome P450 (CYP) enzymes, CYP1A1, CYP1A2, and CYP1B1, which in turn modulate AhR activity by detoxification or production of AhR ligands (Denison & Heath-Pagliuso, 1998). Another key target gene of the AhR is the AhR repressor (AhRR), which suppresses the activity of AhR by dimerizing with ARNT and binding to XREs (Mimura *et al*, 1999; Hahn *et al*, 2009). In contrast to the AhR, the AhRR lacks a ligand-binding and transactivation domain. Thus, the AhRR counteracts AhR-dependent gene expression to result in a temporal control of AhR signaling by negative feedback regulation. Similarly, HIF-1 α can outcompete AhR for its interaction with ARNT, thereby interfering with the AhR pathway (Mascanfroni *et al*, 2015; Zhang *et al*, 2022). The TCDD-inducible poly-adenosine diphosphate (ADP)-ribose polymerase (TiPARP) catalyzes the ADP-ribosylation of the AhR to promote its nuclear export and subsequent proteasomal degradation (MacPherson *et al*, 2013, 2014).

Apart from this canonical pathway, the AhR directly interacts with several transcription factors that are crucial for regulating cell differentiation and immune responses. For example, the AhR binds signal transducers and activators of transcription (STATs), the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) subunit RelB, c-Maf, the estrogen receptor, and the retinoic acid receptor, thereby modulating their activity (Hankinson, 2005).

1.2.2. The diversity of AhR ligands

The AhR was initially identified as a receptor for environmental pollutants like carcinogenic dioxins such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) or polycyclic aromatic hydrocarbons such as benzo(a)pyrene (BaP) and 3-methylcholanthrene (3MC) (Naruse *et al*, 2002; Denison & Nagy, 2003; Barouki *et al*, 2012). In addition to its activation by xenobiotic compounds, metabolic and immune defects in AhR-deficient mice as well as the upregulation of AhR target genes during development indicated the relevance of physiological AhR agonists (Schmidt *et al*, 1996; Fernandez-Salguero *et al*, 1995; Campbell *et al*, 2005). A major source of naturally occurring AhR ligands is the diet. Flavonoids in vegetables, fruits, and herbs represent the largest group of AhR ligands with either agonistic or antagonistic effects on AhR activation (Denison & Nagy, 2003). Cruciferous vegetables like cauliflower and broccoli contain large amounts of the glucosinolate glucobrassicin, which is enzymatically degraded into the AhR agonist precursor indole-3-carbinol (I3C). In the presence of gastric acid

in the stomach, I3C is further converted into the high-affinity ligands indolo-[3,2-b]carbazole (ICZ) and 3,3'-diindolylmethane (DIM) (Loub *et al*, 1975; Bjeldanes *et al*, 1991). Apart from dietary intake, indole and its derivatives are the main metabolites produced by gut microbiota from tryptophan (Jin *et al*, 2014; Hubbard *et al*, 2015). Additional endogenous AhR ligands are formed by the host metabolism of tryptophan. The enzymes indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase (TDO) catalyze the breakdown of tryptophan into kynurenine, which was shown to exert immunosuppressive functions in inflammation and cancer despite of its low affinity to the AhR (Mezrich *et al*, 2010; Opitz *et al*, 2011). Kynurenine can be further metabolized into AhR agonists such as kynurenic acid and anthranilic acid. The exposure of tryptophan to light results in the formation of the photometabolite 6-formylindolo[3,2-b]carbazole (FICZ), a highly potent ligand with a similar affinity to the AhR as TCDD (Rannug *et al*, 1987). The synthetic compound CH223191 is a specific antagonist of AhR signaling with a ligand-selective activity and low affinity to the AhR (Kim *et al*, 2006).

1.2.3. AhR and AhRR expression in different cell types

The AhR is ubiquitously expressed although its expression levels vary between tissues and cell types. According to its function as an environmental sensor, high basal AhR expression levels were found in the liver and barrier tissues such as the lung, the skin, and the gut using microarray analysis (Frericks *et al*, 2007). Within the hematopoietic lineage, myeloid cells, such as macrophages and DCs, ILCs, antibody-secreting plasma cells, splenic marginal zone B cells, and subsets of T cells show the highest AhR expression (Frericks *et al*, 2007; Qiu *et al*, 2012; Sherr & Monti, 2013). Here, no or low AhR expression is detected in naive T, T_h1, T_h2 and T_{reg} cells, whereas the AhR is highly expressed by T_h17, type 1 T_{reg} (T_r1), and innate TCR $\gamma\delta^+$ T cells, thus impacting their effector functions (Veldhoen *et al*, 2008; Gandhi *et al*, 2010; Apetoh *et al*, 2010; Martin *et al*, 2009). Moreover, the AhR is expressed by hematopoietic stem cells (HSCs) and is shown to modulate HSC homeostasis in the bone marrow and periphery, since AhR-deficient mice display an enhanced proliferation and an altered lineage differentiation potential of HSCs (Singh *et al*, 2011; Vaughan *et al*, 2021). In addition, also non-hematopoietic cell types, such as epithelial, endothelial, and stromal cells, in various organs express the AhR (Metidji *et al*, 2018; Wang *et al*, 2022b; Agbor *et al*, 2011; Wiggins *et al*, 2023; Lkhagva-Yondon *et al*, 2023).

In contrast to the AhR, less is known about the expression and function of the AhRR in different tissues. Using an *AhRR* reporter mouse line, our group demonstrated that the AhRR does not strictly mimic the expression pattern of the AhR, as *AhRR* expression is mainly restricted to immune cells of barrier sites. Especially, myeloid cells, ILC3s, T cells, and intraepithelial lymphocytes (IELs) in the intestine as well as epidermal Langerhans cells and dermal DCs show the highest *AhRR* expression. Unlike the AhR, the AhRR is barely expressed in the liver. Thus, AhR activation appears to be regulated by the AhRR in a cell type- and tissue-specific manner (Brandstätter *et al*, 2016).

1.2.4. Functions of AhR in immune responses to infections

The following section is adapted from Mayer et al., 2024 (Mayer M, Cengiz-Dartenne SC,(...), Förster I, Weighardt H. Dysregulation of stress erythropoiesis and enhanced susceptibility to Salmonella Typhimurium infection in aryl hydrocarbon receptor-deficient mice. The Journal of Infectious Diseases. 2024 Jun 6;jiae304.)

The AhR regulates the differentiation and maintenance of immune cell populations and modulates innate as well as adaptive immune responses, but also regulates the host response to pathogens (Kimura *et al*, 2008; Veldhoen *et al*, 2009; Li *et al*, 2011; Qiu *et al*, 2012, 2013). AhR signaling was shown to directly interfere with TLR-induced signal transduction (Kimura *et al*, 2009) and AhR-deficient mice are highly susceptible to acute infections with *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, and *Listeria monocytogenes* (Moura-Alves *et al*, 2014; Shi *et al*, 2007; Kimura *et al*, 2014). In contrast, host resistance to *Streptococcus pneumonia* infection is not impaired in AhR-deficient mice (Vorderstrasse & Lawrence, 2006), highlighting that AhR deficiency does not generally lead to reduced host defense after infections. Nevertheless, treatment with TCDD improved survival of mice infected with nasal *S. pneumoniae* by reducing bacterial burden in the lung (Vorderstrasse & Lawrence, 2006) and activation by benzo(a)pyrene favored the outcome of systemic *Salmonella enterica* infection in mice by enhancing early host defense functions (Fueledner *et al*, 2022).

1.3. *Salmonella* infection in the mouse model

The following section is adapted from Mayer et al., 2024 (Mayer M, Cengiz-Dartenne SC,(...), Förster I, Weighardt H. Dysregulation of stress erythropoiesis and enhanced susceptibility to Salmonella Typhimurium infection in aryl hydrocarbon receptor-deficient mice. The Journal of Infectious Diseases. 2024 Jun 6:jiae304.)

Infection with *S. enterica* serovar Typhimurium (STM) in mice leads to a lethal systemic disease that resembles human typhoid fever caused by *S. enterica* serovar Typhi. Typhus in humans is characterized by sustained fever, weakness, stomach pain, headache, diarrhea, or constipation. In addition, splenomegaly, hepatomegaly, and anemia have been noticed in typhoid fever (Bhandari *et al*; Ndako *et al*, 2020; Sahu *et al*, 2016). To monitor such long-term immune responses to STM in mice, attenuated strains with reduced *in vivo* growth capacity are used, which can induce a protective adaptive immune response through the activation of IFN- γ -producing CD4⁺ T cells and the development of immune memory (Santos *et al*, 2001; Kupz *et al*, 2014; Benoun *et al*, 2018; Peres *et al*, 2021; Wang *et al*, 2023). Chronic infections with STM in mice cause infection-induced anemia that is compensated by extramedullary erythropoiesis (EME) in the spleen with a large increase of erythroid precursor cells, driving a splenomegaly in response to infection (Jackson *et al*, 2010; Brown *et al*, 2010; Rosche *et al*, 2015; Li *et al*, 2016; Wang *et al*, 2023).

1.4. Immune regulation in the intestine

The gastrointestinal tract harbors the largest mucosal surface in the body that separates the external environment from the internal host milieu. By acting as a semipermeable barrier, the intestinal mucosa allows the absorption of essential nutrients and immune sensing, while it prevents the passage of potentially harmful molecules and microorganisms from the intestinal lumen to the host circulation. Only a fine-tuned balance within the mucosal network between immune defense against pathogens and immune tolerance to commensal microbiota and dietary antigens maintains the intestinal barrier integrity and immune homeostasis (Okumura & Takeda, 2016; Vancamelbeke & Vermeire, 2017; Di Sabatino *et al*, 2023).

1.4.1. Components of the small intestinal barrier

The mucosa is the innermost layer of the small intestine. It is composed of an outer mucus layer colonized by commensal microbiota, a continuous monolayer of epithelial cells, an underlying lamina propria (LP), where innate and adaptive immune cells reside, and the muscularis mucosae, promoting the motility of the intestinal mucosa (Mowat & Agace, 2014) (Figure 1.4). The mucus layer above the small intestinal epithelium is composed of highly glycosylated mucin proteins, predominantly mucin 2 (MUC2), secreted by specialized epithelial cells called goblet cells (Rodríguez-Piñeiro *et al*, 2013). These mucin biopolymers form a gel-like structure that is permeable to water and small particles, but limits the direct contact between bacteria and epithelial cells (Pelaseyed *et al*, 2014). The release of AMPs, mainly α -defensins, into the mucus by both epithelial Paneth cells and enterocytes further reinforces the antimicrobial activity (Ayabe *et al*, 2000). In addition, secretory IgA dimers, which are produced by LP plasma cells and secreted into the gut lumen by transcytosis across the epithelium, can neutralize pathogens and toxins (Rojas & Apodaca, 2002). IgA also coats commensal bacteria, thereby controlling the commensal colonization and maintaining the intestinal host-bacterial homeostasis (Mathias & Corthésy, 2011). By the production of a variety of metabolites, such as short-chain fatty acids and vitamins, commensal microbiota are implied to affect the host metabolism and immune system (Tremaroli & Bäckhed, 2012; Brestoff & Artis, 2013).

Below the mucus layer, a polarized single layer of interconnected intestinal epithelial cells (IECs) forms the major component of the intestinal barrier, separating the lumen from the LP. Tight junctions, adherens junctions, and desmosomes between IECs seal the intracellular space to maintain the epithelial integrity and polarity (Farquhar & Palade, 1963). To maximize the surface area for the absorption of nutrients, the intestinal epithelium is folded into numerous finger-like luminal protrusions called villi. The crypts between these villi are populated by leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5)-positive pluripotent intestinal stem cells (ISCs) (Barker *et al*, 2007). Adjacent to ISCs, Paneth cells, and mesenchymal cells secrete essential niche factors including the epidermal growth factor (EGF), WNT agonists such as R-Spondin, Notch delta-like ligands (DLLs), and bone morphogenetic protein (BMP) antagonists to regulate ISC self-renewal and differentiation (Sato *et al*, 2011a). ISCs generate rapidly proliferating transit-amplifying (TA) progenitors, which

subsequently give rise to various IEC populations (Barker *et al*, 2007). Whereas the atonal bHLH transcription factor 1 (ATOH1/MATH1) drives the differentiation of IECs into secretory lineages like goblet cells, Paneth cells, enteroendocrine cells (EECs), and tuft cells, Notch-induced expression of the hairy and enhancer of split 1 (HES1) represses ATOH1 to promote the maturation into absorptive enterocytes (Yang *et al*, 2001; VanDussen & Samuelson, 2010; VanDussen *et al*, 2012). During differentiation, IECs move upwards along the crypt-villus axis until they are shed from the villus tip into the gut lumen (Krndija *et al*, 2019). Constant generation of new progenitors ensures a rapid renewal of the epithelium every 3-5 days in order to sustain an optimal tissue function. Within the epithelium, IELs, predominantly unconventional TCR $\alpha\alpha^+$, TCR $\alpha\beta^+$, and TCR $\gamma\delta^+$ T cells, act as sentinels and provide the first line of cellular defense against luminal microbes (Olivares-Villagómez & Van Kaer, 2018).

The LP is a layer of loose connective tissue located underneath the epithelium and highly vascularized with blood and lymph vessels. Various kinds of immune cells such as T and B cells, DCs, ILCs, macrophages, eosinophils, and mast cells populate the LP. Organized lymphoid structures of the small intestinal mucosa belong to the gut-associated lymphoid tissue (GALT) and include mesenteric lymph nodes (mLNs), Peyer's patches (PPs), and isolated lymphoid follicles (ILFs) (Mowat & Agace, 2014). Microfold (M) cells are specialized cells found in the follicle-associated epithelium of PPs and ILFs and play a key role in mucosal immune surveillance by mediating the uptake of luminal antigens via endocytosis (Neutra *et al*, 1996). The transepithelial transport and presentation of antigens to underlying immune cells within lymphoid follicles initiates an immune response, for example leading to the development of IgA-secreting plasma cells. Besides, adaptive immune responses are induced by antigen-loaded DCs, which migrate via lymphatics to draining mLNs. In addition to immune cells, fibroblasts contribute to extracellular matrix production and remodeling in the LP (Chalkidi *et al*, 2022). Upon inflammation, fibroblasts can secrete pro-inflammatory factors to support immune cell infiltration and function. Neurons and glia cells of the enteric nervous system are also described as potent modulators of the intestinal microbiota, the epithelial barrier integrity, and the immune system (Wang *et al*, 2022a; Progzsky & Pachnis, 2022).

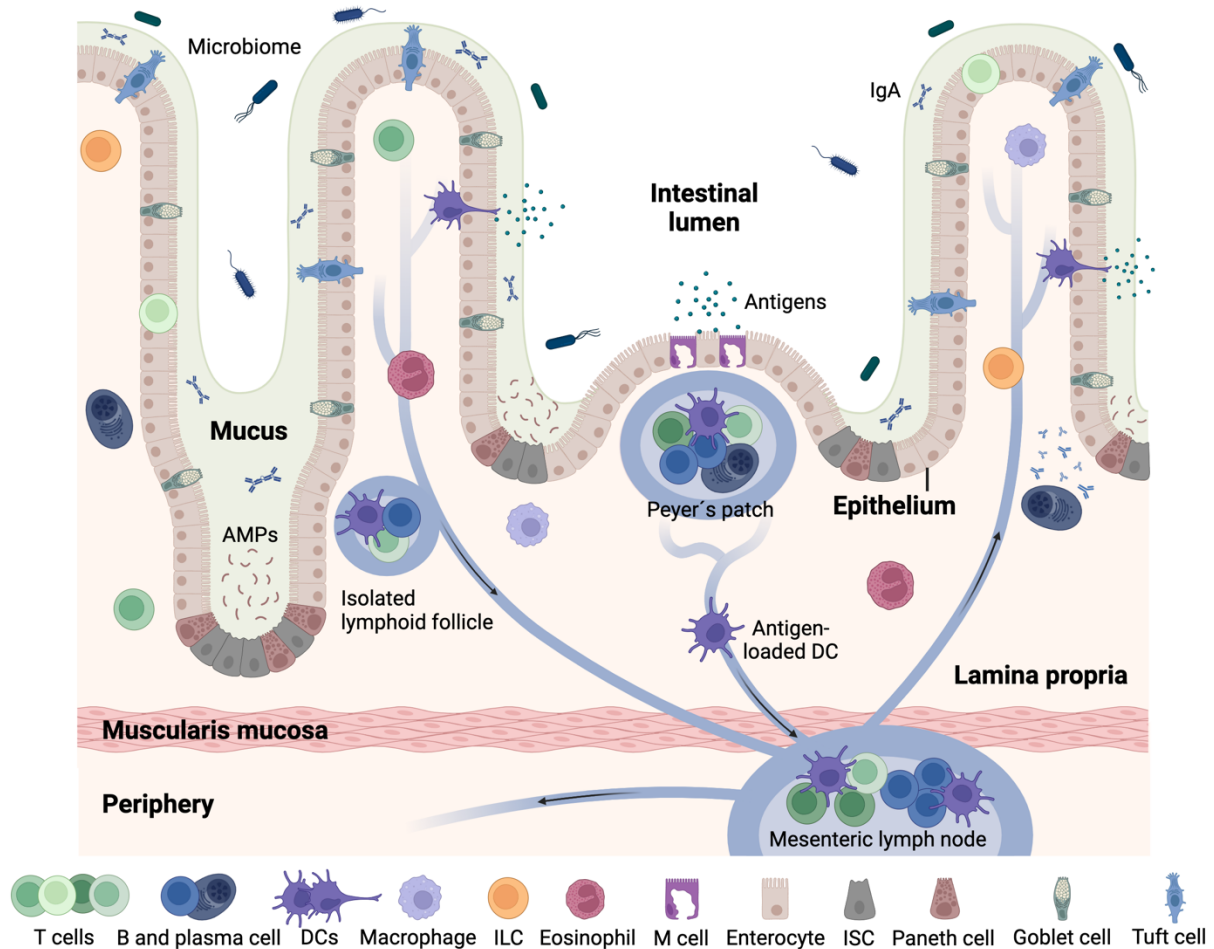


Figure 1.4 | Structure of the small intestinal mucosa

The small intestinal barrier consists of the mucus layer, the epithelium, the lamina propria (LP), and the muscularis mucosa. The mucus acts as a physical barrier and harbors the microbiome, antimicrobial peptides (AMPs), and immunoglobulin A (IgA). The epithelium is continuously renewed by intestinal stem cells (ISC), which can differentiate into absorptive enterocytes or secretory Paneth cells, goblet cells, and tuft cells. Also, intraepithelial lymphocytes reside within the epithelium. Underneath, various innate and adaptive immune cells such as innate lymphoid cells (ILCs), macrophages, eosinophils or antibody-secreting plasma cells are localized in the LP. Antigen sampling by macrophages, dendritic cells (DCs), and microfold (M) cells, overlaying Peyer's patches, initiates T and B cell activation in isolated lymphoid follicles or draining mesenteric lymph nodes (adapted from Tian *et al*, 2024).

1.4.2. The small intestinal tuft cell-ILC2 circuit

Tuft cells are rare chemosensory cells in the intestinal epithelium with an abundance of about 0.3 % and are crucial for initiating type 2 immune responses to parasitic helminths and protists in the small intestine. Because of its distinct morphology in form of a bottle-shaped cell body with an apical microvillus tuft, this cell type was first described as “the peculiar cell” in the mouse stomach in 1956 (Jarvi & Keyrilainen, 1956). Over the last decades, tuft cells were found throughout the body, for example in the epithelium of bronchi, the trachea, the gall bladder, the pancreas, and the gut

(Watson & Brinkman, 1964; Rhodin & Dalhamn, 1956; Luciano, L. Reale, 1969; Kugler *et al*, 1994; Isomaki, 1973). Across diverse tissues tuft cells can be identified based on characteristic markers such as the lineage-defining transcription factor POU class 2 homeobox 3 (POU2F3) and the doublecortin-like kinase 1 (DCLK1) (O’Leary *et al*, 2019).

In addition to its tuft protruding into the lumen, the expression of canonical taste signaling components led to the hypothesis that tuft cells could act as chemosensory sentinels for luminal cues (Howitt *et al*, 2016). Similar to type II taste cells, which are located in taste buds on the tongue and express sweet, bitter, and umami receptors, intestinal tuft cells use G-protein-coupled receptors such as bitter taste receptors of the TAS2R family and the succinate receptor 1 (SUCNR1) for environmental sensing (Howitt *et al*, 2016; Nadjombati *et al*, 2018; Schneider *et al*, 2018; Lei *et al*, 2018; Luo *et al*, 2019) (Figure 1.5). Herewith, tuft cells can detect luminal microbial metabolites like succinate derived from *Tritrichomonas* protists or bacteria. Moreover, helminth parasites, such as *Nippostrongylus brasiliensis*, *Heligmosomoides polygyrus*, and *Trichinella spiralis*, or their excretory/secretory products are able to activate tuft cells via so far unidentified receptors. Whereas the further signal transduction relies on the taste-specific G-protein subunit α -gustducin (GNAT3) and the phospholipase C β 2 (PLC β 2) upon succinate stimulation, the detection of *N. brasiliensis* is SUCNR1- and GNAT3-independent (Schneider *et al*, 2019; Nadjombati *et al*, 2018). Next, increased cytosolic calcium (Ca²⁺) levels in the tuft cell activate the transient receptor potential cation channel subfamily M member 5 (TRPM5), causing a sodium (Na⁺) influx. The resulting depolarization of the cell body finally leads to the secretion of the cytokine IL-25 and the neurotransmitter acetylcholine (ACh). Dependent on the stimulus, tuft cells also release, through unknown mechanisms, other effector molecules such as allergy-related eicosanoids (O’Leary *et al*, 2019).

By using IL-25-tdTomato reporter mice, tuft cells have been identified as the unique source of IL-25 in the mouse intestine (von Moltke *et al*, 2016). They display constant IL25 mRNA expression levels, which are unaffected by stimulation. However, the post-transcriptional regulation and the secretion of IL-25 are strictly dependent on tuft cell activation. Upon stimulation, tuft cell-derived IL-25 initiates the activation of ILC2s in the LP, orchestrating intestinal type 2 immune responses (Gerbe *et al*, 2016; von Moltke *et al*, 2016; Howitt *et al*, 2016) (Figure 1.5). IL-25 directly binds to the cytokine-

specific receptor subunit IL-17RB on the surface of ILC2s in the LP, resulting in ILC2 activation and the release of IL-13. In turn, IL-13 activates IL-4 receptor- α (IL-4R α) and STAT6 signaling in crypt progenitor cells to drive tuft and goblet cell differentiation, further boosting this tuft cell-ILC2 circuit. IL-13-mediated tuft cell expansion requires the activity of SRY (sex-determining region Y)-box transcription factor (SOX) 4 (Gracz *et al*, 2018). An induced goblet cell hyperplasia, increased mucus and AMP production, and smooth muscle hypercontractility facilitate the expulsion of parasites by a “weep and sweep” response.

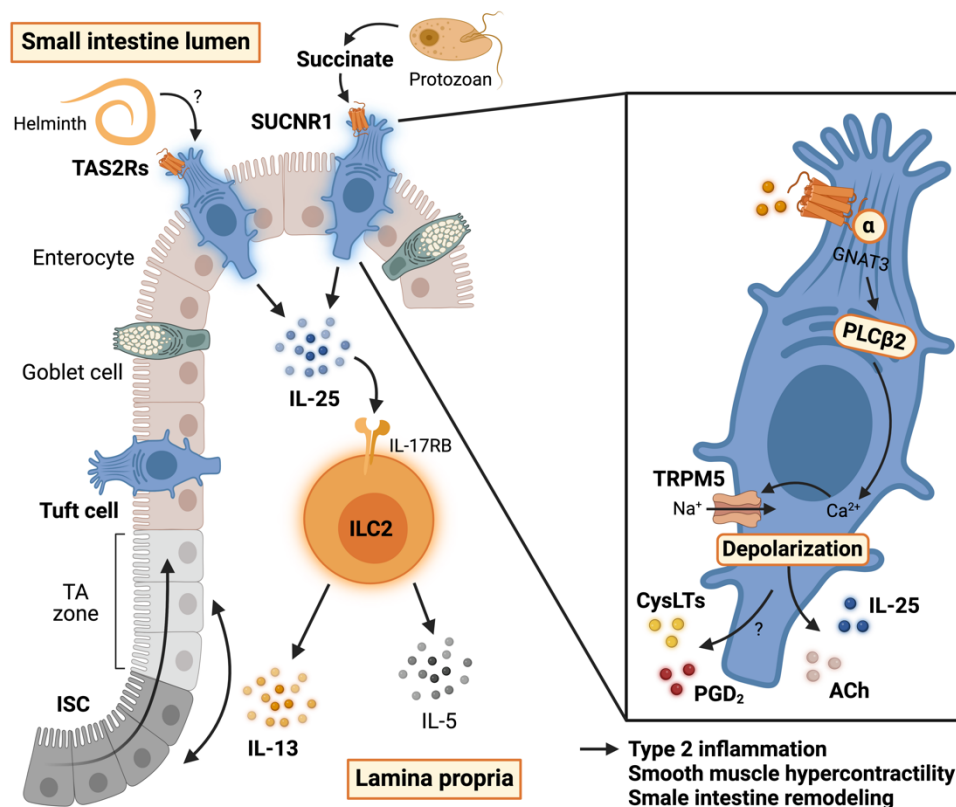


Figure 1.5 | The small intestinal tuft cell-ILC2 circuit

Elevated luminal succinate levels or the presence of intestinal helminths activate tuft cells through the succinate receptor 1 (SUCNR1), bitter taste receptors of the TAS2R family or unknown receptors. A signaling cascade via the G-protein subunit α -gustducin (GNAT3), the phospholipase C β 2 (PLC β 2), and the transient receptor potential cation channel subfamily M member 5 (TRPM5) results in cell depolarization and the release of IL-25 and acetylcholine (ACh). Based on the stimulus, activated tuft cells also produce prostaglandin D₂ (PGD₂) or cysteinyl leukotrienes (CysLTs). Tuft-derived IL-25 acts on group 2 innate lymphoid cells (ILC2s) via the IL-25 receptor IL-17RB. Activated ILC2s increase their IL-13 secretion, which in turn promotes the differentiation of intestinal stem cells (ISCs) into tuft and goblet cells, further reinforcing the circuit. The induced type 2 immune response is accompanied by an enhanced mucus production and smooth muscle hypercontractility to drive parasitic expulsion. TA zone: transit-amplifying cell zone (adapted from Schneider *et al*, 2019).

In addition, tuft cells express key enzymes involved in the biogenesis of eicosanoid lipid mediators, including cyclooxygenase 1 (COX1), COX2, and arachidonate 5-lipoxygenase (ALOX5), critical for prostaglandin D₂ (PGD₂) and cysteinyl leukotriene (CysLT) synthesis, respectively (Oyesola *et al*, 2021; McGinty *et al*, 2020). PGD₂ binds to its receptor chemoattractant receptor-homologous molecule expressed on T_h2 cells (CRTH2) to promote anti-helminth effects of type 2 immune cells and additionally enhance the mucus secretion by goblet cells. Together with IL-25, CysLTs contribute to an optimal ILC2 response to helminths via nuclear factor of activated T cells (NFAT)-dependent signaling downstream of CysLT receptor stimulation on ILC2. The synthesis of the neurotransmitter ACh from extracellular choline and acetyl-coenzyme A is mediated by the choline acetyltransferase (ChAT), a well-established tuft cell marker (Schneider *et al*, 2019). ACh acts on neighboring IECs, expressing muscarinic ACh receptors (mAChRs), to stimulate fluid secretion as part of the “weep” response to helminths (Ndjim *et al*, 2024). Furthermore, tuft cells directly release ACh into the gut lumen, thereby damming worm reproduction (Billipp *et al*, 2024).

1.4.3. The role of AhR signaling in gut immunity

Several studies have shown that AhR activation in various intestinal cell types is indispensable for maintaining barrier integrity and inducing effective immune responses. Here, the AhR not only influences mucosal immune populations but also the function of epithelial and endothelial cells. Within the immune compartment, the AhR regulates the balance between ILC2s and ILC3s in the LP, which are key regulators of the intestinal homeostasis. Whereas the AhR promotes the maintenance of IL-22-producing ILC3s, it inhibits ILC2 function by suppressing the expression of the IL-33 receptor ST2 and the ILC2 effector molecules IL-5 and IL-13 (Qiu *et al*, 2012; Li *et al*, 2018). Consequently, AhR deficiency in mice was demonstrated to enhance the anti-helminth response to *H. polygyrus* (Li *et al*, 2018). In contrast, AhR-deficient mice show enhanced susceptibility to *Citrobacter rodentium* infection due to a loss in ILC3s and a resulting decrease in IL-22-induced secretion of AMPs by IECs (Qiu *et al*, 2012; Li *et al*, 2018). Over the course of infection, CD4⁺ T cells become major IL-22 producers and help to protect against *C. rodentium* infection (Basu *et al*, 2012; Schiering *et al*, 2017). In T_h17 cells, AhR expression fosters the secretion of IL-22, but is not required for their differentiation (Veldhoen *et al*, 2008). To limit the inflammatory response, the AhR drives the conversion of T_h17 cells into IL-10-producing T_r1 cells during resolution

of parasitic and bacterial infections (Gagliani *et al*, 2015). The lack of AhR in intestinal eosinophils impacts their tissue adaptation and function but also alters the T cell compartment in naive and helminth-infected mice (Diny *et al*, 2022). Furthermore, AhR activity promotes the survival of IELs to favor the outcome of dextran sodium sulfate (DSS)-induced colitis or infections and contribute to epithelial barrier integrity (Li *et al*, 2011).

Since the intestinal epithelium is directly exposed to diet- or microbial-derived AhR ligands, also cell-intrinsic roles of AhR signaling in IECs have been described. AhR activation is known to regulate ISC proliferation in intestinal crypts by restraining WNT signaling (Metidji *et al*, 2018). Thus, IEC-specific ablation of AhR leads to an unrestricted expansion of ISCs and defects in epithelial cell differentiation, causing compromised immunity against *C. rodentium* infection and the promotion of tumorigenesis. Moreover, AhR stimulation with I3C in mice drives the differentiation of Paneth cells and particularly goblet cells by suppressing Notch signaling (Park *et al*, 2018). As a result, the expression of the Notch target HES1 was downregulated, limiting the maturation of enterocytes, whereas ATOH1-mediated differentiation into secretory lineages was increased. More recently, a direct transcriptional regulation of *Atoh1* by the AhR in a XRE-dependent manner has been shown (Yagishita *et al*, 2023). The AhR can further strengthen the intestinal barrier by upregulation of tight junction proteins in IECs upon inflammation (Yu *et al*, 2018). Lastly, the sensing of AhR ligands by endothelial cells is essential for limiting excess inflammatory responses upon enteric infections (Wiggins *et al*, 2023).

2. Aims of the thesis

The AhR integrates environmental, microbial, and dietary cues to control numerous biological processes in the immune system. In experimental models, the absence of the AhR has been shown to impact the host response to microbial infections and, in turn, can result in a higher susceptibility to infections with certain pathogens. However, the influence of the AhR on the outcome of infectious diseases is highly context-specific and requires further examination for different infection models. Therefore, this thesis aimed to gather more insights into the function of AhR signaling in the host response to STM infection and in the tuft cell-mediated intestinal immunity to parasites.

Previous results from our laboratory have demonstrated an increased susceptibility of AhR-deficient (AhR^{-/-}) mice to chronic systemic infection with the attenuated STM strain TAS2010, a mouse model for human typhoid fever (Cengiz, 2020). In contrast to wildtype (WT) mice, which cleared the infection within 5 weeks, AhR^{-/-} mice displayed reduced bacterial clearance and elevated mortality upon TAS2010 infection. Furthermore, we could observe an unrestrained EME in the spleen of infected AhR^{-/-} mice with a vast expansion of immature erythroid cells, accompanied by a disruption of the splenic microarchitecture. Thus, the first part of this thesis aimed to further unravel how AhR deficiency in mice impacts the regulation of stress erythropoiesis in response to STM infection. To check how this enhanced EME was fueled and if it was associated with a reduced bone marrow erythropoiesis and anemia upon infection, precursor and mature red blood cell (RBC) subsets were further analyzed in the spleen, the whole blood, and the bone marrow of infected AhR^{-/-} mice.

In the second part of the thesis, it was studied how modulation of AhR signaling affects the differentiation of tuft cells in the small intestine *in vivo* and in mouse intestinal organoids *in vitro*. To understand the role of the AhR in the intestinal type 2 immunity to parasites, the tuft cell-ILC2 circuit activation was analyzed in AhR^{-/-} mice compared to WT mice in a sterile infection model via the administration of succinate-supplemented drinking water. Furthermore, this study investigated the regulation of AhR signaling by its repressor AhRR in intestinal homeostasis by examining the selective expression pattern of AhRR *in vivo* compared to *in vitro* conditions.

In summary, this thesis aimed to contribute to a better understanding of how the AhR signaling pathway influences type 1 and type 2 host responses to infections.

3. Materials and Methods

3.1. Materials

3.1.1. Equipment

Table 3.1 | Equipment

Equipment	Company
Accu-jet [®] pro pipette controller	Brand, Wertheim, Germany
ADVIA [®] 2120i Hematology System	Siemens Healthineers, München, Germany
Balance 440-35A	Kern & Sohn, Balingen, Germany
BD FACSymphony [™] A5	BD Biosciences, Heidelberg, Germany
BDK [®] Laminar Flow workbench	BDK, Sonnenbühl-Genkingen, Germany
Beaker glasses	SIMAX Bohemia Cristal, Selb, Germany
Centrifuge 5415R	Eppendorf, Hamburg, Germany
Centrifuge 5810R	Eppendorf, Hamburg, Germany
Centrifuge Allegra [®] X-15R	Beckman Coulter, Brea, USA
Cryopreservation 800 series-190 MVE	Thermo Fisher Scientific, Waltham, USA
Cryostat CM3050 S	Leica Microsystems, Wetzlar, Germany
Feeding needles	AgnTho's, Lidingö, Sweden
Forceps	Medical Highlights Germany GmbH, Rohrdorf, Germany
Freezer (-80 °C) CryoCube F740hiw	Eppendorf, Hamburg, Germany
Freezer (-80 °C) New Brunswick Ultra-Low Temperature Freezer	Eppendorf, Hamburg, Germany
Gel electrophoresis PerfectBlue Gel System	Peqlab, Erlangen, Germany
Heating block Thermostat TH21	HLC BioTech, Bovenden, Germany
Hemocytometer Neubauer improved	Marienfeld Superior, Lauda-Königshofe, Germany
Ice machine Scotsman Flockeneisbereiter AF200	Hubbard Systems, Birmingham, USA
Incubator CB 150	Binder, Tuttlingen, Germany

Incubator ECOCELL	MMM Group, Planegg/München, Germany
Incubator shaker New Brunswick Innova® 44/44R	Eppendorf, Hamburg, Germany
Incubator shaker Stuart SI500	Cole-Parmer, Stone, UK
Magnetic stirrer IKA RCT basic	IKA-Werke, Staufen, Germany
Measuring beakers/cylinders	VWR International, Radnor, USA
Microscope Nikon Eclipse TS100	Nikon, Melville, USA
Microscope Keyence BZ-9000	KEYENCE DEUTSCHLAND GmbH, Neu-Isenburg, Germany
Microscope confocal Zeiss LSM 710	Carl Zeiss AG, Oberkochen, Germany
Microwave NN-E235M	Panasonic, Osaka, Japan
Mr. Frosty® freezing container	Thermo Fisher Scientific, Waltham, USA
Multichannel pipettes	Cole-Parmer, Stone, UK
pH meter FiveEasy FE20-Basic	Mettler Toledo, Columbus, USA
Pipettes	Eppendorf, Hamburg, Germany
PowerPac™ Basic Power Supply	BioRad, Hercules, USA
Precision balance ABJ 220-4M	Kern & Sohn, Balingen, Germany
Rocking platform shaker DUOMAX 1030	Heidolph Scientific Products GmbH, Schwabach, Germany
Scissors	Medical Highlights Germany GmbH, Rohrdorf, Germany
Spectrophotometer BioPhotometer 6131	Eppendorf, Hamburg, Germany
Staining chamber StainTray™	Simport Scientific, Saint-Mathieu-de-Beloeil, Canada
Stomacher® 80 Biomaster	Seward Limited, Worthing, UK
Thermal Cycler T100™	BioRad, Hercules, USA
Threaded bottles/Erlenmeyer Flasks	Schott, Mainz, Germany
Transilluminator Biostep Dark Hood DH-40/50	Biostep, Burkhaldtsdorf, Germany
Transport Box DuraPorter®	Biozym Scientific, Oldendorf, Germany
Vortex shaker Genie 2	Scientific Industries, New York, USA
Water bath WNB 22	Memmert GmbH, Schwabach, Germany

3.1.2. Consumables

Table 3.2 | Consumables

Item	Company
8-well chamber μ -slide uncoated	ibidi GmbH, Gräfelfing, Germany
Bottle-top vacuum filter Filtropur V50, 500 ml, 0.1 μ m	Sarstedt, Nümbrecht, Germany
Cell strainer nylon (70 μ m, 100 μ m)	VWR International, Radnor, USA Starlab International GmbH, Hamburg, Germany
Conical tubes (15 ml, 50 ml)	Greiner, Kremsmünster, Austria
Cover slips (24x60 μ m)	Epredia, Kalamazoo, USA
CryoPure Tube	Sarstedt, Nümbrecht, Germany
Cryostat blades	A. Hartenstein GmbH, Würzburg, Germany
Cultubes™ Sterile Culture Tubes	Simport Scientific, Saint-Mathieu-de-Beloeil, Canada
Culture plates (6-well, 12-well, 24-well)	Greiner, Kremsmünster, Austria
Disposal bags	Carl Roth, Karlsruhe, Germany
Filter papers	Whatman, Maidstone, UK
Flow cytometry tubes	Sarstedt, Nümbrecht, Germany
Micro tube 1.1 ml Z-Gel	Sarstedt, Nümbrecht, Germany
Microplates 96-well (F/U)	Greiner, Kremsmünster, Austria
Microscope slides Superfrost™Plus	Epredia, Portsmouth, UK
Microvette®	Sarstedt, Nümbrecht, Germany
Parafilm®	Heathrow Scientific, Vernon Hills, USA
PCR tubes	Sarstedt, Nümbrecht, Germany
Petri dishes	Greiner, Kremsmünster, Austria
Precision wipes	Kimberly-Clark, Irving, USA
Reagent reservoirs	Diversified Biotech, Dedham, USA
Safe seal reaction tubes (0.5 ml, 1.5 ml, 2.0 ml)	Sarstedt, Nümbrecht, Germany
Serological pipettes	Greiner, Kremsmünster, Austria
Sterican® needles	Braun, Melsungen, Germany

Sterile applicators	Boettiger, Bodenmais, Germany
Syringe filters Filtropur S, 0.45 µm	Sarstedt, Nümbrecht, Germany
Syringes Injekt® Luer Solo	Braun, Melsungen, Germany
Tissue-Tek® Cryomolds	Sakura Finetek USA, Inc., Torrance
UVette®	Eppendorf, Hamburg, Germany
Weighing pans	Carl Roth, Karlsruhe, Germany

3.1.3. Chemicals reagents, enzymes and recombinant proteins

Table 3.3 | Chemical reagents, enzymes and recombinant proteins

Reagent	Company
100 bp DNA Ladder	New England BioLabs, Ipswich, USA
10x TAE buffer	Invitrogen, Waltham, USA
2-Mercaptoethanol	Sigma-Aldrich, St. Louis, USA
3MC (3-Methylcholanthrene)	Sigma-Aldrich, St. Louis, USA
4-hydroxytamoxifen	Sigma-Aldrich, St. Louis, USA
Acetone	VWR International, Radnor, USA
Advanced DMEM (Dulbecco's Modified Eagle Medium)/F-12 Medium	Gibco™ by Life Technologies, Carlsbad, USA
Bacto™ Agar	BD Biosciences, Franklin Lakes, USA
B-27™ supplement (50x)	Gibco™ by Life Technologies, Carlsbad, USA
BSA (Bovine serum albumin)	SERVA Electrophoresis GmbH, Heidelberg, Germany
Cell Activation Cocktail with Brefeldin A (500x)	BioLegend, Fell, Germany
CH223191	Sigma-Aldrich, St. Louis, USA
Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-Free, LDEV-Free	Corning, Corning, USA
DAPCO (Triethylenediamine)	Sigma-Aldrich, St. Louis, USA
DAPI (4-6-Diamidino-2-phenylindole)	Sigma-Aldrich, St. Louis, USA
DMSO (Dimethylsulfoxide)	Merck, Darmstadt, Germany

DNase I	Merck, Darmstadt, Germany
DPBS (Dulbecco's phosphate-buffered saline), w/o: Ca and Mg	PAN-Biotech GmbH, Aidenbach, Germany
DTT (Dithiothreitol), molecular grade	Promega, Fitchburg, USA
Eosin	Merck, Darmstadt, Germany
Ethanol 70 % (methylated)	Carl Roth, Karlsruhe, Germany
Ethanol ROTIPURAN® >99.8 %	Carl Roth, Karlsruhe, Germany
Euparal	Carl Roth, Karlsruhe, Germany
FACS Clean Solution	BD Biosciences, Franklin Lakes, USA
FACS Rinse Solution	BD Biosciences, Franklin Lakes, USA
FCS (Fetal calf serum)	PAN-Biotech GmbH, Aidenbach, Germany
FICZ (6-Formylindolo(3,2-b)carbazole)	Enzo Life Sciences, Lörrach, Germany
Fixable Viability Dye eFluor™ 780	eBioscience, San Diego, USA
GlutaMax™ (100x)	Gibco™ by Life Technologies, Carlsbad, USA
Glycerol	Carl Roth, Karlsruhe, Germany
HBSS (Hank's balanced salt solution), w or w/o: Ca and Mg	PAN-Biotech GmbH, Aidenbach, Germany
Hydrogen chloride (HCl) 37 %	Carl Roth, Karlsruhe, Germany
Hematoxylin solution (Mayer's)	Sigma-Aldrich, St. Louis, USA
HEPES (4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid)	Sigma-Aldrich, St. Louis, USA
Human R-Spondin 1 Recombinant Protein	PeproTech, Cranbury, USA
Isopropanol	Carl Roth, Karlsruhe, Germany
LB (Luria-Bertani) Broth, high salt	Sigma-Aldrich, St. Louis, USA
L-Glutamine 200 mM (100x)	Gibco™ by Life Technologies, Carlsbad, USA
Liberase™	Merck, Darmstadt, Germany
L-Lysine HCl	Sigma-Aldrich, St. Louis, USA
Mouse BD Fc Block™	BD Biosciences, Franklin Lakes, USA

Mouse EGF (Epidermal Growth Factor) Recombinant Protein	PeproTech, Cranbury, USA
Mouse IL-13 (Interleukin-13) Recombinant Protein	PeproTech, Cranbury, USA
Mouse Noggin Recombinant Protein	PeproTech, Cranbury, USA
Mowiol®	Sigma-Aldrich, St. Louis, USA
N-2 supplement (100x)	Gibco™ by Life Technologies, Carlsbad, USA
N-acetyl-L-cysteine	Sigma-Aldrich, St. Louis, USA
Normal goat serum	Sigma-Aldrich, St. Louis, USA
OneComp/UltraComp eBeads™	Invitrogen, Waltham, USA
OneTaq Quick-Load 2x Master Mix with Standard Buffer	New England BioLabs, Ipswich, USA
PFA (Paraformaldehyde)	Merck, Darmstadt, Germany
Penicillin-Streptomycin	Gibco™ by Life Technologies, Carlsbad, USA
PeqGOLD Universal Agarose	Peqlab, Erlangen, Germany
Precision Count beads™	BioLegend, Fell, Germany
Proteinase K	Sigma-Aldrich, St. Louis, USA
ROTI®-Histofix 4 %	Carl Roth, Karlsruhe, Germany
RPMI 1640 (Roswell Park Memorial Institute) Medium	PAN-Biotech GmbH, Aidenbach, Germany
Saponin	Sigma-Aldrich, St. Louis, USA
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich, St. Louis, USA
Sodium chloride (NaCl)	Carl Roth, Karlsruhe, Germany
Sodium hydrogen carbonate	Sigma-Aldrich, St. Louis, USA
Sodium periodate	Sigma-Aldrich, St. Louis, USA
Sorenson´s Salt (Disodium-hydrogen- orthophosphate-dihydrate)	Sigma-Aldrich, St. Louis, USA
Sterile Water for cell culture	PAN-Biotech GmbH, Aidenbach, Germany
Streptomycin sulfate	Carl Roth, Karlsruhe, Germany
Succinic acid	Sigma-Aldrich, St. Louis, USA

Sucrose	Sigma-Aldrich, St. Louis, USA
SYBR TM Safe DNA Gel Stain	Invitrogen, Waltham, USA
Tissue-Tek [®] O.C.T TM Compound	A. Hartenstein GmbH, Würzburg, Germany
Toothpicks	Wenco, Hennef, Germany
TRIS-HCl	Carl Roth, Karlsruhe, Germany
Triton X-100	Sigma-Aldrich, St. Louis, USA
Trypan Blue	Sigma-Aldrich, St. Louis, USA
TrypLE TM Express	Gibco TM by Life Technologies, Carlsbad, USA
UltraPure TM 0.5 M EDTA (Ethylene-diaminetetraacetic acid), pH 8.0	Invitrogen, Waltham, USA
UltraPure TM Distilled Water, DNase/RNase Free	Invitrogen, Waltham, USA
Xylol	Carl Roth, Karlsruhe, Germany
Zombie Violet TM Fixable Viability Kit	BioLegend, Fell, Germany

3.1.4. Solutions and buffers

Table 3.4 | Solutions and buffers

Solution/Buffer	Content
2x Freezing medium (Organoids)	FCS 20 % DMSO
Basal organoid medium	Advanced DMEM/F-12 medium 1 % GlutaMax TM (100x) 1 % HEPES 100 U/ml Penicillin 100 µg/ml Streptomycin
Blocking buffer (Histology)	Tissue staining buffer 5 % Normal goat serum
Buffer 1 (Intestinal digestion)	HBSS w/o: Ca and Mg 5 % FCS 10 mM HEPES

	10 mM DTT 10 mM EDTA
Buffer 2 (Intestinal digestion)	HBSS w/o: Ca and Mg 2 % FCS 10 mM HEPES 10 mM DTT 10 mM EDTA
Complete RPMI 1640 medium	RMPI 1640 medium 10 % FCS 2 mM L-Glutamine 100 U/ml Penicillin 100 µg/ml Streptomycin 0.05 mM 2-mercaptoethanol
Digestion buffer (Intestinal digestion)	HBSS w: Ca and Mg 3 % FCS 10 mM HEPES 30 µg/ml DNase I 100 µg/ml Liberase
ENR medium	Basal organoid medium 2 % B-27™ supplement (50x) 1 % N-2 supplement (100x) 1 mM N-acetyl-L-cysteine (solved in sterile water) 50 ng/ml Mouse EGF (solved in 0.5 % BSA in PBS) 100 ng/ml Mouse Noggin (solved in 0.5 % BSA in PBS) 100 ng/ml Human R-Spondin 1 (solved in 0.5 % BSA in PBS)
FACS buffer	PBS 0.5 % BSA 2 mM EDTA
Lysis buffer (tail lysis)	Distilled water 100 mM Tris HCl, pH 8.5

	5 mM EDTA, pH 8.0 0.2 % SDS 200 mM NaCl 0.1 mg/ml Proteinase K
PFA, 4 %	100 ml PBS 4 g PFA pH 7.4
PLP (Periodate-Lysine-Paraformaldehyde) solution	Distilled water 75 mM L-Lysine HCl 10 mM Sorenson's Salt 1.25 % PFA 10 mM Sodium periodate pH 7.2
Pre-freeze wash solution (Organoids)	Advanced DMEM/F-12 medium 10 mM HEPES 100 U/ml Penicillin 100 µg/ml Streptomycin
Post-thaw wash solution (Organoids)	Advanced DMEM/F-12 medium 1 % BSA 10 mM HEPES 100 U/ml Penicillin 100 µg/ml Streptomycin
Rinse buffer (Intestinal digestion)	HBSS w: Ca and Mg 2 % FCS 10 mM HEPES
Tissue staining buffer (Histology)	PBS 1 % BSA 0.3 % Triton X-100
Trypan blue working solution	0.25 % Trypan blue in PBS

3.1.5. Kits and assays

Table 3.5 | Kits and assays

Kit/assay	Company
ADVIA® 2120i Hematology System	Siemens Healthineers, München, Germany
DuoSet® ELISA Mouse IFN-γ	R&D Systems, Minneapolis, USA
eBioscience™ Foxp3/Transcription Factor Staining Buffer Set	eBioscience, San Diego, USA
LEGENDplex™ Mouse HSC Erythroid Panel	BioLegend, Fell, Germany

3.1.6. Antibodies

Table 3.6 | Flow cytometry antibodies and streptavidins

Antigen/ Streptavidin	Clone	Conjugate	Dilution	Company
B220	RA3-6B2	APC, FITC	1:200	BioLegend
CD105	MJ7/18	PE	1:200	BioLegend
CD11b	M1/70	BUV395, PE	1:200	BD Biosciences
CD11b	M1/70	BV605	1:200	BioLegend
CD11b	M1/70	PE-Cy7	1:200	eBioscience
CD11c	N418	PE	1:200	eBioscience
CD133	315-2C11	PE/Dazzle™ 594	1:200	BioLegend
CD150	TC15-12F12.2	BV510	1:200	BioLegend
CD19	eBio1D3	PE	1:200	eBioscience
CD24	M1/69	Biotin	1:300	BioLegend
CD24	M1/69	PE-Cy7	1:200	BioLegend
CD3	145-2C11	APC	1:200	BioLegend
CD34	SA376A4	PE-Cy7	1:200	BioLegend
CD4	RM4-5	PE/Dazzle™ 594, PerCP/Cyanine5.5	1:200	BioLegend

CD45	30-F11	BUV395, BUV661	1:200 - 1:300	BD Biosciences
CD45	30-F11	BV510, BV711, PE-Cy7	1:200 - 1:300	BioLegend
CD71	RI7217	BV421	1:200	BioLegend
CD8	53-6.7	BUV805	1:200	BD Biosciences
CD8	53-6.7	PE, PE-Cy7	1:200	BioLegend
ChgA	N/A	AF647	1:200	In house production Schneider Lab
c-Kit	2B8	PerCP/Cyanine5.5	1:200	BioLegend
DCLK1 (intracellular)	polyclonal	unconjugated, host: rabbit	1:1000	Abcam
EpCAM	G8.8	APC, PE, PerCP/Cyanine5.5	1:100 - 1:200	BioLegend
F4/80	BM8	PE	1:200	eBioscience
GATA-3 (intracellular)	TWAJ	PE-eFluor610	1:50	eBioscience
IFN- γ (intracellular)	XMG1.2	APC	1:100	BioLegend
IFN- γ (intracellular)	XMG1.2	PE	1:100	BD Biosciences
Ki-67 (intracellular)	16A8	PE-Cy7	1:200	BioLegend
KLRG1	2F1	BUV395	1:200	BD Biosciences
Ly6G	1A8	PE, PE-Cy7	1:200	BioLegend
Ly6G	1A8	PE-CF594	1:200	BD Biosciences
LysP	N/A	unconjugated, host: rabbit	1:200	In house production Schneider Lab
NK1.1	PK136	PE	1:200	BD Biosciences
Rabbit IgG	polyclonal	AlexaFluor488, AlexaFluor594	1:1000	Invitrogen
Rabbit IgG	polyclonal	AlexaFluor647	1:1000	BioLegend
Siglec-F	E50-2440	APC-Cy7, PE	1:200	BD Biosciences

Siglec-F	S17007L	PE/Dazzle™ 594	1:200	BioLegend
ST2	U29-93	BV605	1:200	BD Biosciences
Streptavidin	-	APC, BV605	1:300	BioLegend
TCR β	H57-597	BUV737	1:200	BD Biosciences
Ter119	TER-119	APC, APC-Cy7	1:200	BioLegend

Table 3.7 | Histology antibodies and streptavidins

Antigen	Clone	Conjugate	Host	Dilution	Company
B220	RA3-6B2	unconjugated	rat	1:100	eBioscience
Rat IgG	polyclonal	AlexaFluor647	goat	1:300	BioLegend
TCR β	H57-597	Biotin	hamster	1:100	BD Biosciences
Streptavidin	-	AlexaFluor594	-	1:300	Invitrogen
CD71	YTA74.4	unconjugated	rat	1:300	BioRad
Rat IgG	polyclonal	AlexaFluor647	goat	1:300	BioLegend
SIGN-R1	eBio22D1	unconjugated	hamster	1:300	Invitrogen
Hamster IgG	polyclonal	AlexaFluor594	goat	1:300	Invitrogen
Siglec-1	3D6.112	unconjugated	rat	1:300	BioLegend
Rat IgG	polyclonal	AlexaFluor647	goat	1:300	BioLegend
DCLK1	polyclonal	unconjugated	rabbit	1:1000	Abcam
Rabbit IgG	polyclonal	AlexaFluor647	donkey	1:1000	BioLegend
EpCAM	G8.8	unconjugated	rat	1:300	BD Biosciences
Rat IgG	polyclonal	AlexaFluor594	donkey	1:1000	Invitrogen

3.1.7. Bacterial strains

Table 3.8 | *Salmonella* Typhimurium strains

Strain	Resistance	Characteristics	Source/Reference
TAS2010	Streptomycin	Living, attenuated strain (Δ edd Δ pfkA Δ pfkB)	Prof. R. Strugnell, Melbourne, Australia (Kupz <i>et al</i> , 2013)

3.1.8. Mouse lines

Table 3.9 | Mouse lines

Mouse line	Strain	Reference	Supplier
Wildtype	C57BL/6JRcc	-	LIMES-GRC
AhR ^{-/-}	C57BL/6JRcc	(Schmidt <i>et al</i> , 1996)	Prof. I. Förster
AhRR ^{E/E}	C57BL/6JRcc	(Brandstätter <i>et al</i> , 2016)	Prof. I. Förster
TripleCyp ^{-/-} AhRR ^{E/E}	C57BL/6JRcc	unpublished	Prof. I. Förster
Atoh1-CreER ^{T2} ; R26-Ai14/Ai14	C57BL/6	Crossing of Atoh1-CreER ^{T2} line (RRID: IMSR_JAX:007684) and Ai14 line (RRID: IMSR_JAX:007914)	Prof. C. Schneider, Zürich, Switzerland

3.1.9. Software

Table 3.10 | Software

Software	Company
ADVIA [®] 2120i Hematology System Software	Siemens Healthineers, München, Germany
BioRender	BioRender, Toronto, Canada
BZ-II Analyzer	KEYENCE DEUTSCHLAND GmbH, Neu-Isenburg, Germany
FACS Diva	BD Biosciences, Franklin Lakes, USA
FlowJo 10.5.3	TreeStar, Inc., Ashland, USA
GraphPad Prism 10.1.1	GraphPad, La Jolla, USA
ImageJ (Fiji)	Rasband, W.S., ImageJ, National Institutes of Health, Bethesda, USA
Mendeley Software	Elsevier, Amsterdam, Netherlands
Microsoft Office 2016 for Mac	Microsoft, Redmond, USA

3.2. Methods

3.2.1. Mouse housing conditions

Mice were housed in the Genetic Resources Center of the Life and Medical Sciences Institute (LIMES-GRC), University of Bonn, under specific-pathogen-free conditions in individually ventilated cages at 22 °C with a 12h/12h dark/light cycle and *ad libitum* access to water and food. All experiments were performed with age-matched female WT, AhR^{-/-}, AhRR^{E/E}, or TripleCyp^{-/-}AhRR^{E/E} mice backcrossed for >10 generations to the C57BL/6JRccHsd background. Genotypes of mice were defined by polymerase-chain reaction. Animal experiments were conducted according to the German law of animal protection and approved by the government of North Rhine-Westphalia (Az 81-02.04.2018.A094 and Az 81-02.04.2023.A326).

3.2.2. *Salmonella* infection model

A single colony of TAS2010 was grown in 10 ml LB broth with 50 µg/ml streptomycin at 37 °C overnight. On the following day, the culture was transferred in 90 ml fresh LB broth at 37 °C. Based on a previously determined growth curve, bacterial concentration was calculated after 4 h by measuring optical density at 600 nm ($OD_{600}1 = 5 \times 10^8$ bacteria/ml). 5×10^7 bacteria were resuspended in 500 µl LB/glycerol mix (1:1), transferred to cryovials and stored at -80 °C. The remaining TAS2010 culture was used for oral infection of mice.

WT and AhR^{-/-} mice were infected with 2.5×10^9 colony-forming units (CFUs) of TAS2010 in a volume of 200 µl of LB broth/10 % sodium bicarbonate solution (1:1) by oral gavage. To determine the time course of infection, animals were sacrificed 2 or 5 weeks postinfection. Bacterial load in spleen and liver homogenates was quantified by plating serial dilutions on LB agar plates supplemented with 50 µg/ml streptomycin.

3.2.3. Blood/serum analysis

Blood samples were collected into EDTA tubes for blood analysis or tubes with gel separator and clot activator for serum preparation. After centrifugation at 16,000 g for 5 min, serum levels of cytokines were analyzed by IFN-γ ELISA or LEGENDplex™ Mouse HSC Erythroid Panel for EPO, SCF, IL-6, TPO, CXCL12, and TGF-β according to manufacturer's protocol. Whole blood was directly diluted 1:10 in FACS buffer (1 % FCS 2 mM EDTA in PBS) and analyzed by ADVIA® 2120i Hematology System to

measure hematocrit, hemoglobin concentration, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) of RBC, or prepared for flow cytometry analysis as described in section 3.2.13.

3.2.4. Preparation of single-cell suspensions from bone marrow and spleen

Bone marrow cells were isolated from murine hind leg tibiae and femurs by centrifugation at 10,000 g for 30 sec. After washing with FACS buffer and filtering through a 70 μ m strainer, samples were analyzed by ADVIA® 2120i Hematology System, or by flow cytometry.

For preparation of splenic single-cell suspensions, tissue was mashed through a 100 μ m strainer. Cells were washed in FACS buffer and prepared for flow cytometry analysis.

3.2.5. Succinate treatment

A 100 mM solution of succinic acid was prepared in tap water. The pH of the solution was adjusted to that of only tap water, which was given to the control group. Both, succinate as well as control water, were sterile filtered before being used as drinking water. WT and AhR^{-/-} mice were provided with the experimental drinking water *ad libitum*. After 7 days, mice were sacrificed and the small intestine was harvested for analysis. The most proximal 1 cm was discarded, the next 7 cm were collected for flow cytometry and the following 7 cm harvested for immunofluorescence analysis.

3.2.6. Preparation of single-cell suspensions from small intestine

To analyze the small intestine of WT, AhR^{-/-}, AhRR^{E/E}, or TripleCyp^{-/-}AhRR^{E/E} mice by flow cytometry, tissues were collected as described in section 3.2.5. The isolation of intestinal cells was performed according to a published protocol (O'Leary *et al*, 2021). In summary, the small intestine was flushed with cold PBS using a 10 ml syringe and a gavage needle. The surrounding fat tissue as well as Peyer's patches were removed. The intestine was opened longitudinally and cut into 1-2 cm-long pieces. These pieces were transferred into a 50 ml conical tube containing 20 ml prewarmed buffer 1 and incubated with rocking at 100 rpm for 15 min at 37 °C. After vortexing at maximum speed for 30 sec, the supernatant including epithelial cells was filtered through a 100 μ m cell strainer into a 50 ml conical tube containing 10 ml cold FACS buffer. Next, 10 ml prewarmed buffer 2 were added to the tissue. Following incubation with rocking

at 100 rpm for 15 min at 37 °C, the sample was vortexed at maximum speed for 30 sec. The supernatant was collected in the same 50 ml conical tube as before and the cell strainer was rinsed with 5 ml FACS buffer. The epithelial cell fraction was centrifuged at 400 g for 5 min at 4 °C and the cell pellet was resuspended in 1 ml FACS buffer. For flow cytometry, 250 µl each were used for surface and intracellular tuft cell staining as described in section 3.2.13.

The remaining gut pieces were incubated in 20 ml prewarmed rinse buffer with rocking at 100 rpm for 5 min at 37 °C. The supernatant was discarded and the tissue was minced with blunt end scissors in 1 ml digestion buffer. Additional 4 ml of digestion buffer were added and the tissue was digested with rocking at 120 rpm for 20 min at 37 °C. After strongly pipetting up and down, the cell suspension containing LP cells was filtered through a 100 µm filter into a 50 ml conical tube filled with 10 ml cold FACS buffer. The cell strainer was rinsed with 5 ml FACS buffer. Following centrifugation at 400 g for 5 min at 4 °C, isolated LP cells were used for co-culture with intestinal crypts or were restimulated for flow cytometry analysis.

3.2.7. Restimulation of LP cells

Isolated LP cells were resuspended in 2 ml complete RPMI medium and the cell count was determined. Cells were stimulated at 3×10^6 cells/ml in 10 ml complete RPMI medium supplemented with Cell Activation Cocktail with Brefeldin A for 4 h at 37 °C. After washing with FACS buffer, the complete sample was stained for ILC2s as described in section 3.2.13. and analyzed by flow cytometry.

3.2.8. Isolation of small intestinal crypts

Crypts from the small intestine of WT, AhR^{-/-}, AhRR^{E/E}, TripleCyp^{-/-}AhRR^{E/E} and Atoh1-CreER^{T2}; R-26-Ai14/Ai14 mice were isolated according to a published protocol (Sato & Clevers, 2013). For this purpose, the first 14 cm of the small intestine were harvested and the surrounding fat tissue was removed. The intestine was opened longitudinally, excessively washed in cold PBS, and cut into very fine pieces. These gut fragments were resuspended in 15 ml cold PBS, transferred to a 50 ml conical tube, and pipetted up and down with a prewetted 25 ml pipette. After letting the tissue sink down, the upper liquid phase was carefully taken off. This washing step was repeated 10-15 times until the supernatant was clear. Next, pieces were incubated in 25 ml

2 mM EDTA in PBS with rocking for 30 min at 4 °C. The supernatant was discarded and 20 ml cold PBS were added to the sample. To solve crypts, gut pieces were strongly pipetted up and down 3-4 times with a prewetted 10 ml pipette. The upper liquid phase was filtered through a 70 µm cell strainer into a 50 ml conical tube. This step was repeated 3 times to get four different fractions with increasing purity. Following centrifugation at 300 g for 5 min at 4 °C, crypts of the fourth fraction were washed in 10 ml cold basal organoid medium and spun down at 100 g for 5 min at 4 °C. Isolated crypts were used for small intestinal organoid culture or for co-culture with intestinal LP cells.

3.2.9. Small intestinal organoid culture

Organoid culture medium was prepared by supplementing basal organoid medium with 1x B-27TM supplement, 1x N-2 supplement, 1 mM N-acetyl-L-cysteine, 50 ng/ml mouse EGF, 100 ng/ml mouse Noggin and 100 ng/ml human R-Spondin 1 (ENR medium). Matrigel Matrix was thawed overnight at 4 °C. Approximately 200-300 crypts per well were resuspended in cold 50 µl Matrigel/ENR medium mix (1:1) and added to the well center of a prewarmed 24-well plate. The plate was incubated at 37 °C for at least 15 min to allow the polymerization of Matrigel. Next, 500 µl ENR medium were carefully added to each well without disturbing the domes. The culture medium was replaced every 3-4 days and organoids were passaged after 7 days of culture. To this end, organoids were dissociated by strong mechanical pipetting for 10-15 times in 1.5 ml cold 0.5 mM EDTA in PBS. Dissociated organoids from up to six wells were collected in one 15 ml conical tube and centrifuged at 100 g for 5 min at 4 °C. The sample was washed in 5 ml cold basal organoid medium and centrifuged at 100 g for 5 min at 4 °C. The pellet was resuspended in 50 µl Matrigel/ENR medium mix per well to split organoids 1:2.

All experiments were performed with organoids from the third passage on. Organoid lines derived from different mice of the same genotype were handled as biological replicates. To drive tuft cell expansion, freshly passaged organoids were treated with 50 ng/ml IL-13 in ENR medium for 4 days for flow cytometry analysis. AhR activation was induced by stimulation with 1 µM 3MC or FICZ, while the addition of 1 µM CH223191 inhibited AhR signaling in organoids. DMSO-treated samples served as unstimulated controls. Organoids derived from Atoh1-CreER^{T2}; R26-Ai14/Ai14 mice

were cultured with 0.5 μ M 4-hydroxytamoxifen solved in DMSO to induce tdTomato reporter signal in *Atoh1*-expressing cells.

3.2.10. Cryopreservation of small intestinal organoids

First, the number of mature organoids in each well was determined. The culture medium was removed and replaced by 1.5 ml cold PBS. By strong mechanical pipetting for 10-15 times, Matrigel was solved and organoids were fragmented. To achieve 200-300 organoids per cryovial, the content of multiple well was combined in one 15 ml conical tube and centrifuged at 280 g for 5 min at 4 °C. The sample was washed once in 7 ml cold pre-freeze wash solution and centrifuged at 100 g for 5 min at 4 °C. The pellet was resuspended in 500 μ l cold FCS and transferred to cryovials. Additional 500 μ l 2x freezing medium were added and gently mixed with the organoid suspension. Cryovials were placed in a freezing container with 500 ml isopropanol and stored in a -80 °C freezer for 24 h, before being transferred to liquid nitrogen for long-term storage.

Cryopreserved organoids were rapidly thawed by placing the cryovial in a 37 °C water bath. 1 ml of post-thaw wash solution was directly added to the vial and the complete content was transferred to a 15 ml conical tube containing 7 ml post-thaw wash solution. The cryovial was washed again with 1 ml post-thaw wash solution. After centrifugation at 280 g for 5 min at 4 °C, fragments of approximately 100 frozen organoids were resuspended in cold 50 μ l Matrigel/ENR medium mix (1:1) and added to the well center of a prewarmed 24-well plate. The plate was incubated at 37 °C for at least 15 min to allow the polymerization of Matrigel. Next, 500 μ l ENR medium were carefully added to each well without disturbing the domes. The culture medium was replaced every 3-4 days and organoids were passaged after 7 days of culture. Previously frozen organoids were passaged two times before being used for experiments.

3.2.11. Co-culture of crypts and LP cells

Approximately 200-300 crypts from WT, AhRR^{E/E}, and TripleCyp^{-/-}AhRR^{E/E} mice were mixed with 5 x 10⁵ isolated LP cells of matching genotype in cold 50 μ l Matrigel/ENR medium mix and added to the well center of a prewarmed 24-well plate. The plate was incubated at 37 °C for at least 15 min to allow the polymerization of Matrigel. Co-cultures were stimulated with 1 μ M 3MC in 500 μ l ENR medium for 20 h. DMSO-

treated samples served as unstimulated controls. To generate conditioned medium, 5×10^5 LP cells were cultured in 50 μ l Matrigel/ENR medium mix in 500 μ l ENR medium with 1 μ M 3MC or DMSO. On the next day, the conditioned medium (LP-CM DMSO/3MC) was collected and each supplemented with 1 μ M 3MC or DMSO. Approximately 200-300 crypts, which were started to be cultured in 50 μ l Matrigel/ENR medium mix in 500 μ l ENR medium on the day before, were then stimulated with 500 μ l conditioned medium of LP cells for 20 h. Direct and indirect co-cultures were finally analyzed for *AhRR*-EGFP expression by flow cytometry.

3.2.12. Dissociation of small intestinal crypts and organoids

The cell culture medium was aspirated and intestinal crypts and organoids were washed out of the Matrigel dome by pipetting up and down 10-15 times in 1.5 ml cold PBS. After centrifugation at 400 g for 5 min at 4 °C, the pellet was resuspended in 300 μ l TrypLE Express reagent plus 10 μ g/ml DNaseI per well collected. Crypts and organoids were dissociated by harshly pipetting up and down more than 20 times during 10 min incubation at 37 °C. The incubation was stopped by adding 2 ml FACS buffer and the cell suspension was centrifuged at 400 g for 5 min at 4 °C. Cells were resuspended in FACS buffer, transferred to a 96-well U-bottom plate, and stained for tuft cell analysis by flow cytometry as described in section 3.2.13.

3.2.13. Flow cytometry

The staining for flow cytometry analysis was performed in U-bottom plates. To avoid unspecific binding, cells were incubated in 50 μ l FACS buffer containing Fc-Block™ for 10 min. After blocking, 50 μ l surface antibody mix (listed in Table 3.6) in FACS buffer were directly added and cells were stained for 30 min at 4 °C in the dark. Subsequently, cells were washed in FACS buffer, centrifuged at 400 g for 5 min at 4 °C, and resuspended in 150 μ l FACS buffer. Before measurement, samples were filtered and 10 μ l counting beads were added to each sample to enable the calculation of total cell counts. BD CompBeads were used for compensation. Data were collected with a BD FACSymphony™ A5 and analyzed with the FlowJo™ software.

The intracellular staining was performed following surface marker staining. For intracellular cytokine analysis, splenocytes were fixed with 2 % PFA for 20 min and permeabilized with 0.5 % Saponin/BSA in PBS. Intestinal epithelial cells were fixed in

4 % PFA for 5 min, whereas LP cells were fixed and permeabilized using the eBioscience™ FoxP3/transcription factor staining kit according to manufacturer's instructions. Cells were stained with 50 µl intracellular antibody mix (listed in Table 3.6) in permeabilization buffer for 30 min or overnight at 4 °C in the dark. If necessary, after washing in 100 µl permeabilization buffer at 400 g for 5 min at 4 °C, a secondary antibody staining was done in 50 µl permeabilization buffer for 15 min at 4°C in the dark. Finally, cells were first washed using 100 µl permeabilization buffer followed by washing in FACS buffer at 400 g for 5 min at 4°C. For acquisition, samples were resuspended in 150 µl FACS buffer, filtered and analyzed as described above.

3.2.14. Histology

The spleen and the liver of control and TAS2010-infected WT or AhR^{-/-} mice were fixed overnight in a freshly prepared PLP solution containing 1.25 % PFA at 4 °C and dehydrated in 20 % sucrose. The small intestine of WT, AhR^{-/-}, AhRR^{E/E}, or TripleCyp^{-/-}AhRR^{E/E} mice was harvested as described in section 3.2.5. and was cut open longitudinally on PBS-soaked filter paper using scissors. The luminal content was rinsed with PBS and the sample was fixed between folded filter paper in 4 % PFA overnight at 4 °C. After 3 times washing in PBS, the tissue was dehydrated in 30 % sucrose solution overnight at 4 °C. A swiss-roll was formed by rolling the intestine from top to bottom over a toothpick.

Samples were embedded in cryomolds filled with Tissue-Tek® O.C.T.™ and stored at -80 °C. Several 7 µm sections per tissue at different tissue planes were prepared using a cryostat. Slides were air-dried before staining. For the H&E staining, hydrated sections were incubated in hematoxylin for 5 min, rinsed with water, then counterstained with eosin for 3 min, and washed with distilled water. Following dehydration, samples were mounted in Euparal.

For the immunofluorescence staining, sections were rehydrated in PBS for 10 min and permeabilized in tissue staining buffer for 10 min. To prevent unspecific binding, samples were incubated with 200 µl blocking buffer for 2 h at room temperature in a darkened humidity chamber. Subsequently, sections were stained with 100 µl primary antibodies (listed in Table 3.7) diluted in tissue staining buffer overnight at 4 °C. After washing 3 times in PBS for 10 min, slides were incubated with 100 µl secondary antibodies (listed in Table 3.7) diluted in PBS for 2 h at room temperature. Finally,

samples were counterstained with 0.2 µg/ml DAPI in PBS for 5 min, rinsed 3 times in PBS for 10 min, and mounted by using Mowiol® plus DABCO. Stainings were analyzed with a Keyence BZ-9000 digital fluorescence microscope and the BZ-II Viewer and Analyzer software. Processing, stitching, and quantitative analysis of images were performed using the Fiji software ImageJ. To calculate the mean liver granuloma size, at least 15 different images per mouse were analyzed by outlining granulomas using freehand line selection. Similarly, areas of splenic B cell zones were determined for at least 3 images per mouse. Expression levels of CD71 in spleen were quantified as mean grey value (MGV) of 3-4 different images per mouse. Tuft cell numbers per crypt-villus axis were quantified for three sections à four images per mouse.

For the immunofluorescence staining of organoids, freshly passaged organoids were cultured in 30 µl Matrigel/ENR medium mix in 300 µl ENR medium plus 50 ng/ml IL-13 in an 8-well chamber slide. AhR activation was induced by stimulation with 1 µM 3MC or FICZ. DMSO-treated samples served as unstimulated controls. After 3 days, medium was taken off and organoids were fixed in 300 µl 4 % PFA for 20 min. Fixative was removed and wells were washed in 300 µl PBS. The PBS was discarded and 300 µl 0.5 % Triton X-100 in PBS were added for 20 min at room temperature to permeabilize cell membranes. Organoids were blocked in 300 µl blocking buffer for 1 h at room temperature. Primary antibodies (listed in Table 3.7) were diluted in 150 µl blocking buffer and incubated overnight at 4 °C. On the next day, samples were washed 3 times in PBS for 10 min. Organoids were stained with secondary antibodies (listed in Table 3.7) and 0.02 µg/ml DAPI diluted in 150 µl blocking buffer for 2 h at room temperature in the dark. Each well was washed 3 times in PBS for 10 min. After the last washing step, organoids were left in a small volume of PBS. Immunofluorescence stainings were analyzed with a Zeiss LSM 710 confocal laser scanning microscope and the ZEN 2010 software. Processing and maximum intensity projections of images were performed using the Fiji software ImageJ.

3.2.15. Statistical analysis

Statistical analyses were performed with GraphPad Prism 10.1.1. Data was plotted as mean values ± standard error of the mean (SEM) or standard deviation (SD). All data points are reflecting biological replicates and n-values are indicated in each figure legend. The statistical significance was determined by p-value calculation using

unpaired *t* test, log-rank test (Mantel-Cox) for survival curves, and one-way or two-way ANOVA with Tukey's test for multiple comparison. A p-value of ≤ 0.05 was considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

4. Results

4.1. Dysregulation of stress erythropoiesis and enhanced susceptibility to *Salmonella* Typhimurium infection in AhR-deficient mice

The following chapter is adapted from Mayer et al., 2024 (Mayer M, Cengiz-Dartenne SC,(...), Förster I, Weighardt H. Dysregulation of stress erythropoiesis and enhanced susceptibility to Salmonella Typhimurium infection in aryl hydrocarbon receptor-deficient mice. The Journal of Infectious Diseases. 2024 Jun 6;jiae304.)

4.1.1. AhR^{-/-} mice display increased susceptibility to TAS2010 infection

As it has previously been shown that the AhR ligand benzo(a)pyrene favors the outcome of systemic *S. enterica* infection in mice by enhancing early host defense functions (Fueledner *et al*, 2022), we aimed at elucidating the impact of AhR deficiency on the long-term host response to chronic invasive STM infection in mice, mimicking typhus in humans. Therefore, WT and AhR^{-/-} mice were orally infected with 2.5×10^9 CFU of the attenuated STM strain TAS2010. Based on previous observations that WT mice display the highest TAS2010 levels in systemic organs 2 weeks postinfection and largely clear the infection after approximately 5 weeks (Wang *et al*, 2023), we monitored the survival of WT and AhR^{-/-} mice for 5 weeks. Whereas all WT mice survived, only 20 % of AhR^{-/-} mice survived 5 weeks after infection (Figure 4.1 A). AhR^{-/-} mice showed an exacerbated bacterial load in spleen and liver as compared with WT mice 2 and 5 weeks after infection. Whereas CFU in WT mice dropped from week 2 to week 5 postinfection in both organs, indicating a clearance of the infection, they remained elevated in AhR^{-/-} mice (Figure 4.1 B). Histopathology of typhoid fever includes the presence of typhoid nodules in host tissues, in which STM reside to establish a persistent infection (Avraham, 2023; Bharadwaj *et al*, 2009). In line, TAS2010 led to granuloma formation in the liver of WT and AhR^{-/-} mice 5 weeks after infection, with significantly larger granuloma size in AhR^{-/-} mice (Figure 4.1 C). As high levels of IFN- γ were shown to correlate with disease severity rather than clearance of chronic infections (Kupz *et al*, 2014), serum IFN- γ levels were measured 2 and 5 weeks postinfection. At both time points, significantly higher IFN- γ levels could be detected in AhR^{-/-} mice versus WT mice (Figure 4.1 D), in line with the finding that bacteria were not effectively cleared in the absence of the AhR.

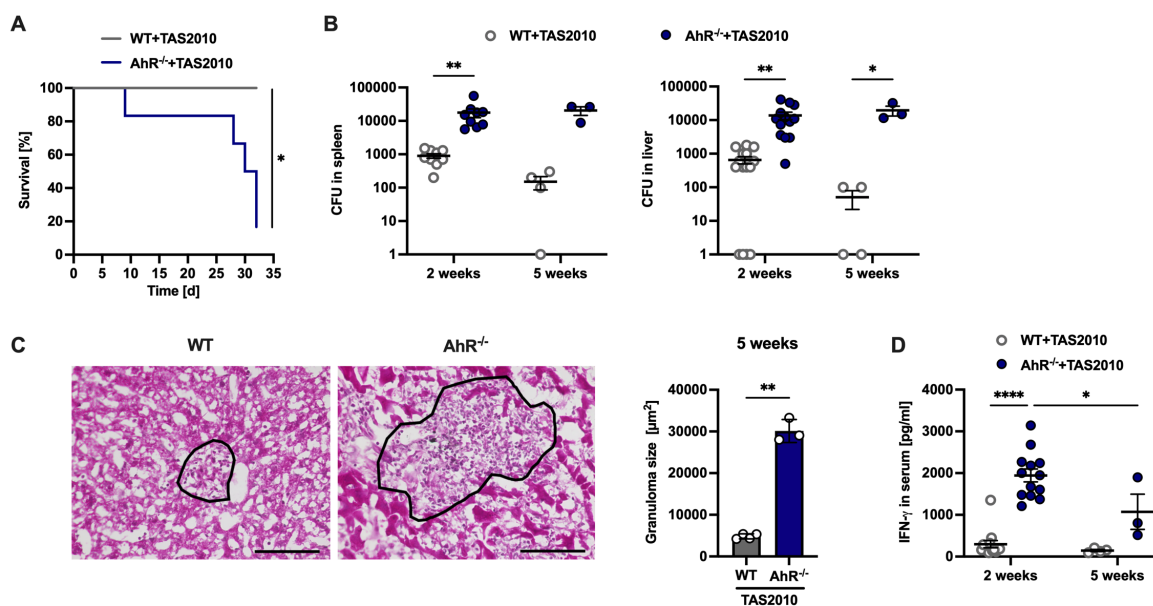


Figure 4.1 | AhR deficiency in mice results in higher susceptibility to TAS2010 infection

(A) WT and AhR^{-/-} mice were orally infected with 2.5×10^9 attenuated *Salmonella* Typhimurium TAS2010, and survival was monitored for 32 days: $n = 4$, WT; $n = 6$, AhR^{-/-}. (B) CFU in spleen and liver of WT and AhR^{-/-} mice. Spleen: $n = 9$ (WT/AhR^{-/-}, 2 weeks), $n = 4$ (WT, 5 weeks), $n = 3$ (AhR^{-/-}, 5 weeks). Liver: $n = 15$ (WT, 2 weeks), $n = 13$ (AhR^{-/-}, 2 weeks), $n = 4$ (WT, 5 weeks), $n = 3$ (AhR^{-/-}, 5 weeks). (C) Representative images of hematoxylin- and eosin–stained liver sections of WT and AhR^{-/-} mice 5 weeks after TAS2010 infection. Black line marks the granuloma outline. Scale bar: 100 μm. At least 15 images per mouse were analyzed to calculate the mean granuloma size: $n = 4$ (WT), $n = 3$ (AhR^{-/-}). (D) IFN-γ levels in serum of infected WT and AhR^{-/-} mice measured by enzyme-linked immunosorbent assay: $n = 14$ (WT, 2 weeks), $n = 13$ (AhR^{-/-}, 2 weeks), $n = 4$ (WT, 5 weeks), $n = 3$ (AhR^{-/-}, 5 weeks). Data are represented as mean $\pm$ SEM, and significance was calculated by (A) log-rank test (Mantel-Cox), (B, D) two-way analysis of variance with a Tukey multiple-comparison test, and (C) Welch *t* test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

4.1.2. TAS2010 infection leads to increased splenomegaly and altered splenic architecture in AhR^{-/-} mice

Another hallmark of systemic *Salmonella* infection in murine and human hosts is the development of splenomegaly, which was shown to correlate with reduced bacterial clearance (Bhandari *et al*; Jackson *et al*, 2010; Li *et al*, 2016). Indeed, spleens of WT mice were slightly enlarged 2 and 5 weeks after TAS2010 infection, whereas spleen weights were almost 3-fold higher in AhR^{-/-} mice (Figure 4.2 A), correlating with a markedly increased splenic cellularity after infection. Next, we tested whether AhR deficiency affects splenic cell composition upon TAS2010 infection. Infected AhR^{-/-} mice showed enhanced numbers of macrophages, B cells, CD4⁺ T cells, and CD8⁺ T cells in comparison with WT mice 2 weeks postinfection and uninfected mice (Figure 4.2 B and Appendix Figure 7.1 A). Macrophage as well as B cell numbers were

already higher in uninfected AhR^{-/-} mice as compared with WT mice. Although absolute immune cell counts were increased in infected AhR^{-/-} mice, correlating with increased splenic cellularity, the frequencies of immune cells were not altered in infected AhR^{-/-} mice when compared with WT mice (Appendix Figure 7.1 B). Cell numbers of macrophages and T cells declined to near preinfection levels 5 weeks postinfection, whereas B cell counts were reduced in infected WT and AhR^{-/-} mice as compared with uninfected mice. At this time point, numbers of all immune cell subsets were comparable between WT and AhR^{-/-} mice. Unlike that for WT mice, analysis of infected AhR^{-/-} mice revealed that the number of IFN- γ -producing CD4⁺ T cells was significantly increased at both time points (Figure 4.2 C). Although the frequency of IFN- γ ⁺CD8⁺ T cells was not significantly altered in either mouse line, CD8⁺ T cells of uninfected and infected AhR^{-/-} mice tended to produce more IFN- γ , indicating a more inflammatory milieu. This enhanced IFN- γ production of AhR^{-/-} splenic T cells might contribute to the augmented systemic IFN- γ levels (Figure 4.1 D).

STM infection is known to disrupt the splenic microarchitecture, specifically in T and B cell follicles and in the marginal zone (Rosche *et al*, 2015; Marcial-Juárez *et al*, 2023). TAS2010 infection led to a reduction of the white pulp with altered T and B cell compartments (Figure 4.2 D). Whereas in WT mice some T cell follicles were still intact at 2 weeks after infection and a reconstitution of the structure was obvious 5 weeks postinfection, AhR^{-/-} mice showed a complete loss of T cell zones, resulting in a relocation of T cells to the red pulp. Also, the B cell follicles were significantly smaller in AhR^{-/-} mice when compared with WT mice, a phenomenon that was already observed in uninfected controls to a lower extent. Nevertheless, TAS2010 infection further reduced the size of B cell zones in both genotypes. In addition, the structure of the marginal zone was affected during TAS2010 infection with a decrease of SIGN-R1⁺ marginal zone macrophages (MZM Φ s) and Siglec-1⁺ marginal metallophilic macrophages (MMM Φ s) at 5 weeks postinfection (Appendix Figure 7.1 C). Both subsets were more strongly affected in AhR^{-/-} mice, as evident by an almost complete loss of MZM Φ s and enhanced distribution of MMM Φ s into the white pulp.

Together, this demonstrated that unlike WT mice, AhR^{-/-} mice were not able to control TAS2010 infection, as indicated by a prolonged infection-induced remodeling of the spleen. However, the remarkable splenomegaly in AhR^{-/-} mice upon infection could not be explained solely by changes in leukocyte numbers.

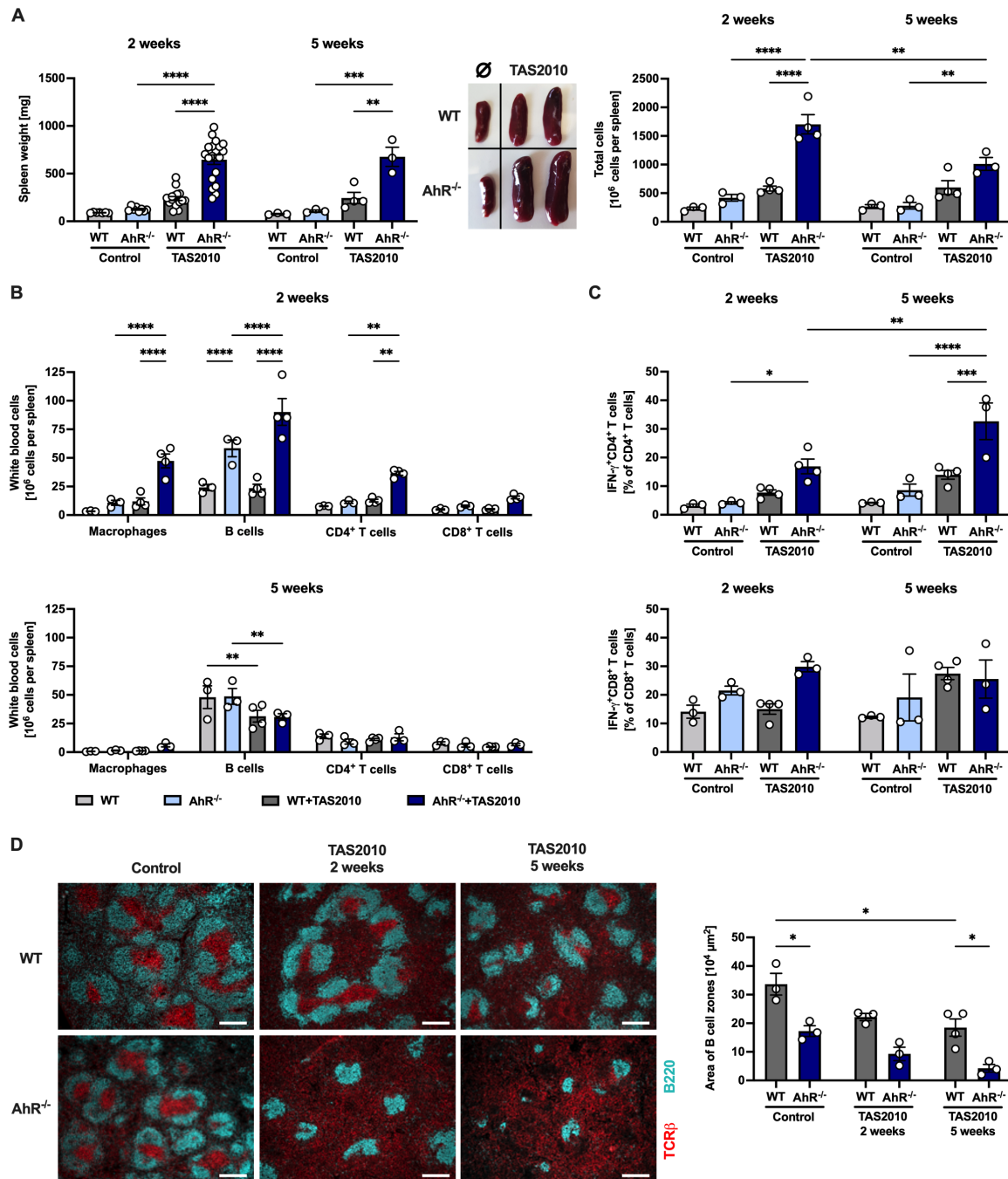


Figure 4.2 | AhR^{-/-} mice display increased splenomegaly and remodeling of splenic immune cell compartments upon infection

(A) Weight and representative images of spleens of uninfected and infected WT and AhR^{-/-} mice. Control: n = 7 (WT, 2 weeks), n = 8 (AhR^{-/-}, 2 weeks), n = 3 (WT/AhR^{-/-}, 5 weeks). TAS2010: n = 17 (WT, 2 weeks), n = 19 (AhR^{-/-}, 2 weeks), n = 4 (WT, 5 weeks), n = 3 (AhR^{-/-}, 5 weeks). Total cell count per spleen of uninfected and infected WT and AhR^{-/-} mice. Control: n = 3 (WT/AhR^{-/-}, 2 weeks/5 weeks). TAS2010: n = 4 (WT/AhR^{-/-}, 2 weeks), n = 4 (WT, 5 weeks), n = 3 (AhR^{-/-}, 5 weeks). (B) Absolute numbers of macrophages and B and T cells per spleen of uninfected and infected WT and AhR^{-/-} mice. Control: n = 3 (WT/AhR^{-/-}, 2 weeks/5 weeks). TAS2010: n = 4 (WT/AhR^{-/-}, 2 weeks), n = 4 (WT, 5 weeks), n = 3 (AhR^{-/-}, 5 weeks). (C) Frequencies of IFN- γ ⁺ CD4⁺ and CD8⁺ T cells in spleen of uninfected and infected WT and AhR^{-/-} mice. Control: n = 3 (WT/AhR^{-/-}, 2 weeks/5 weeks). TAS2010: n = 4 (WT/AhR^{-/-}, 2 weeks), n = 4 (WT, 5 weeks), n = 3 (AhR^{-/-}, 5 weeks). (D) Representative images of

immunofluorescence analysis of TCR β ⁺ T cells and B220⁺ B cells in spleen of uninfected and infected WT and AhR^{-/-} mice 2 and 5 weeks after infection with TAS2010. Scale bar: 400 μ m. At least 3 images per mouse were analyzed to calculate the mean area of B cell zones. Control: n = 3 (WT/AhR^{-/-}). TAS2010: n = 3 (WT/AhR^{-/-}, 2 weeks), n = 4 (WT, 5 weeks), n = 3 (AhR^{-/-}, 5 weeks). Data are represented as mean $\pm$ SEM, and significance was calculated by two-way analysis of variance with a Tukey multiple-comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

4.1.3. AhR^{-/-} mice show macrocytic anemia and reduced bone marrow erythropoiesis upon infection

Besides increases in the number of leucocytes, splenomegaly is known to result from the induction of stress erythropoiesis in the spleen in response to reduced bone marrow erythropoiesis and anemia upon infection (Jackson *et al*, 2010; Brown *et al*, 2010; Rosche *et al*, 2015; Li *et al*, 2016; Hyland *et al*, 2005). To study the stress erythropoiesis response, precursor and mature RBC subsets in whole blood and bone marrow were analyzed by expression of Ter119 and CD71 (Socolovsky *et al*, 2001).

Proerythroblasts were identified as Ter119^{med}CD71^{hi} cells, basophilic and polychromatic erythroblasts as Ter119^{hi}CD71^{hi} cells, orthochromatic erythroblasts and reticulocytes as Ter119^{hi}CD71^{med}, and mature RBCs as Ter119^{hi}CD71^{low} (Appendix Figure 7.2 D). A significant reduction of RBCs in hematocrit and hemoglobin levels was detected in infected AhR^{-/-} mice 2 weeks postinfection as compared with infected WT and uninfected AhR^{-/-} mice, indicative of infection-induced anemia (Figure 4.3 A). AhR^{-/-} mice also showed enhanced absolute counts and frequency of blood reticulocytes (Appendix Figure 7.2 A, B). Thus, increased mean corpuscular volume of RBCs and decreased mean corpuscular hemoglobin concentration caused by reticulocytosis indicated macrocytic anemia in AhR^{-/-} mice (Appendix Figure 7.2 C). Furthermore, reduced erythropoiesis was detected in the bone marrow of infected WT and AhR^{-/-} mice. Here, a decline in RBCs could be observed with a more pronounced reduction of late erythroblasts, reticulocytes, and mature erythrocytes when compared with early erythroblasts (Figure 4.3 B-D).

In sum, reduced bone marrow erythropoiesis could be detected in infected WT and AhR^{-/-} mice, leading to macrocytic anemia in AhR^{-/-} but not WT mice 2 weeks after TAS2010 infection.

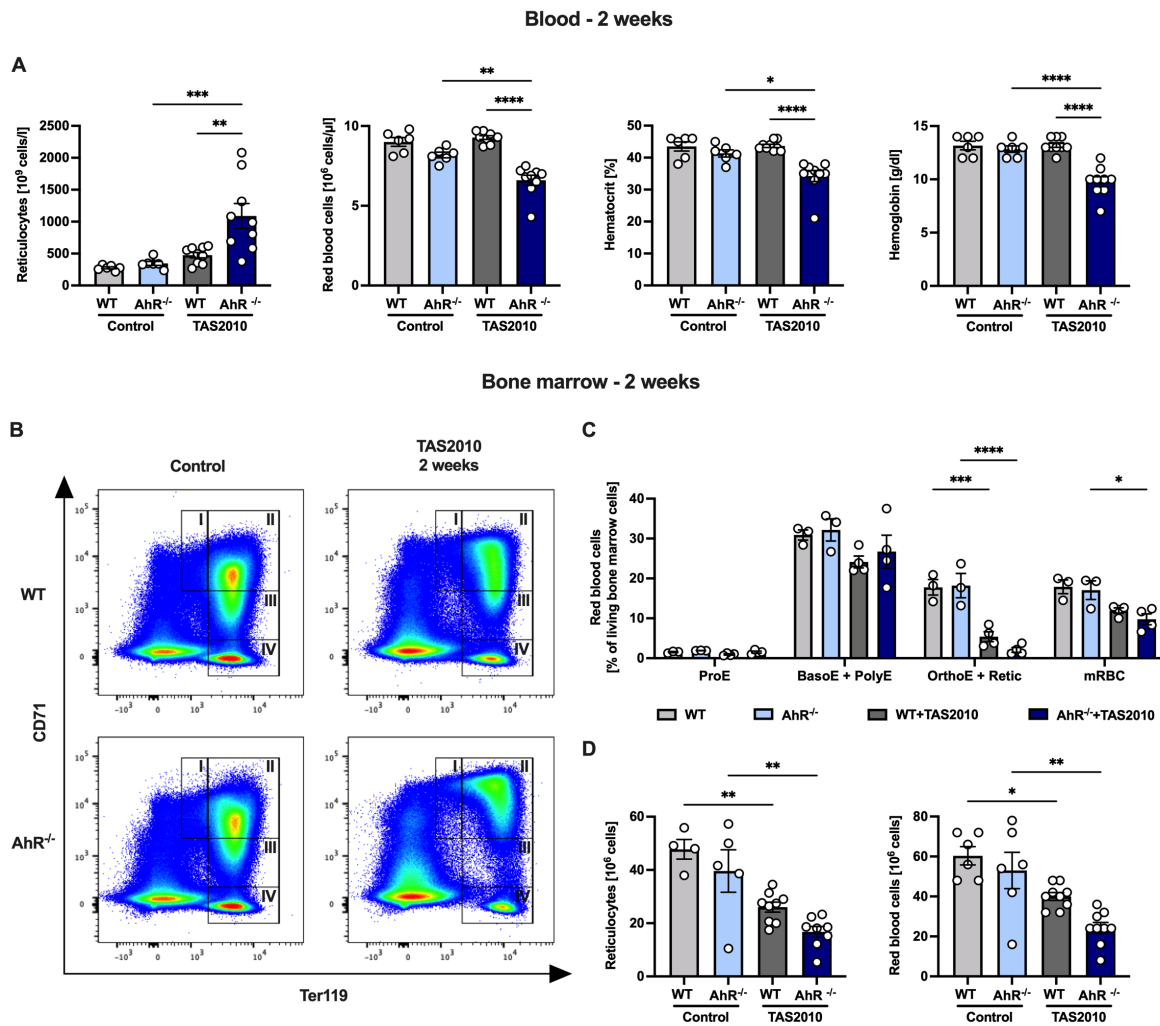


Figure 4.3 | TAS2010 infection results in macrocytic anemia and reduced bone marrow erythropoiesis in $AhR^{-/-}$ mice

(A) Numbers of red blood cells and reticulocytes in blood of uninfected and infected WT and $AhR^{-/-}$ mice. Control: $n = 6$ (WT/ $AhR^{-/-}$). TAS2010: $n = 9$ (WT/ $AhR^{-/-}$). Blood hematocrit and hemoglobin concentration of uninfected and infected WT and $AhR^{-/-}$ mice. Control: $n = 6$ (WT/ $AhR^{-/-}$). TAS2010: $n = 9$ (WT/ $AhR^{-/-}$). (B) Representative flow cytometry profiles of uninfected and infected WT and $AhR^{-/-}$ bone marrow erythroid cell populations. I: Ter119^{med}CD71^{hi} proerythroblasts (ProE). II: Ter119^{hi}CD71^{hi} basophilic and polychromatic erythroblasts (BasoE + PolyE). III: Ter119^{hi}CD71^{med} orthochromatic erythroblasts and reticulocytes (OrthoE + Retic). IV: Ter119^{hi}CD71^{low} mature red blood cells (mRBCs). (C) Frequencies of ProE, BasoE + PolyE, OrthoE + Retic, and mRBCs in the bone marrow of uninfected and infected WT and $AhR^{-/-}$ mice. Control: $n = 3$ (WT/ $AhR^{-/-}$). TAS2010: $n = 4$ (WT/ $AhR^{-/-}$). (D) Absolute numbers of red blood cells and reticulocytes in bone marrow per femur and tibia of uninfected and infected WT and $AhR^{-/-}$ mice. Reticulocytes, control: $n = 4$ (WT), $n = 5$ ($AhR^{-/-}$). TAS2010: $n = 9$ (WT), $n = 8$ ($AhR^{-/-}$). Red blood cells, control: $n = 6$ (WT/ $AhR^{-/-}$). TAS2010: $n = 9$ (WT/ $AhR^{-/-}$). Data are represented as mean $\pm$ SEM, and significance was calculated by two-way analysis of variance with a Tukey multiple-comparison test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

4.1.4. Reduced bone marrow erythropoiesis in AhR^{-/-} mice correlates with an increase in stress erythropoiesis upon TAS2010 infection

Since increases in immune cell populations could not solely explain the infection-induced splenomegaly in AhR^{-/-} mice, we tested whether anemia might fuel EME, additionally driving splenomegaly. We found a major increase of immature RBC precursors in the spleen upon infection. As expected, the frequencies and numbers of immature erythroid cells from a basophilic erythroblast to reticulocyte state were low in uninfected mice, whereas mature erythrocytes constituted the largest proportion of RBC populations (Figure 4.4 A, B and Appendix Figure 7.3 A). Two weeks after infection, the frequencies and counts of immature RBC populations increased significantly in AhR^{-/-} mice as compared with WT mice. In contrast, the frequency of mature RBCs in the spleen dropped to around 45 % in WT mice and only 25 % in AhR^{-/-} mice, even though total erythrocyte counts were significantly increased. Thus, we found an enhanced production of reticulocytes and a block in their transition to mature erythrocytes in infected AhR^{-/-} mice. Five weeks postinfection, the frequency of erythrocytes was still reduced in AhR^{-/-} mice when compared with WT mice, whereas absolute numbers were comparable 2 and 5 weeks postinfection in AhR^{-/-} mice. In addition, the proportions and numbers of immature RBC populations recovered to steady state levels in WT mice but remained high in AhR^{-/-} mice. Mainly late erythroblasts and reticulocytes stayed significantly elevated, reflecting enhanced splenic erythropoiesis in AhR^{-/-} mice upon TAS2010 infection as compared with WT mice. Immunostaining for CD71, a marker for immature RBCs as erythroblasts and reticulocytes but not mature erythrocytes (Paulson *et al*, 2020), clearly showed that CD71⁺ cells localized to erythroid niches within the red pulp (Figure 4.4 C). In line, TAS2010 infection enhanced CD71 expression levels in infected mice, but this was significant only in AhR^{-/-} mice.

Stress erythropoiesis in the spleen is initiated by unique stress erythroid progenitors (SEPs) (Paulson *et al*, 2020). In steady state, bone marrow hematopoietic stem cells migrate to the spleen, giving rise to self-renewing immature c-Kit⁺CD133⁺CD34⁺SEP1s. Inflammatory signals induce the expansion of transiently amplifying SEP2s, which downregulate CD34 expression. Erythropoietin (EPO) signaling induces their differentiation into more committed stress BFU-E cells (burst-forming units-erythroid cells), leading to CFU-E cells (colony-forming units-erythroid cells), which terminally

mature to erythrocytes. Interestingly, uninfected $AhR^{-/-}$ mice already showed increased frequencies of SEP1s when compared with WT mice, indicating an enhanced capacity for splenic stress erythropoiesis after an infection insult (Figure 4.5 A). At 2 weeks after infection, the ratio of immature SEP1s decreased, whereas the frequencies of SEP2s, BFU-E, and CFU-E increased in both genotypes. This effect was more pronounced in $AhR^{-/-}$ mice, which could explain the enhanced stress erythropoiesis in $AhR^{-/-}$ mice.

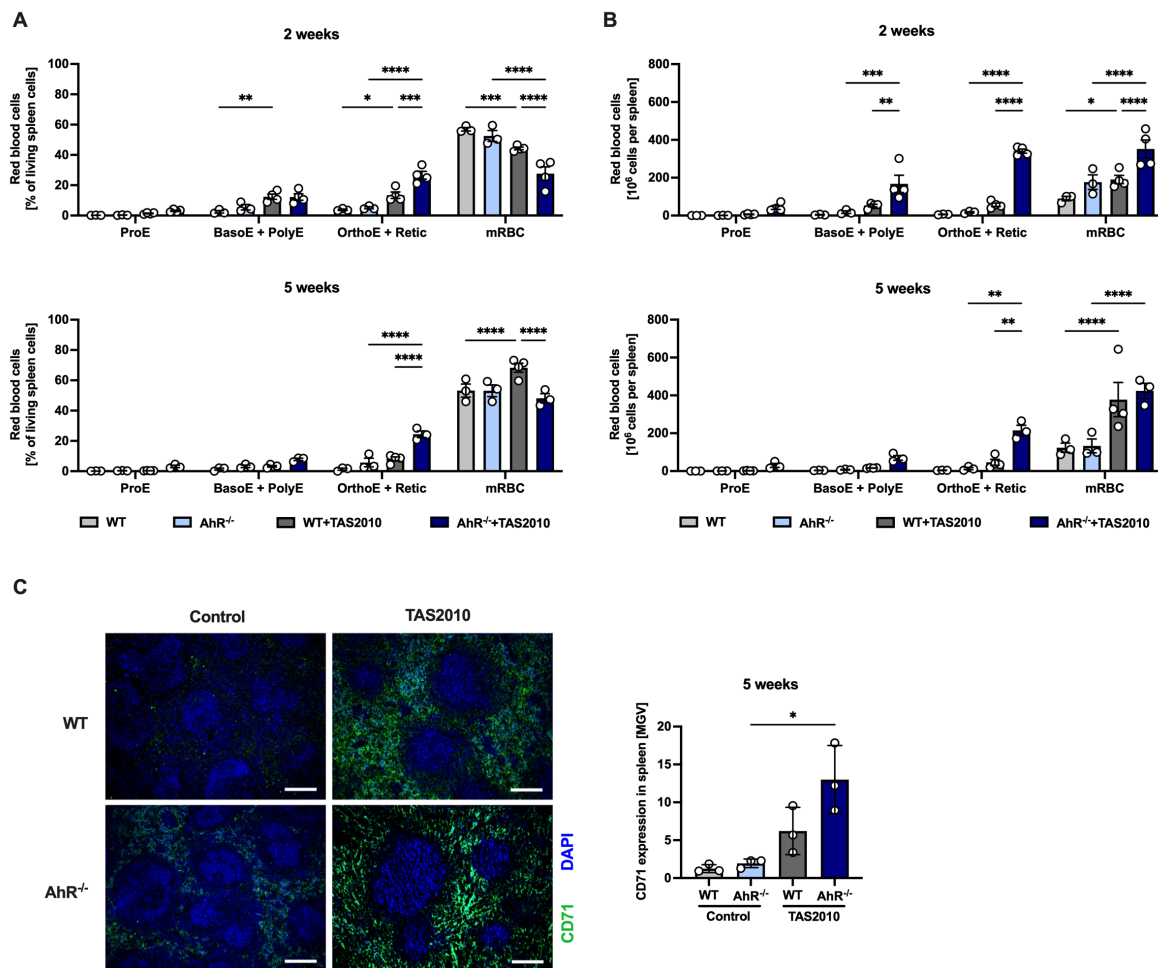


Figure 4.4 | Splenomegaly in $AhR^{-/-}$ mice is mainly caused by enhanced stress erythropoiesis

(A) Frequencies of proerythroblasts (ProE), basophilic and polychromatic erythroblasts (BasoE + PolyE), orthochromatic erythroblasts and reticulocytes (OrthoE + Retic), and mature red blood cells (mRBCs) in spleen of uninfected and infected WT and $AhR^{-/-}$ mice analyzed by flow cytometry. Control: $n = 3$ (WT/ $AhR^{-/-}$, 2 weeks/5 weeks). TAS2010: $n = 4$ (WT/ $AhR^{-/-}$, 2 weeks), $n = 4$ (WT, 5 weeks), $n = 3$ ($AhR^{-/-}$, 5 weeks). (B) Absolute numbers of ProE, BasoE + PolyE, OrthoE + Retic, and mRBCs per spleen of uninfected and infected WT and $AhR^{-/-}$ mice analyzed by flow cytometry. Control: $n = 3$ (WT/ $AhR^{-/-}$, 2 weeks/5 weeks). TAS2010: $n = 4$ (WT/ $AhR^{-/-}$, 2 weeks), $n = 4$ (WT, 5 weeks), $n = 3$ ($AhR^{-/-}$, 5 weeks). (C) Representative images of immunofluorescence analysis of CD71⁺ cells in spleen of uninfected and infected WT and $AhR^{-/-}$ mice after 5 weeks. Scale bar: 200 μ m. Expression levels of CD71 were quantified as mean gray value (MGV) of 3 or 4 images per mouse ($n = 3$). Data are represented as mean $\pm$ SEM, and significance was calculated by two-way analysis of variance with a Tukey multiple-comparison test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Whereas EPO levels of WT mice were not altered after TAS2010 infection, AhR^{-/-} mice showed significantly elevated EPO levels 2 and 5 weeks after infection (Figure 4.5 B). Other niche factors important for EME, such as stem cell factor (SCF), thrombopoietin (TPO), CXCL12, and transforming growth factor (TGF)- β (Lee & Zhan, 2024; Inra *et al*, 2015; Di Giandomenico *et al*, 2020; Perry *et al*, 2007), were not significantly altered in either genotype (Appendix Figure 7.3 B). Since IL-6 is known to contribute to anemia in chronic inflammation by impairing the development of late-stage erythroid progenitors (Akchurin *et al*, 2019; Prince *et al*, 2012; McCranor *et al*, 2014), serum IL-6 levels were examined. IL-6 concentrations were already increased in uninfected AhR^{-/-} mice as compared with WT mice and were increased further after infection in both genotypes. Of note, AhR^{-/-} mice showed a 4-fold induction of IL-6 when compared with WT mice 2 weeks postinfection, which increased to a 20-fold difference after 5 weeks, suggesting that besides elevated EPO production, the enhanced IL-6 levels might contribute to elevated stress erythropoiesis and infection-induced anemia in AhR^{-/-} mice.

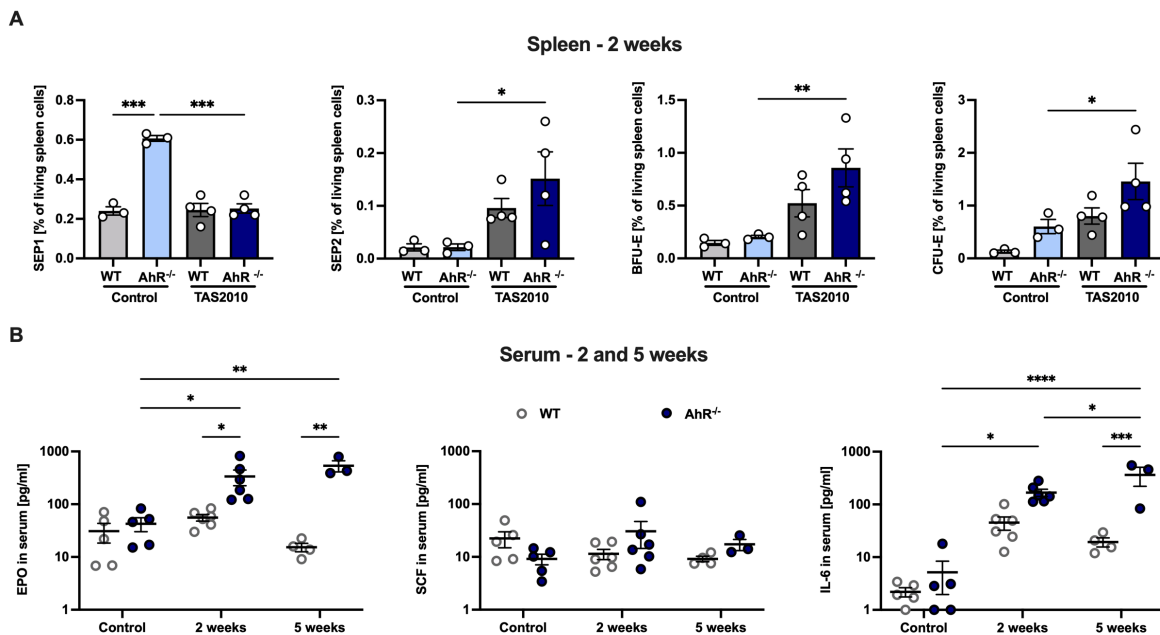


Figure 4.5 | Expansion of splenic stress erythroid progenitors and elevated EPO and IL-6 serum levels in AhR^{-/-} mice upon TAS2010 infection

(A) Frequencies of stress erythroid progenitors 1 (SEP1), SEP2, burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) in spleen of uninfected and infected WT and AhR^{-/-} mice. Control: n = 3 (WT/AhR^{-/-}). TAS2010: n = 4 (WT/AhR^{-/-}). (B) Levels of EPO, SCF, and IL-6 in serum of uninfected and infected WT and AhR^{-/-} mice. Control: n = 5 (WT/AhR^{-/-}). TAS2010: n = 6 (WT/AhR^{-/-}, 2 weeks), n = 4 (WT, 5 weeks), n = 3 (AhR^{-/-}, 5 weeks). Data are represented as mean $\pm$ SEM, and significance was calculated by two-way analysis of variance with a Tukey multiple-comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

4.2. Modulation of AhR signaling influences the tuft cell-mediated immunity in the mouse small intestine

The AhR pathway is critical for maintaining intestinal homeostasis and regulating immune responses to intestinal infections (Lamas *et al*, 2018; Stockinger *et al*, 2021; Wiggins *et al*, 2023). The AhR affects the function of various immune cell populations in the intestine, but is also ubiquitously expressed in the intestinal epithelium. As the activation of AhR is shown to suppress the intestinal ILC2 function and promote the differentiation of ISCs into secretory goblet and Paneth cell lineages (Li *et al*, 2018; Metidji *et al*, 2018; Park *et al*, 2018; Alvarado *et al*, 2019; Zhou *et al*, 2023), AhR signaling could potentially also influence the small intestinal tuft cell-ILC2 circuit that mediates type 2 immune responses to helminth infections (Gerbe *et al*, 2016; Howitt *et al*, 2016; von Moltke *et al*, 2016) and can be activated by the tuft cell agonist succinate (Lei *et al*, 2018; Nadjisombati *et al*, 2018; Schneider *et al*, 2018).

To test this hypothesis, WT, AhR^{-/-}, AhRR^{E/E}, and TripleCyp^{-/-}AhRR^{E/E} mice were used. Whereas AhR signaling is lost in AhR^{-/-} mice, the deletion of AhRR and CYP1 enzymes, which serve as AhR feedback inhibition mechanisms, reinforces the AhR activity in AhRR^{E/E} and TripleCyp^{-/-}AhRR^{E/E} mice. To examine if these modulations of the AhR pathway might affect the tuft cell-mediated immunity, the expansion of tuft cells by IL-13 treatment was studied in intestinal organoids derived from WT, AhR^{-/-}, AhRR^{E/E}, and TripleCyp^{-/-}AhRR^{E/E} mice upon addition of different AhR ligands. Furthermore, tuft cell frequencies as well as ILC2 proliferation were investigated *in vivo* in the small intestine under steady state conditions or after treatment with succinate.

Moreover, AhRR^{E/E} and TripleCyp^{-/-}AhRR^{E/E} mice served as *AhRR* reporter mice, which allow to explore the expression of *AhRR*-EGFP in different cell types. Our group previously showed that in these reporter mice AhR-dependent *AhRR*-EGFP expression is restricted to immune cells located in the LP of the small intestine and is absent in IECs (Brandstätter *et al*, 2016). Also, *in vivo* AhR ligand application could not significantly enhance *AhRR*-EGFP expression by IECs (Iriady, 2024). Here, the regulation of *AhRR*-EGFP expression was further investigated *in vitro* by using mouse intestinal organoids and co-cultures of intestinal crypts with LP cells in comparison to *ex vivo* analysis of naive small intestines of *AhRR* reporter mice.

4.2.1. AhR activation restricts the IL-13-driven tuft cell expansion and induces *AhRR* expression by IECs in mouse intestinal organoids

In order to study the role of the AhR in intestinal tuft cell function, mouse small intestinal organoids derived from adult somatic intestinal stem cells were used as an *in vitro* model (Sato & Clevers, 2013). Therefore, epithelial crypts containing ISCs were isolated by enzymatic and mechanical digestion from the small intestine of WT, *AhR*^{-/-}, *AhRR*^{E/E}, and *TripleCyp*^{-/-}*AhRR*^{E/E} mice. Isolated crypts were embedded in a dome of Matrigel matrix and differentiated into organoids by a culture medium containing mouse EGF, mouse Noggin, and human R-Spondin 1 (ENR medium). At day 1 of culture, crypts closed and developed into 3D cysts, which started to bud after 3 days to form crypt-like domains (Figure 4.6). As organoids differentiated, the branching of organoids progressed around day 5, while at the same time apoptotic cells were shed into the lumen. After 7 days, the increasing accumulation of luminal debris necessitated the passaging of organoids by mechanical dissociation.

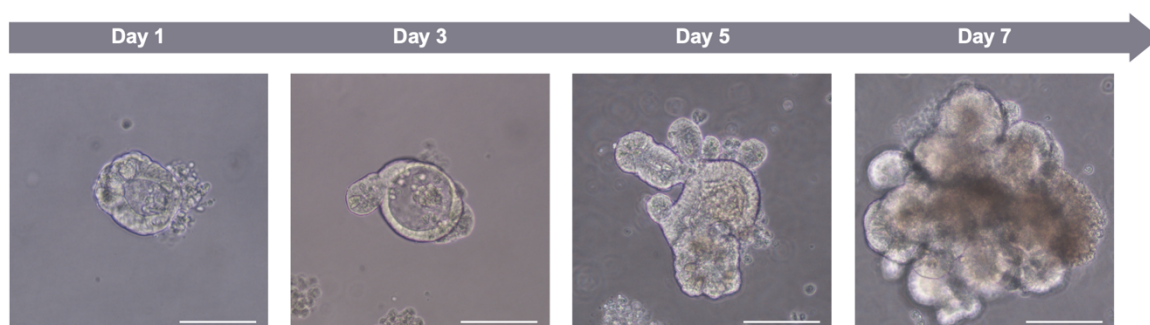


Figure 4.6 | Differentiation of mouse small intestinal organoids derived from adult somatic intestinal stem cells

Mouse epithelial crypts were isolated from the small intestine and embedded in a dome of Matrigel matrix. Representative microscopy images show organoid differentiation in ENR medium for 7 days. Crypts closed at day 1 of culture, began budding after 3 days to form crypt-like domains and finally produced branching organoids around day 5. Passaging of organoids was necessary after 7 days because of increasing accumulation of debris within the organoid lumen. Scale bar = 100 μ m.

To assess if the differentiation of tuft cells might be altered by modulation of the AhR pathway, intestinal organoids from WT, *AhR*^{-/-}, *AhRR*^{E/E}, and *TripleCyp*^{-/-}*AhRR*^{E/E} mice were differentiated in ENR medium with the addition of IL-13 and the AhR agonists 3MC or FICZ for 3 days to induce tuft cell expansion. To examine tuft cell frequencies, organoids were fixed, permeabilized, and immunofluorescently stained for the tuft cell marker DCLK1 (Figure 4.7). In addition, IECs were marked by the expression of

EpCAM on the cell surface, whereas nuclei were counterstained with DAPI. IL-13 treatment clearly led to an increase of tuft cell numbers compared to unstimulated organoids of all genotypes (Figure 4.7 A, B). Interestingly, AhR activation by 3MC or FICZ appeared to restrain the IL-13-mediated tuft cell expansion in WT, AhRR^{E/E}, and TripleCyp^{-/-}AhRR^{E/E} organoids but not in AhR^{-/-} organoids (Figure 4.7 C, D). In the absence of AhR stimulation, a weak AhRR-EGFP signal could only be observed in IECs of TripleCyp^{-/-}AhRR^{E/E} organoids, but not in AhRR^{E/E} organoids (Figure 4.7 A, B).

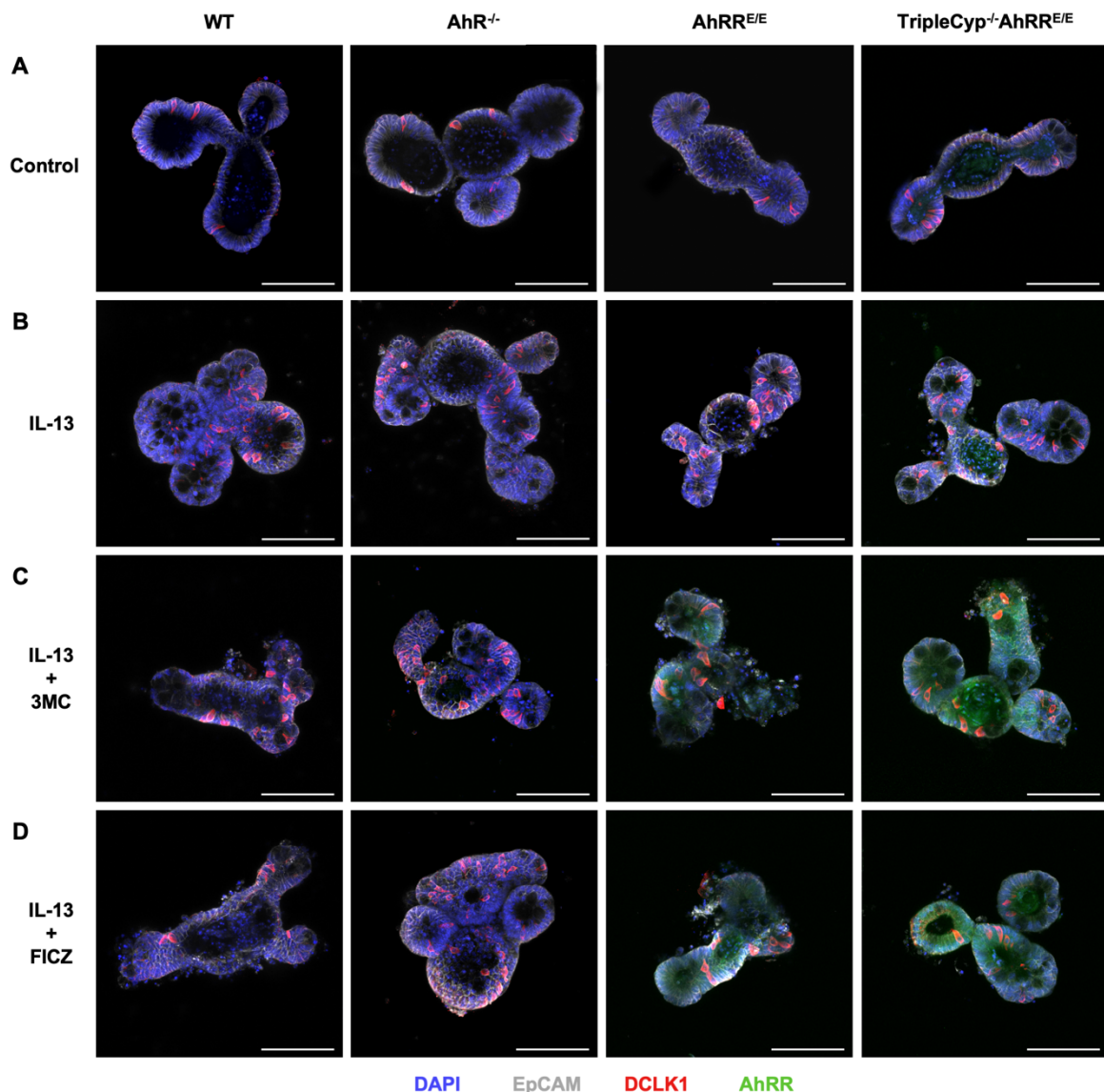


Figure 4.7 | AhR activation appears to limit IL-13-mediated tuft cell expansion and induces *AhRR*-EGFP expression by IECs in intestinal organoids

Intestinal organoids from WT, AhR^{-/-}, AhRR^{E/E}, and TripleCyp^{-/-}AhRR^{E/E} mice were differentiated in ENR medium for 3 days (A) with addition of 50 ng/ml IL-13 (B) plus 1 μ M 3MC (C) or FICZ (D). Organoids were fixed and stained for DCLK1 (red) and EpCAM (grey) expression. EGFP reporter signal indicated AhRR (green) expression. Nuclei were counterstained with DAPI (blue). Representative images are projections of z-stacks. Scale bar = 100 μ m.

In contrast to previous *in vivo* data of our group, *AhRR*-EGFP expression by IECs could be induced by 3MC or FICZ stimulation in organoids from both *AhRR*^{E/E} and *TripleCyp*^{-/-}*AhRR*^{E/E} mice with a more pronounced increase of *AhRR*-EGFP expression if the CYP1 enzymes were additionally deleted (Figure 4.7 C, D).

For a better quantification of tuft cells and the *AhRR*-EGFP signal, intestinal organoids from WT, *AhR*^{-/-}, *AhRR*^{E/E}, and *TripleCyp*^{-/-}*AhRR*^{E/E} mice were differentiated for 4 days and similarly treated with IL-13 and 3MC or FICZ before being dissociated into a single-cell suspension and then investigated via flow cytometry. To analyze the frequencies of tuft cells, the following gating strategy was applied (Figure 4.8): Cells were selected on SSC and FSC properties. Doublets and dead cells were excluded and IECs were gated as CD45⁻EpCAM⁺ cells. Among the IECs, tuft cells were identified by co-expression of CD24 and Siglec-F. Additionally, *AhRR*-EGFP expression by IECs and tuft cells was assessed. In IL-13-untreated organoids with or without 3MC addition, tuft cells constituted a minor fraction of IECs with just about 0.5 % (Figure 4.9 A, B). IL-13 stimulation however resulted in a marked expansion of tuft cells to a frequency of more than 3 % of IECs. Of note, this IL-13-driven tuft cell hyperplasia was significantly restrained by 3MC stimulation in organoids of all genotypes except of *AhR*^{-/-} organoids. Similar results were obtained for the stimulation of intestinal organoids with the more physiological AhR agonist FICZ (Figure 4.9 C).

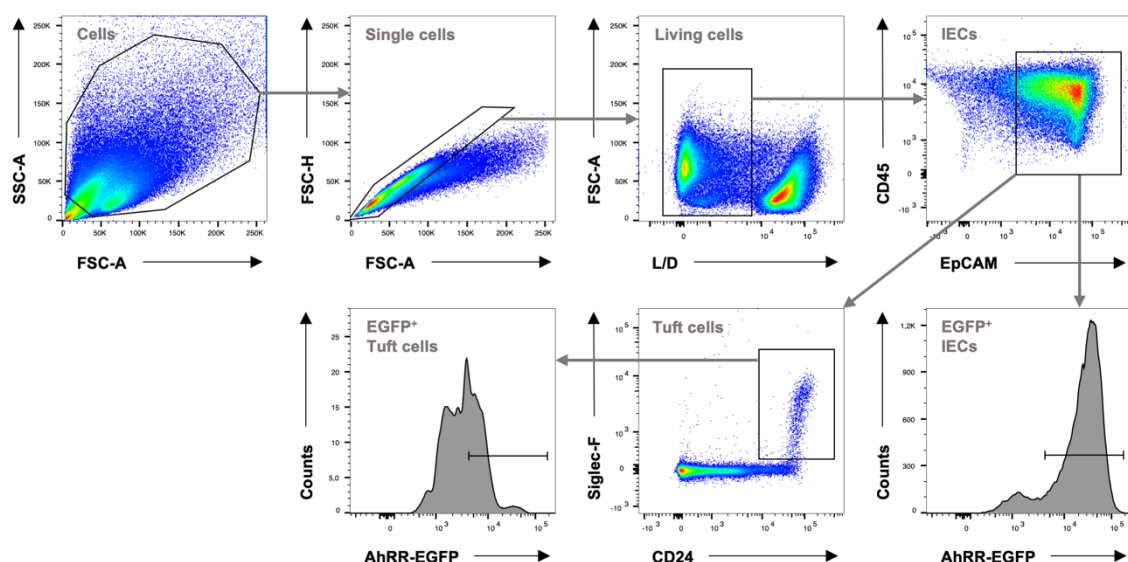


Figure 4.8 | Tuft cell gating strategy of *AhRR* reporter intestinal organoids

Living single cells of dissociated organoids were gated on CD45⁻EpCAM⁺ IECs. Cells were then checked for EGFP expression to identify *AhRR*-expressing IECs. IECs were further analyzed for tuft cells based on CD24 and Siglec-F expression. Moreover, *AhRR*-EGFP expression by tuft cells was assessed.

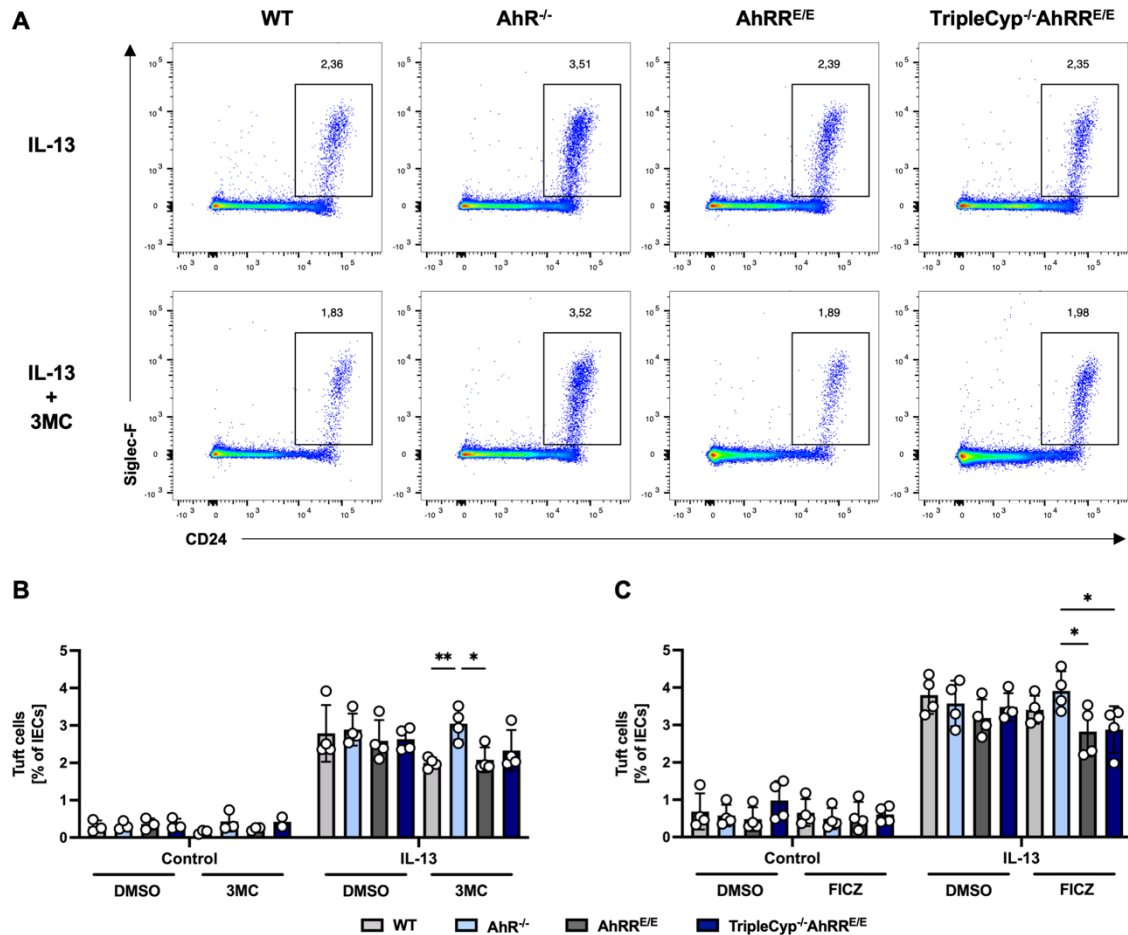


Figure 4.9 | Tuft cell expansion upon IL-13 treatment is restricted by AhR activation in intestinal organoids

Intestinal organoids from WT, AhR^{-/-}, AhRR^{E/E}, and TripleCyp^{-/-}AhRR^{E/E} mice were differentiated in ENR medium for 4 days with addition of 50 ng/ml IL-13 plus 1 μ M 3MC or FICZ. (A) Representative flow cytometry plots of CD24⁺Siglec-F⁺ tuft cells from IL-13-treated organoids after 3MC stimulation. Frequencies of tuft cells from IL-13-treated organoids after 3MC (B) or FICZ (C) stimulation. Data are represented as mean $\pm$ SD (n = 3-4). Statistical significance was tested using two-way ANOVA with Tukey multiple-comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

In line with the immunofluorescence analysis, flow cytometric quantification revealed a strong increase of EGFP expression by IECs in both AhRR reporter organoids upon 3MC treatment (Figure 4.10 A, C). Thus, 80 % and 90 % of IECs were EGFP-positive in 3MC-stimulated AhRR^{E/E} and TripleCyp^{-/-}AhRR^{E/E} organoids, respectively. Even if an induction of AhRR-EGFP expression could be observed for tuft cells, a lower proportion of tuft cells was EGFP-positive compared to the total IEC fraction (Figure 4.10 B, D). Interestingly, IL-13 treatment alone or in combination with 3MC tended to enhance the amount of AhRR-EGFP-expressing IECs and tuft cells in AhRR reporter organoids (Figure 4.10 A-D). Stimulation of organoids with FICZ showed

comparable effects on *AhRR*-EGFP expression by IECs and tuft cells as 3MC (Figure 4.10 E, F).

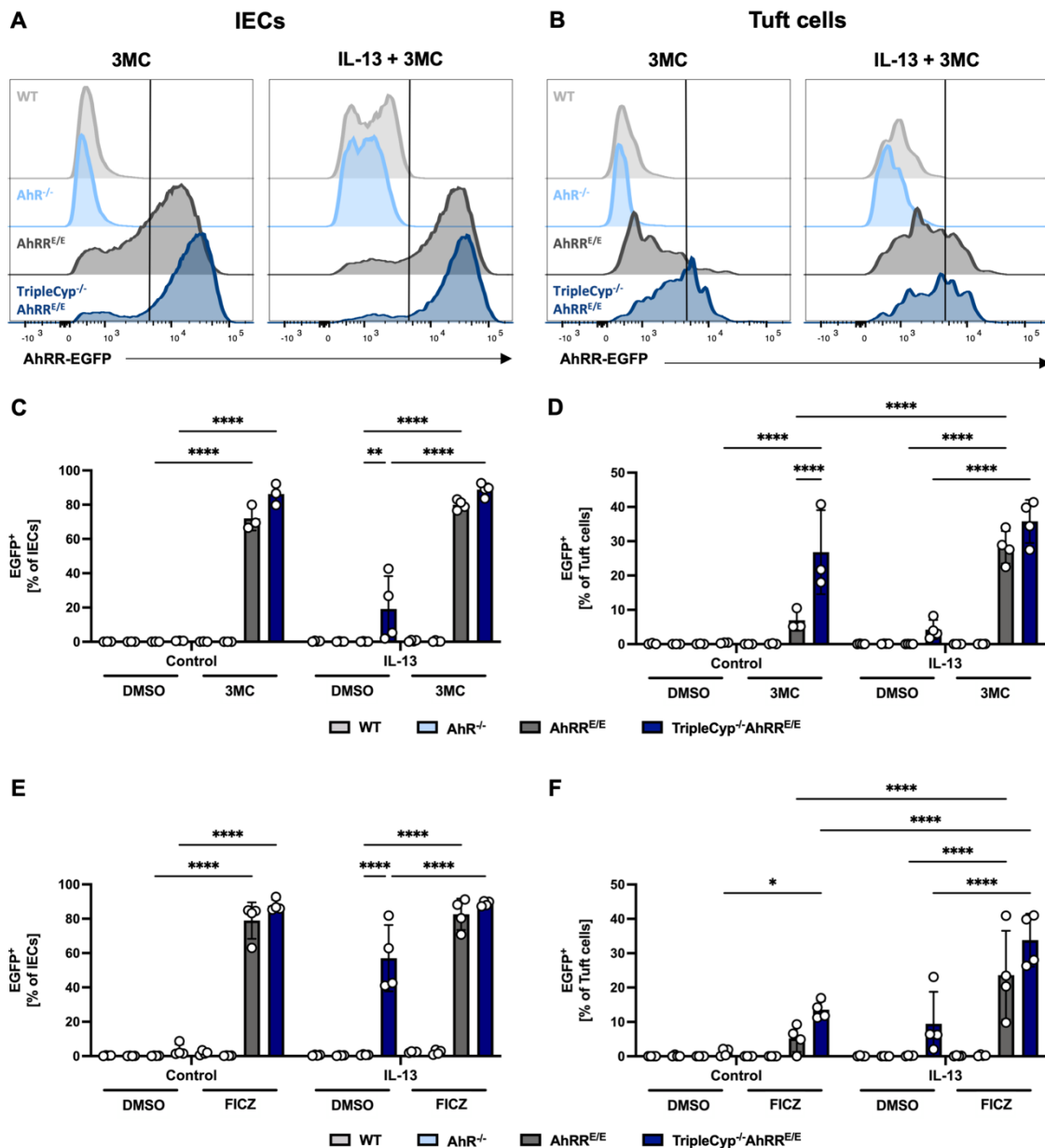


Figure 4.10 | 3MC and FICZ stimulation of intestinal organoids induce *AhRR*-EGFP expression by IECs as well as tuft cells

Intestinal organoids from WT, *AhR*^{-/-}, *AhRR*^{E/E} and *TripleCyp*^{-/-}*AhRR*^{E/E} mice were differentiated in ENR medium for 4 days with addition of 50 ng/ml IL-13 plus 1 μ M 3MC or FICZ. Representative histograms of *AhRR*-EGFP expression by IECs (A) and tuft cells (B) from 3MC-stimulated organoids with or without IL-13 treatment. Frequencies of *AhRR*-EGFP⁺ IECs and tuft cells from 3MC- (C + D) or FICZ-stimulated (E + F) organoids with or without IL-13 treatment. Data are represented as mean $\pm$ SD (n = 3-4). Statistical significance was tested using two-way ANOVA with Tukey multiple-comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

As AhR activation is known to promote the development of secretory epithelial cells (Park *et al*, 2018; Alvarado *et al*, 2019; Zhou *et al*, 2023), IL-13- and 3MC-treated WT, AhR^{-/-}, AhRR^{E/E}, and TripleCyp^{-/-}AhRR^{E/E} organoids were analyzed for the frequencies of Paneth cells and EECs via flow cytometry. Hence, the gating strategy was set as follows (Figure 4.11): Living single cells of dissociated organoids were gated on CD45⁻EpCAM⁺ IECs. Further, Paneth cells and EECs were identified by intracellular staining for the key transcription factors lysozyme-P (LysP) and chromogranin A (ChrgA), respectively. In line with the literature, the proportions of both Paneth cells and EECs are slightly increased by 3MC-induced AhR activation in WT and AhRR^{E/E} organoids, whereas their frequencies appeared to be unchanged in AhR^{-/-} and TripleCyp^{-/-}AhRR^{E/E} organoids (Figure 4.12 A, B). Also, IL-13 treatment had no major impact on Paneth cell and EEC levels.

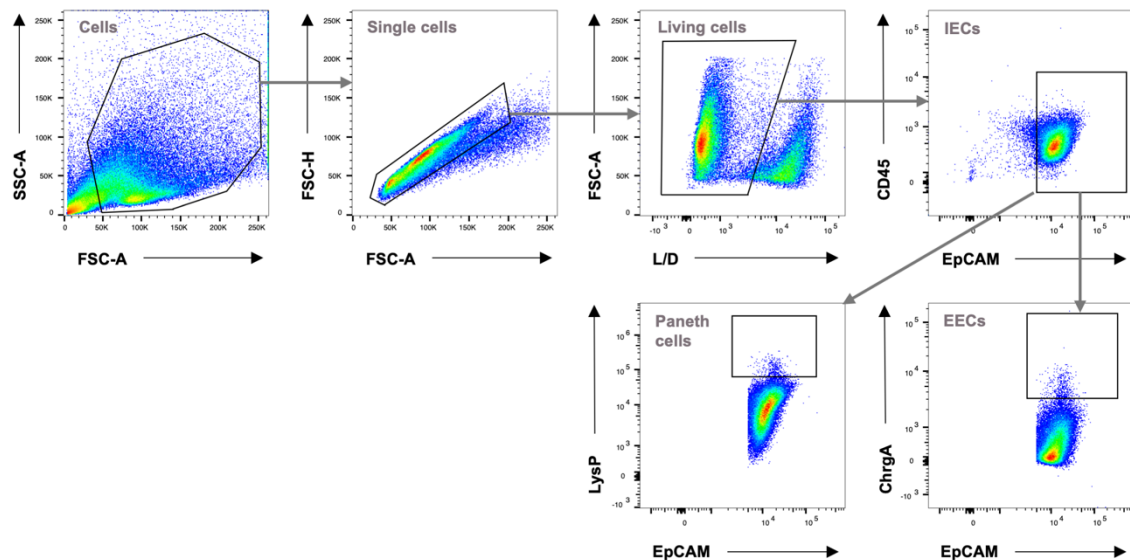


Figure 4.11 | Paneth cell and EEC gating strategy of intestinal organoids

Living single cells ChrgA of dissociated organoids were gated on CD45⁻EpCAM⁺ IECs. IECs were further analyzed for LysP expression to gate on Paneth cells or for ChrgA expression to identify EECs.

All in all, the activation of AhR by its agonists 3MC and FICZ limited IL-13-mediated tuft cell expansion in mouse intestinal organoids. In contrast to previous *in vivo* results of our group, showing no induction of AhRR expression by IECs upon ligand stimulation, 3MC as well as FICZ treatment resulted in a strong increase of AhRR-EGFP expression by IECs in AhRR-deficient/EGFP reporter organoids derived from AhRR^{E/E} and TripleCyp^{-/-}AhRR^{E/E} mice.

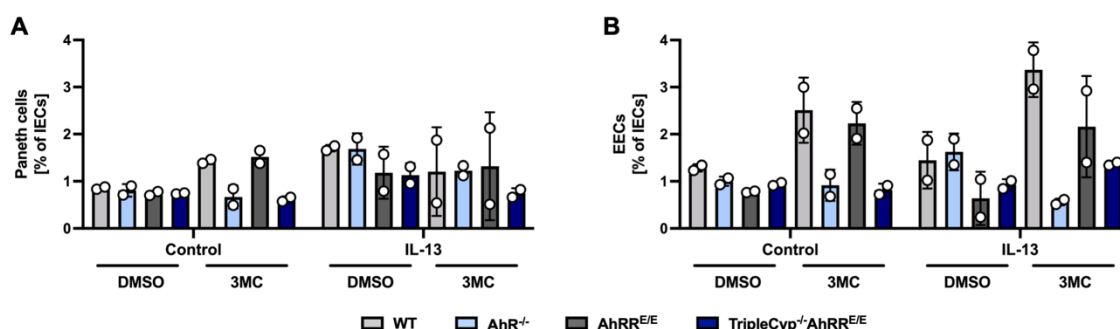


Figure 4.12 | Frequencies of Paneth cells and EECs are not altered upon IL-13 stimulation in intestinal organoids

Intestinal organoids from WT, AhR^{-/-}, AhRR^{E/E}, and TripleCyp^{-/-}AhRR^{E/E} mice were differentiated in ENR medium for 4 days with addition of 50 ng/ml IL-13 plus 1 μ M 3MC. Frequencies of LysP⁺ Paneth cells (A) and ChrgA⁺ EECs (B) from IL-13-treated organoids after 3MC stimulation. Data are represented as mean $\pm$ SD (n = 2).

4.2.2. Modulation of AhR signaling has no effect on *Atoh1* expression by tuft cells in organoids

Previous studies showed that the expression of the transcription factor ATOH1 is essential for the differentiation of mouse ISCs into tuft cells (Gerbe *et al*, 2011). Since AhR has been described to directly regulate *Atoh1* expression in an XRE-dependent manner (Yagishita *et al*, 2023), intestinal organoids derived from *Atoh1*-CreER^{T2}; R26-Ai14/Ai14 mice were analyzed upon AhR activation or inhibition. R26-Ai14/Ai14 mice harbor a loxP-flanked STOP cassette, preventing the transcription of a CAG promoter-driven red fluorescent protein variant (tdTomato), within the *Rosa26* locus. In *Atoh1* reporter organoids, the expression of the recombinase Cre under the control of the *Atoh1* promoter can be induced by tamoxifen treatment, resulting in a tdTomato reporter signal in *Atoh1*⁺ cells.

In order to examine whether AhR signaling might influence *Atoh1*-tdTomato expression by tuft cells and thereby alters the tuft cell differentiation, the tuft cell frequency was assessed in *Atoh1* reporter organoids upon treatment with the AhR agonist 3MC or the antagonist CH223191. The following gating strategy for tuft cell analysis via flow cytometry was used (Figure 4.13): Cells of dissociated organoids were gated based on SSC and FSC properties, followed by exclusion of doublets and dead cells. IECs were identified as CD45-EpCAM⁺ cells and tuft cells were further gated based on CD24 and DCLK1 expression. Moreover, *Atoh1* expression by tuft cells was quantified by the tdTomato reporter signal.

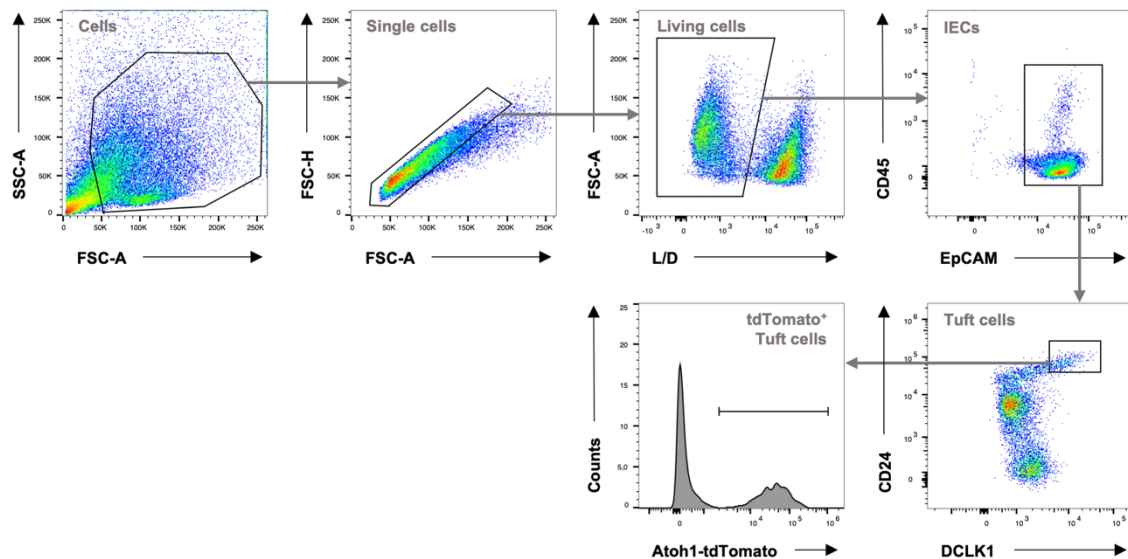


Figure 4.13 | Tuft cell gating strategy of *Atoh1* reporter intestinal organoids

Living single cells of dissociated organoids were gated on CD45⁻EpCAM⁺ IECs. IECs were further analyzed for tuft cells based on CD24 and intracellular DCLK1 expression. Moreover, *Atoh1*-tdTomato expression by tuft cells was assessed.

Consistent with previous findings, AhR activation by 3MC significantly inhibited IL-13-mediated tuft cell expansion in *Atoh1*-CreER^{T2}; R26-Ai14/Ai14 organoids (Figure 4.14 A, B). However, the addition of CH223191 did not alter the frequency of tuft cells compared to DMSO-treated controls. In addition, *Atoh1*-tdTomato expression by tuft cells was affected neither by AhR activation nor by AhR inhibition (Figure 4.14 C, D). Of note, IL-13 stimulation generally led to a marked decrease of tdTomato⁺ tuft cells from over 60 % to about 20 % in organoids of all conditions, indicating that *Atoh1*-negative tuft cells drove the observed IL-13-induced tuft cell hyperplasia.

In summary, *Atoh1*-tdTomato expression by tuft cells was not influenced by modulation of the AhR pathway. Thus, the restrained tuft cell differentiation upon IL-13 treatment by AhR activation was not caused by direct regulation of *Atoh1* expression by AhR signaling.

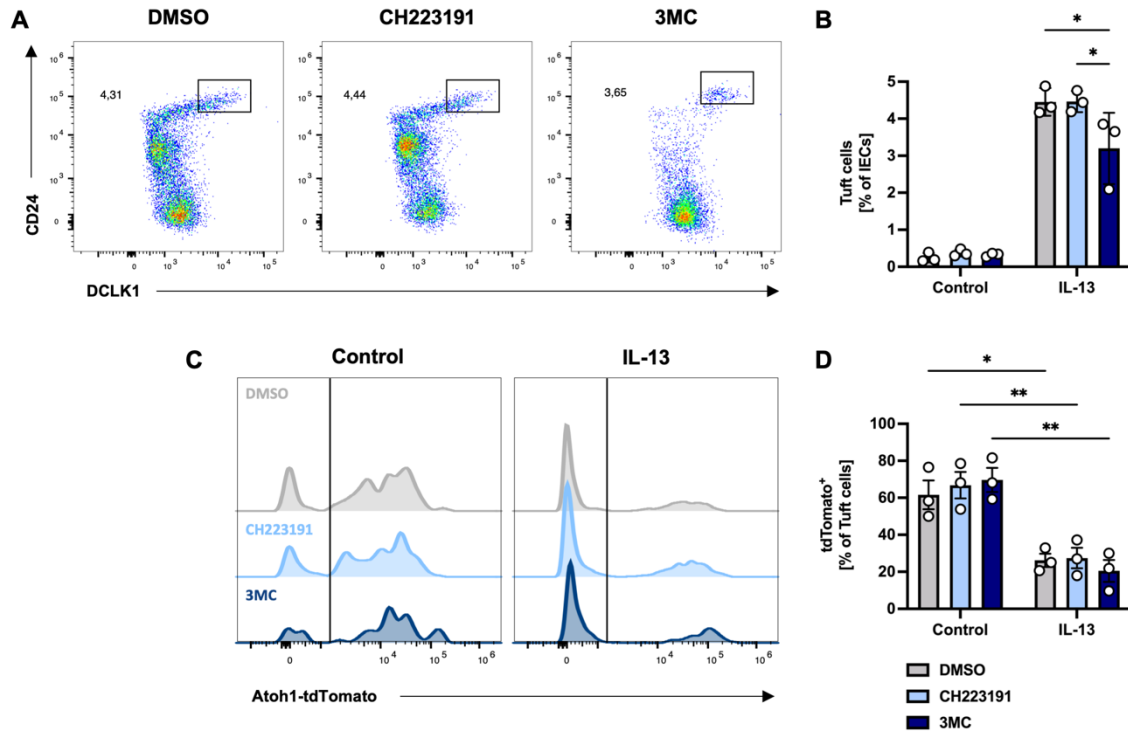


Figure 4.14 | *Atoh1*-tdTomato expression by tuft cells is not influenced by AhR signaling

Intestinal organoids from *Atoh1*-CreER^{T2}; R26-Ai14/Ai14 mice were differentiated in ENR medium for 4 days with addition of 50 ng/ml IL-13 plus 1 μ M CH223191 or 3MC. Simultaneous treatment with 0.5 μ M 4-hydroxytamoxifen induced tdTomato expression by tuft cells upon *Atoh1*-Cre-mediated recombination. Representative flow cytometry plots (A) and frequencies (B) of CD24⁺DCLK1⁺ tuft cells from IL-13-treated organoids after CH223191 or 3MC stimulation. Representative histograms of *Atoh1*-tdTomato expression by tuft cells (C) and frequencies of *Atoh1*-tdTomato⁺ tuft cells (D). Data are represented as mean $\pm$ SD (n = 3). Statistical significance was tested using two-way ANOVA with Tukey multiple-comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

4.2.3. Direct co-culture of intestinal crypts with LP cells suppresses *AhRR* expression by IECs upon AhR activation

Previous experiments of our group indicated that IECs in the small intestine of *AhRR*^{E/E} and *TripleCyp*^{-/-}*AhRR*^{E/E} mice barely expressed *AhRR* even after treatment with AhR agonists. Contrary, IECs in intestinal organoids displayed a strong induction of *AhRR*-EGFP expression upon 3MC and FICZ stimulation. Hence, further analysis aimed to elucidate why the expression of *AhRR* by IECs differed *in vivo* versus *in vitro*. Since intestinal organoids were only composed of a polarized epithelial layer, the crosstalk of IECs with immune cells of the LP was completely missing in this model. To study the impact of LP cells on *AhRR*-EGFP expression by IECs, freshly isolated intestinal crypts from WT, *AhRR*^{E/E}, and *TripleCyp*^{-/-}*AhRR*^{E/E} mice were cultured with LP cells of matching genotypes. In addition to direct co-cultures, crypts were cultured in

conditioned medium from DMSO- or 3MC-treated LP cells (LP-CM DMSO/3MC) to examine the indirect interaction of LP cells with IECs via soluble factors. Crypts in co-culture or cultured in LP-CM were then stimulated with 3MC for 20 hours and *AhRR*-EGFP expression by IECs was analyzed via flow cytometry using the gating strategy described above (Figure 4.8).

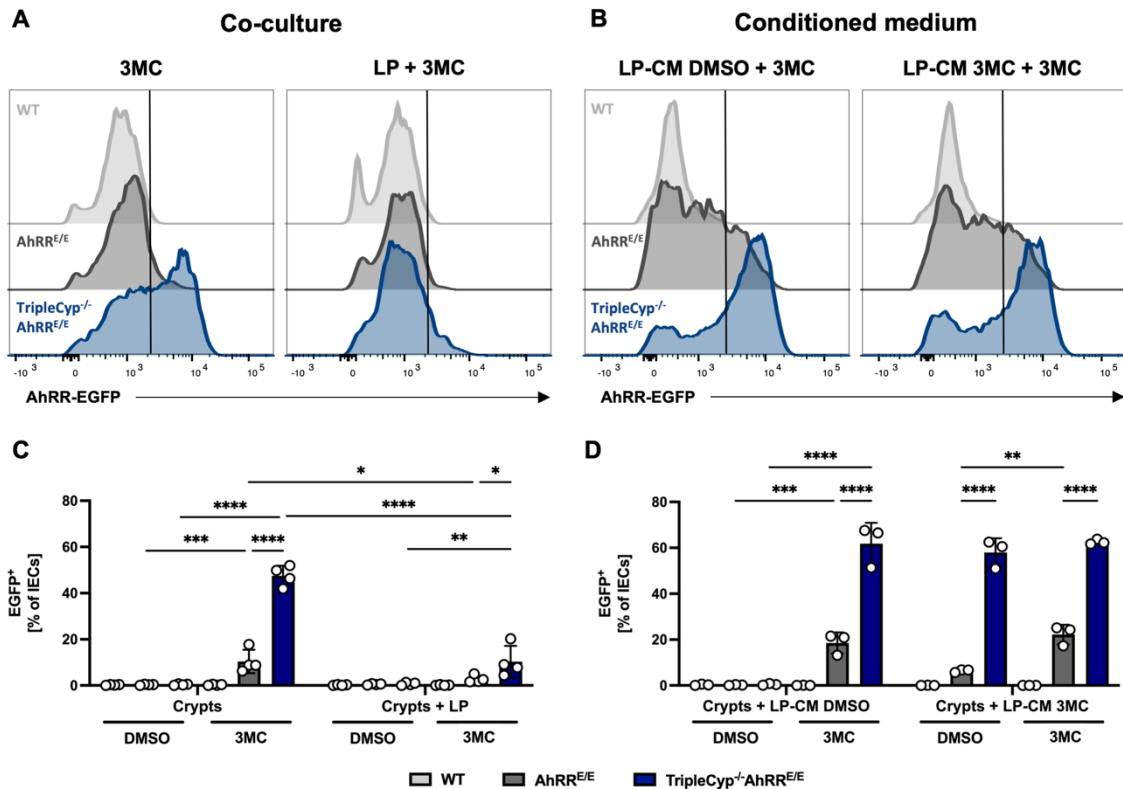


Figure 4.15 | 3MC-induced *AhRR*-EGFP expression by IECs is inhibited by direct co-culture of intestinal crypts with LP cells

Epithelial crypts and LP cells were isolated from the small intestine of WT, AhRR^{E/E}, and TripleCyp^{-/-} AhRR^{E/E} mice. Co-culture of crypts and LP cells or crypts cultured in conditioned medium from DMSO- or 3MC-treated LP cells (LP-CM DMSO/3MC) were stimulated with 1 μ M 3MC for 20 hours. Representative histograms of *AhRR*-EGFP expression by IECs from 3MC-stimulated crypts in co-culture (A) or cultured in LP-CM (B). Frequencies of *AhRR*-EGFP⁺ IECs from 3MC-stimulated crypts in co-culture (C) or cultured in LP-CM (D). Data are represented as mean $\pm$ SD (n = 3-4). Statistical significance was tested using two-way ANOVA with Tukey multiple-comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

In line with the results obtained in assembled organoids, *AhRR*-EGFP expression by IECs was significantly induced by 3MC stimulation in freshly isolated crypts from AhRR^{E/E} mice, but particularly upon loss of the CYP1 enzymes in crypts from TripleCyp^{-/-} AhRR^{E/E} mice (Figure 4.15 A, C). Interestingly, this 3MC-induced *AhRR*-EGFP expression by IECs was clearly suppressed by the direct co-culture with LP cells, as indicated by a marked decrease in the frequency of EGFP⁺ IECs from about

50 % in TripleCyp^{-/-}AhRR^{E/E} crypts without LP cells to 10 % in co-culture. In contrast, a reduction of AhRR-EGFP expressing IECs upon 3MC treatment was not observed by the culture of crypts in CM from DMSO- or 3MC-treated LP cells (Figure 4.15 B, D). Thus, an indirect co-culture with soluble factors contained in the CM was not sufficient to suppress AhRR-EGFP expression.

Taken together, the direct interaction with LP cells restrained the induction of AhRR-EGFP expression by IECs upon 3MC stimulation *in vitro*. This could explain why IECs *in vivo* were not able to enhance AhRR expression after treatment with AhR agonists, but also raises the question how and why the induction of AhRR expression upon AhR activation is suppressed *in vivo*.

4.2.4. Genetic modulation of AhR signaling in mice alters the tuft cell frequency in the small intestinal epithelium

Based on the results obtained from the *in vitro* studies, the role of the AhR in the intestinal tuft cell-ILC2 circuit was further investigated *in vivo* by analyzing the small intestine of naive WT, AhR^{-/-}, AhRR^{E/E}, and TripleCyp^{-/-}AhRR^{E/E} mice. To this end, fixed small intestines were prepared as swiss-rolls, which allowed a wide assessment of proximal to distal segments in one section (Figure 4.16). Immunofluorescence staining for DCLK1 identified tuft cells in the EpCAM-expressing intestinal epithelium and nuclei were counterstained with DAPI. Moreover, AhRR expression was explored by the EGFP signal in the small intestine of AhRR^{E/E} and TripleCyp^{-/-}AhRR^{E/E} reporter mice. To quantify the intestinal tuft cells, four areas of three different sections were imaged in a higher magnification and the numbers of tuft cells per crypt-villus structure were determined (Figure 4.17 A). Consistent with the organoid data, in steady state, the genetic ablation of AhR in mice significantly enhanced the frequency of intestinal tuft cells, whereas an amplification of AhR signaling by loss of the CYP1 enzymes decreased the tuft cell numbers in the TripleCyp^{-/-}AhRR^{E/E} small intestine (Figure 4.17 B). However, the deficiency of AhRR alone had no effect on the intestinal tuft cell frequency. In line with previous data of our group, AhRR-EGFP expression was restricted to the LP of both AhRR reporter mice, but with a brighter EGFP signal in the TripleCyp^{-/-}AhRR^{E/E} small intestine (Figure 4.16 and 4.17).

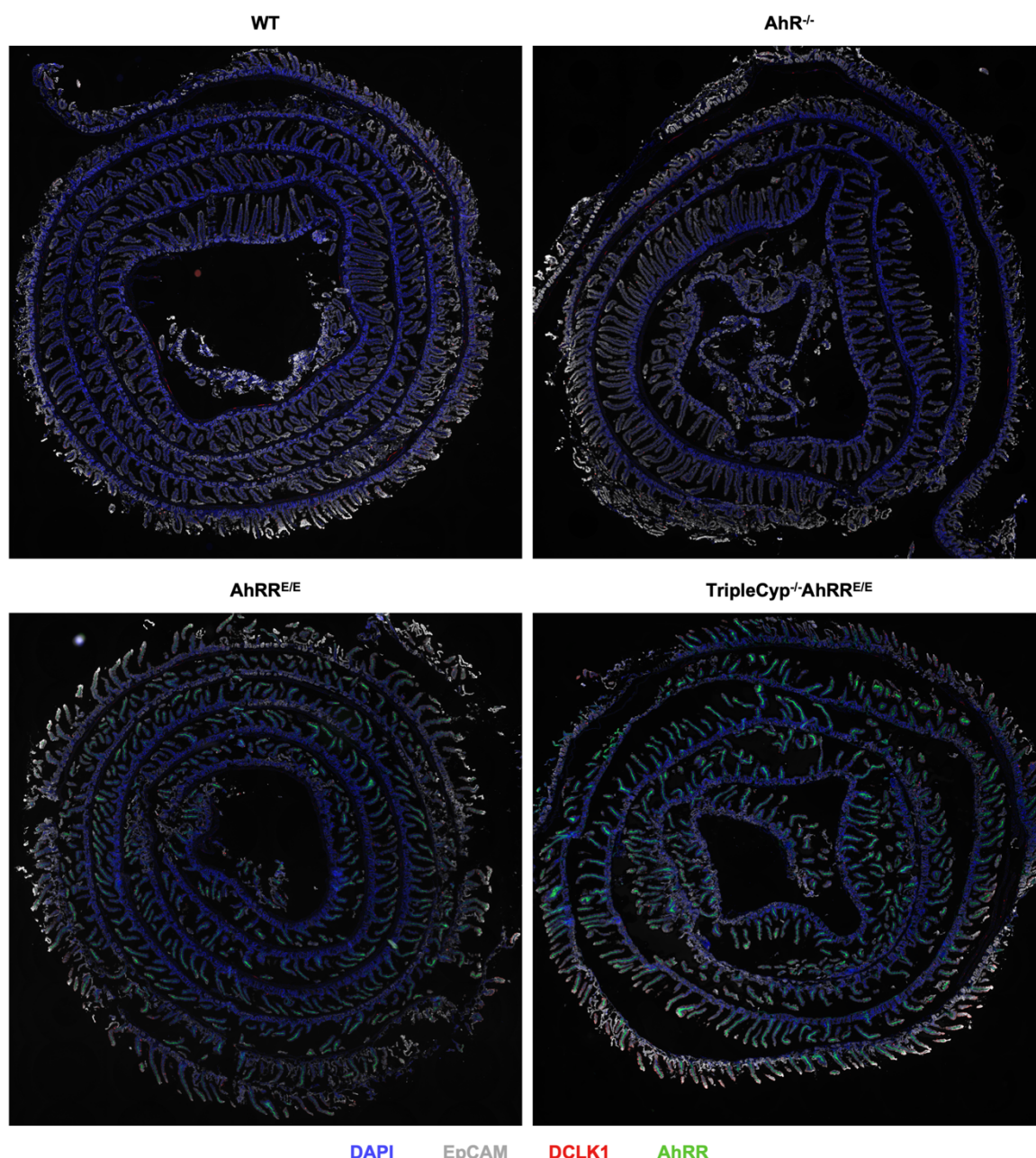


Figure 4.16 | DCLK1 marks tuft cells in the small intestine of WT, *AhR*^{-/-}, *AhRR*^{E/E} and *TripleCyp*^{-/-}*AhRR*^{E/E} mice

Representative stitched images of swiss-roll preparations of the small intestine from WT, *AhR*^{-/-}, *AhRR*^{E/E}, and *TripleCyp*^{-/-}*AhRR*^{E/E} mice stained for DCLK1 (red) and EpCAM (grey). EGFP reporter signal indicated *AhRR* (green) expression. Nuclei were counterstained with DAPI (blue).

In addition to the immunofluorescence staining, *ex vivo* analysis of the small intestine from naive WT, *AhR*^{-/-}, *AhRR*^{E/E}, and *TripleCyp*^{-/-}*AhRR*^{E/E} mice was performed via flow cytometry to examine tuft cells as well as ILC2 populations in more detail. Thus, the following gating strategy was applied to analyze tuft cells in the intestinal epithelial cell suspension (Figure 4.18): Among living single cells, IECs were gated as CD45⁻ EpCAM⁺ cells and tuft cells were further identified by co-expression of CD24 and

Siglec-F. Moreover, *AhRR*-EGFP expression by IECs and tuft cells was checked in *AhRR* reporter mice. Additionally, some of the samples were fixed, permeabilized, and intracellularly stained for DCLK1 to further mark tuft cells. To examine intestinal ILC2s the gating strategy was set as follows (Figure 4.19): Living CD45⁺ immune cells were identified among single cells of the fixed and permeabilized LP cell fraction. Lineage⁻ (CD19, CD11b, CD11c, F4/80, Siglec-F, NK1.1, Ly6G) and CD3⁻ cells were analyzed for co-expression of KLRG1 and the transcription factor GATA-3 to identify ILC2s. Furthermore, the expression of *AhRR*-EGFP and the proliferation marker Ki-67 by ILC2s was assessed.

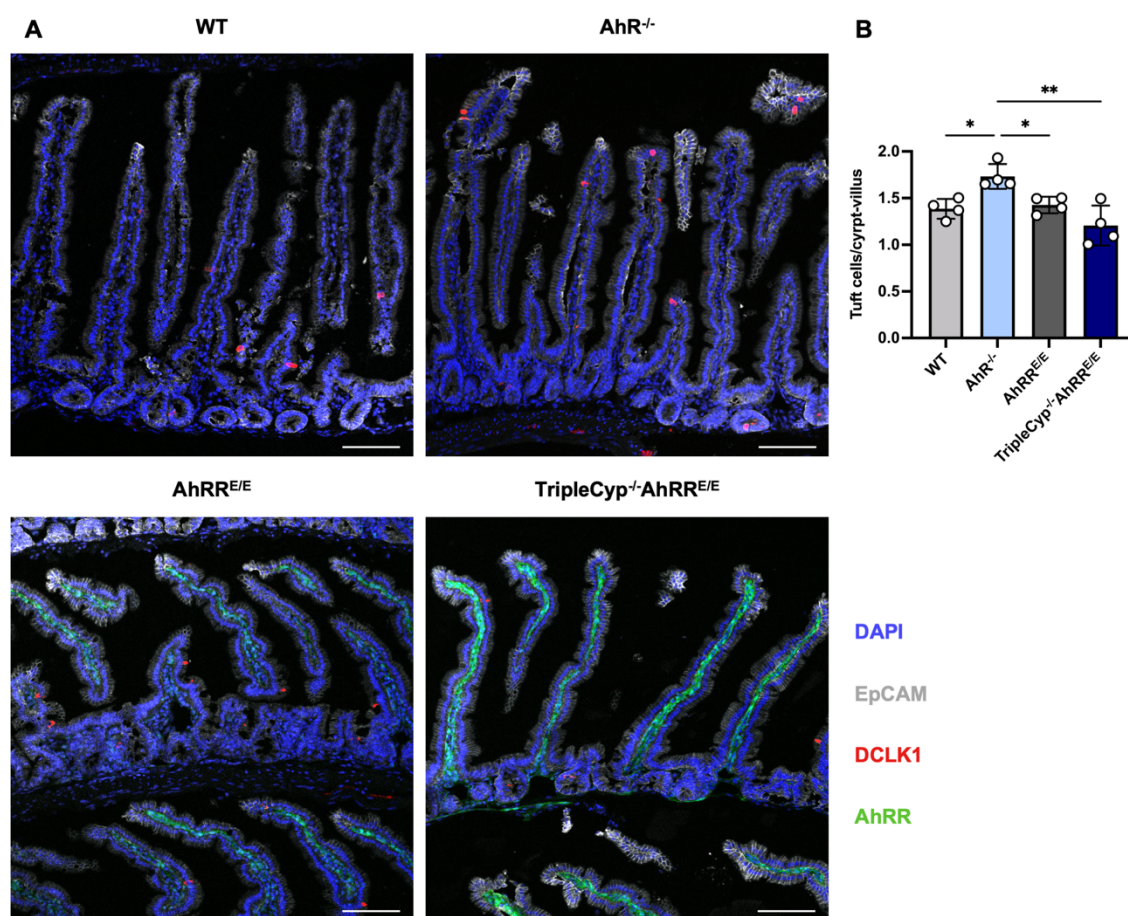


Figure 4.17 | Intestinal tuft cell numbers are altered by genetic modulation of AhR signaling

(A) Representative images of the small intestine from WT, *AhR*^{-/-}, *AhRR*^{E/E}, and *TripleCyp*^{-/-}*AhRR*^{E/E} mice stained for DCLK1 (red) and EpCAM (grey). EGFP reporter signal indicated *AhRR* (green) expression. Nuclei were counterstained with DAPI (blue). Scale bar = 100 μm. (B) Quantification of tuft cell numbers per crypt-villus axis. Three sections à four images per mouse were evaluated. Data are represented as mean ± SD (n = 4). Statistical significance was tested using one-way ANOVA with Tukey multiple-comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

In line with the histological quantification, the frequency of CD24⁺Siglec-F⁺ tuft cells was significantly increased to about 0.45 % of IECs in *AhR*^{-/-} mice compared to 0.33 %

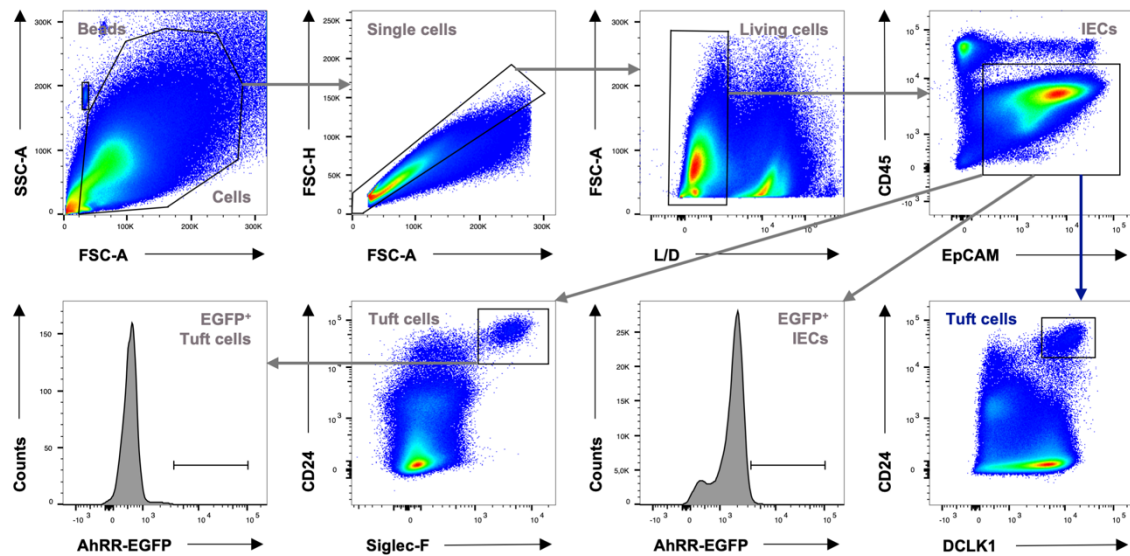


Figure 4.18 | Gating strategy for ex vivo analysis of small intestinal tuft cells

Living single cells of intestinal epithelial cell fraction were gated on $CD45^-EpCAM^+$ IECs. Cells were then checked for EGFP expression to identify *AhRR*-expressing IECs. IECs were further analyzed for tuft cells based on CD24 and Siglec-F expression. Moreover, *AhRR*-EGFP expression by tuft cells was assessed. Additional samples were fixed and intracellularly stained for DCLK1 to identify tuft cells (blue arrow).

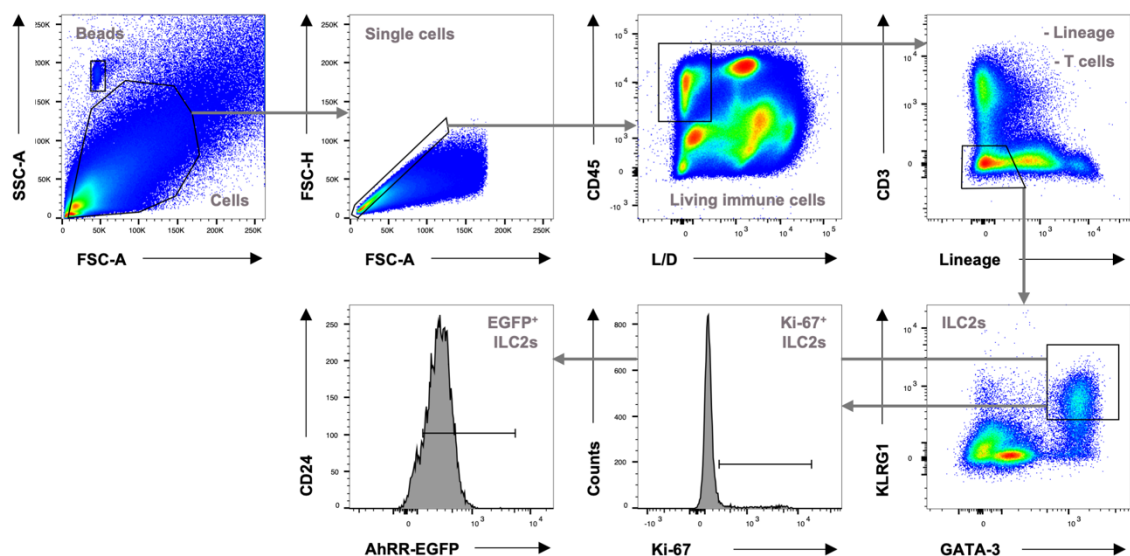


Figure 4.19 | Gating strategy for ex vivo analysis of small intestinal ILC2s

Living single cells of fixed intestinal LP cell fraction were gated on $CD45^+$ immune cells. Cells expressing lineage markers (CD3, CD19, CD11b, CD11c, F4/80, Siglec-F, NK1.1, Ly6G) were further excluded. ILC2s were identified by intracellular expression of GATA-3 and KLRG1. Moreover, expression of proliferation marker Ki-67 and *AhRR*-EGFP reporter signal by ILC2s were assessed.

of IECs in WT mice (Figure 4.20 A, B). Whereas the deletion of AhR alone did not affect the tuft cell frequency, the additional loss of the CYP1 enzymes led to a reduction of tuft cells to about 0.2 % of IECs in TripleCyp^{-/-}AhR^{EE} mice. Even if absolute tuft cell counts were not significantly changed between all genotypes, they displayed a similar tendency as observed for the frequency of tuft cells (Figure 4.20 C).

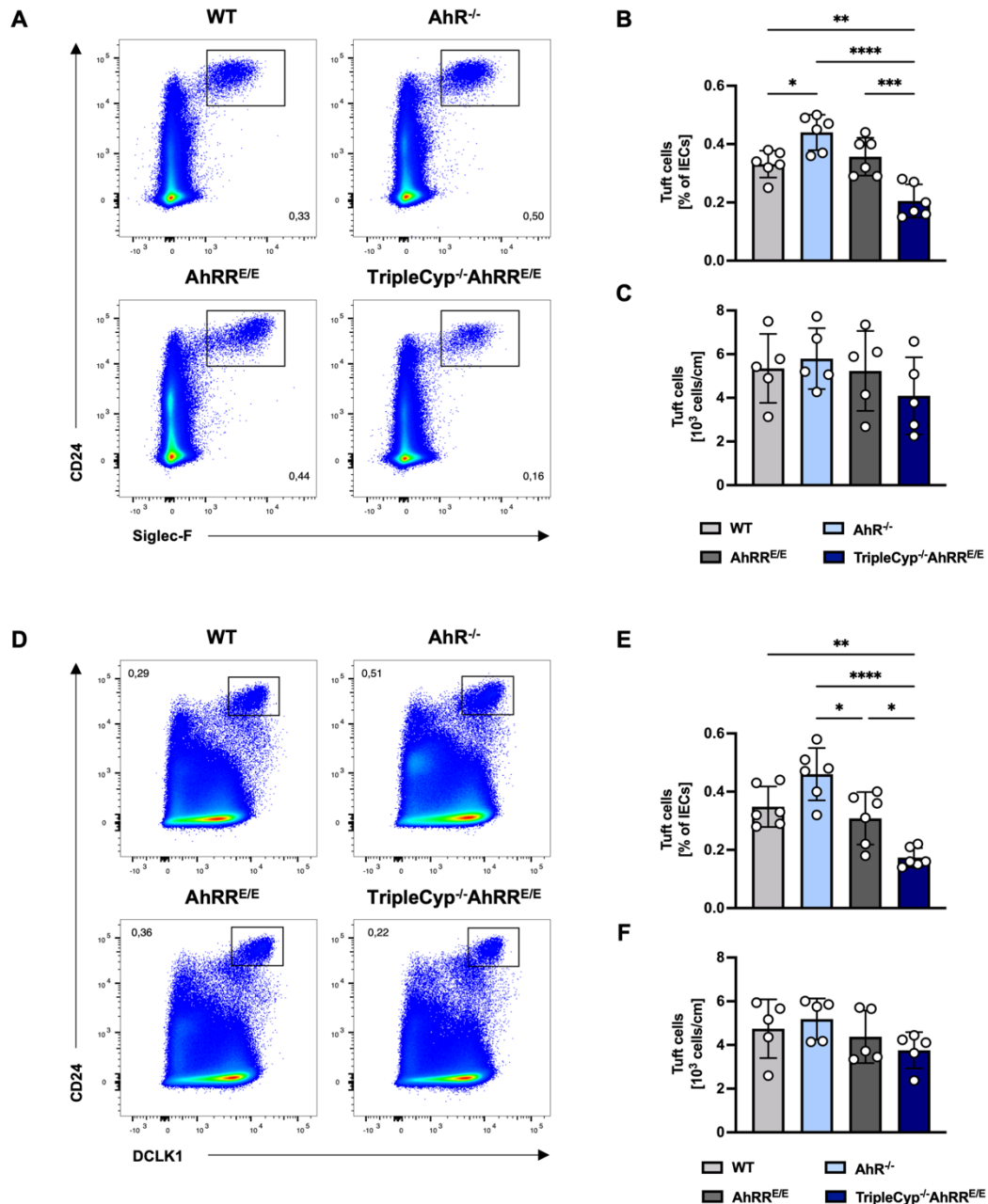


Figure 4.20 | Genetic ablation of AhR enhances whereas loss of CYP1 enzymes decreases the frequency of intestinal tuft cells

Representative flow cytometry plots (A), frequencies (B), and absolute cell counts (C) of CD24⁺Siglec-F⁺ tuft cells from the small intestine of WT, AhR^{-/-}, AhR^{RE/EE}, and TripleCyp^{-/-}AhR^{RE/EE} mice. Representative flow cytometry plots (D), frequencies (E), and absolute cell counts (F) of CD24⁺DCLK1⁺ tuft cells. Data are represented as mean ± SD (n = 5-6). Statistical significance was tested using one-way ANOVA with Tukey multiple-comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

In addition, comparable results were obtained for the intracellular tuft cell staining against DCLK1, indicating that the surface staining can be well used to define tuft cells (Figure 4.20 D-F). As epithelial tuft cells are known as the source of intestinal IL-25, which in turn activates ILC2s in the LP (Gerbe *et al*, 2016; Howitt *et al*, 2016; von Moltke *et al*, 2016), the previously described alterations in tuft cell numbers could influence the ILC2 population. Indeed, corresponding to elevated tuft cell numbers, the frequency and absolute cell count of ILC2s tended to be raised in $AhR^{-/-}$ mice, although these differences were not significant (Figure 4.21 A-C). Neither frequencies nor cell counts of ILC2s were altered in $AhRR^{E/E}$ and $TripleCyp^{-/-}AhRR^{E/E}$ mice compared to WT mice. Moreover, the proliferation of ILC2s measured by the percentage of Ki-67⁺ cells was not significantly different comparing all genotypes (Figure 4.21 D, E).

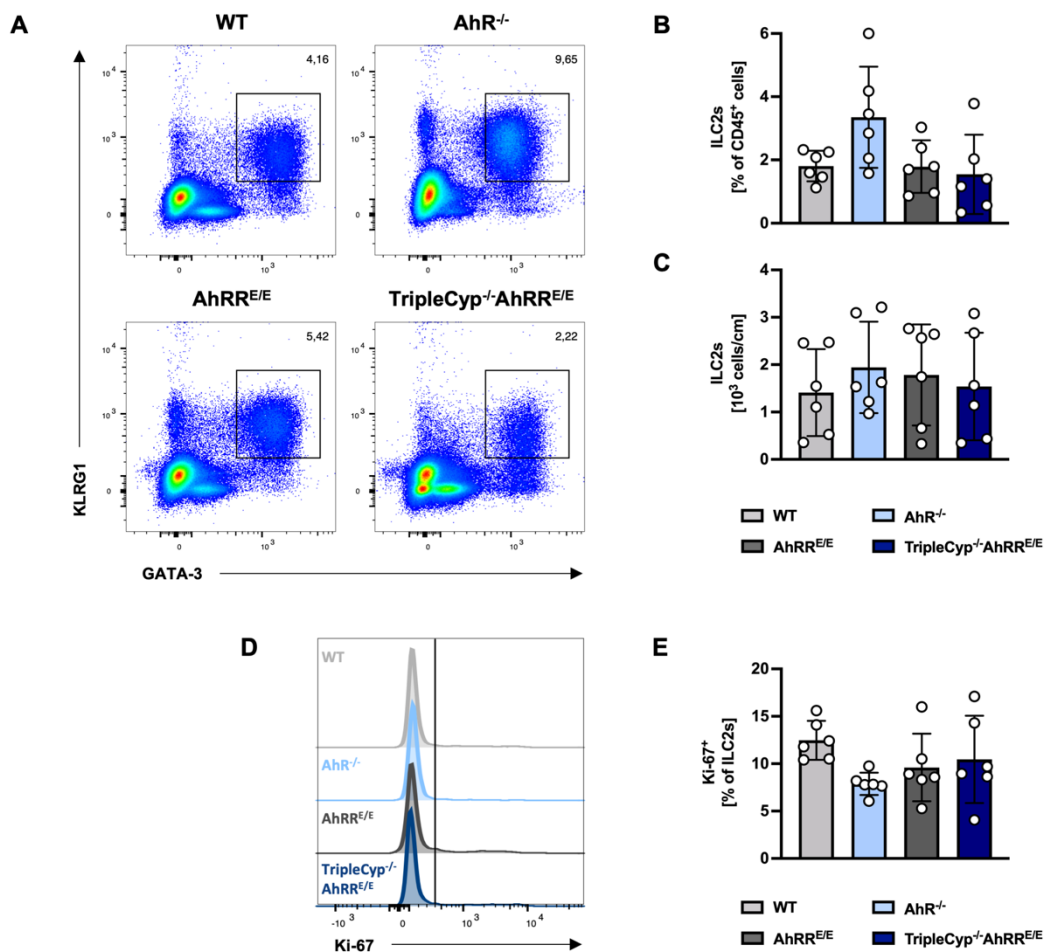


Figure 4.21 | Genetic modulation of AhR signaling does not significantly affect the frequency and proliferation of intestinal ILC2s

Representative flow cytometry plots (A), frequencies (B), and absolute cell counts (C) of KLRG1⁺ GATA-3⁺ ILC2s from the small intestine of WT, $AhR^{-/-}$, $AhRR^{E/E}$, and $TripleCyp^{-/-}AhRR^{E/E}$ mice. Representative histograms of Ki-67 expression by ILC2s (D) and frequencies of Ki-67⁺ ILC2s (E). Data are represented as mean $\pm$ SD (n = 6). Statistical significance was tested using one-way ANOVA with Tukey multiple-comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

As seen by the immunofluorescence analysis, flow cytometry also indicated that *AhRR*-EGFP was barely expressed by IECs of both *AhRR* reporter mice with just about 1 % and 2 % EGFP⁺ IECs in *AhRR*^{E/E} and *TripleCyp*^{-/-}*AhRR*^{E/E} mice, respectively (Figure 4.22 A, D). Tuft cells even showed a lower frequency of EGFP⁺ cells than the total IEC population in *AhRR*^{E/E} and *TripleCyp*^{-/-}*AhRR*^{E/E} mice (Figure 4.22 B, E). In contrast to epithelial cell types, ILC2s isolated from LP displayed a high *AhRR*-EGFP expression in both *AhRR* reporter mouse lines (Figure 4.22 C, F). Of note, the frequency of EGFP⁺ ILC2s (about 75 %) was significantly enhanced in *TripleCyp*^{-/-}*AhRR*^{E/E} mice compared to about 50 % EGFP⁺ ILC2s in *AhRR*^{E/E} mice.

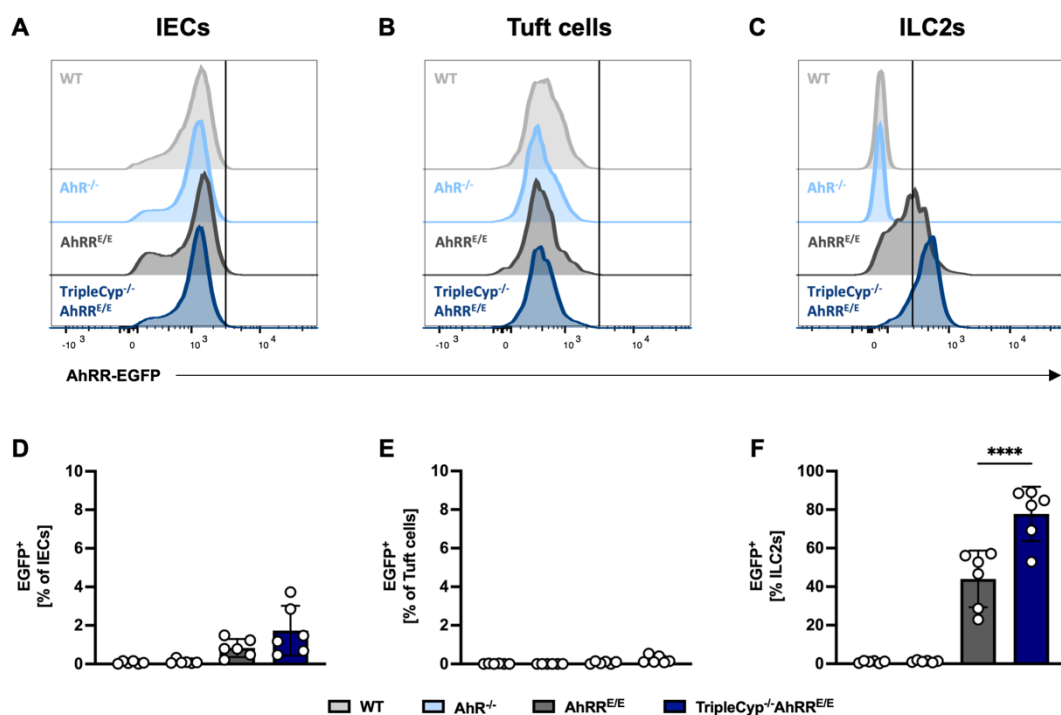


Figure 4.22 | *Ex vivo* isolated IECs including tuft cells barely express *AhRR*-EGFP

Representative histograms of *AhRR*-EGFP expression by IECs (A), tuft cells (B), and ILC2s (C) from the small intestine of WT, *AhR*^{-/-}, *AhRR*^{E/E}, and *TripleCyp*^{-/-}*AhRR*^{E/E} mice. Frequencies of *AhRR*-EGFP⁺ IECs (D), tuft cells (E), and ILC2s (F). Data are represented as mean ± SD (n = 6). Statistical significance was tested using one-way ANOVA with Tukey multiple-comparison test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

To sum up, in steady state, the deficiency of AhR in mice enhanced the frequency of intestinal tuft cells. In contrast, an amplification of AhR signaling by the deletion of *AhRR* alone had no effect, whereas the additional loss of the CYP1 enzymes decreased the tuft cell numbers. In line with previous *in vivo* data of our group as well as the co-culture results, the *AhRR* was highly expressed by LP cells and barely expressed by IECs including tuft cells.

4.2.5. Loss of AhR in mice impairs the succinate-induced activation of the intestinal tuft cell-ILC2 immune circuit

Intestinal tuft cells act as sentinels for helminths and microbial metabolites such as succinate to rapidly induce a type 2 immune response. Therefore, they express a variety of apical receptors including taste receptors and the succinate receptor 1 to react to different luminal stimuli (Lei *et al*, 2018; Nadjisombati *et al*, 2018; Schneider *et al*, 2018; Luo *et al*, 2019). To study the role of the AhR in the intestinal tuft cell-ILC2 immune circuit activation *in vivo*, WT and AhR^{-/-} mice were provided with succinate in drinking water. While ILC2s in the LP are rapidly activated by tuft cell-derived IL-25, the subsequent IL-13-mediated tuft expansion requires a few days, as the intestinal epithelium undergoes turnover every 4-5 days (O'Leary *et al*, 2021; Arike *et al*, 2020). Therefore, the small intestine of WT and AhR^{-/-} mice were analyzed after administration of control or succinate-supplemented drinking water for 7 days. Tuft cells in the intestinal epithelium were identified by immunofluorescence staining for DCLK1 (Figure 4.23). Whereas IECs were marked by the expression of EpCAM, nuclei were counterstained with DAPI. Of note, succinate treatment clearly resulted in tuft cell hyperplasia in WT mice, whereas this succinate-mediated tuft cell expansion in the epithelium was clearly reduced upon deletion of AhR.

For additional quantification by flow cytometry, tuft cell as well as ILC2 populations of control and succinate-treated WT and AhR^{-/-} mice were examined using the gating strategies described above (Figure 4.18 and 4.19). Therefore, tuft cells were again identified by surface staining for CD24 and Siglec-F as well as by intracellular expression of DCLK1. According to the immunofluorescence staining, the frequency of tuft cells markedly raised from about 0.3 % to nearly 4 % of IECs in WT mice upon succinate supplementation (Figure 4.24 A, B, D, E). Of note, the tuft cell percentage was not changed in AhR^{-/-} mice, indicating a significant inhibition of succinate-induced tuft cell expansion upon loss of AhR in mice. Similarly, absolute tuft cell counts were strongly elevated only in WT and not in AhR^{-/-} mice after succinate treatment (Figure 4.24 C, F).

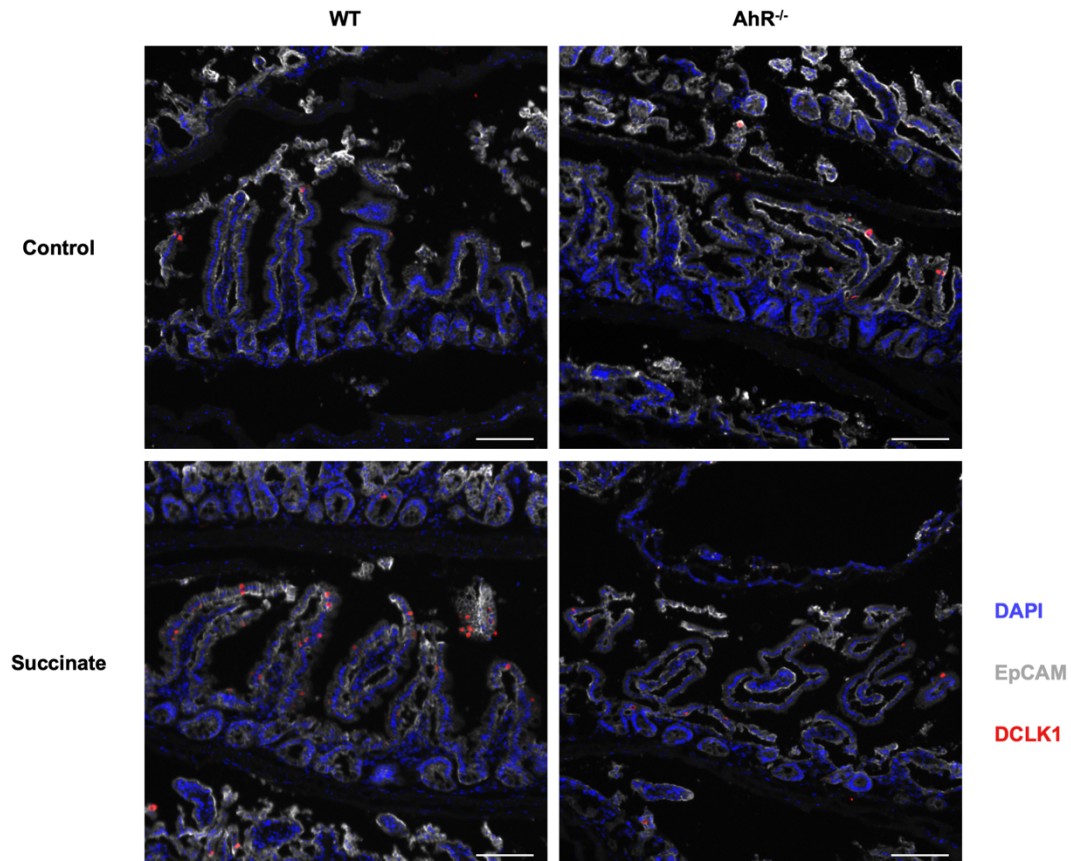


Figure 4.23 | Tuft cell expansion upon succinate treatment is limited in AhR^{-/-} mice

WT and AhR^{-/-} mice were treated with 100 mM succinate or regular drinking water, and the small intestine was analyzed on day 7. Representative images of the small intestine from WT and AhR^{-/-} mice upon succinate treatment stained for DCLK1 (red) and EpCAM (grey). Nuclei were counterstained with DAPI (blue). Scale bar = 100 μ m.

The increased tuft cell numbers upon succinate supplementation *in vivo* could be associated with enhanced IL-13 secretion by activated ILC2s. Therefore, the frequency and the proliferation of ILC2s was checked. In line with the alterations seen in tuft cell numbers, the frequency of ILC2s in WT mice was clearly increased to about 15 % of immune cells in the succinate group compared to about 5 % in controls (Figure 4.25 A, B). In contrast, AhR^{-/-} mice displayed significantly lower ILC2 frequencies compared to WT mice upon succinate administration. Even if absolute ILC2 counts were not significantly changed between all conditions, they displayed a similar tendency as observed for their frequencies (Figure 4.25 C). Moreover, the proliferation of ILC2s shown by the percentage of Ki-67⁺ cells was clearly enhanced only in WT mice, but not in AhR^{-/-} mice after succinate treatment (Figure 4.25 D, E).

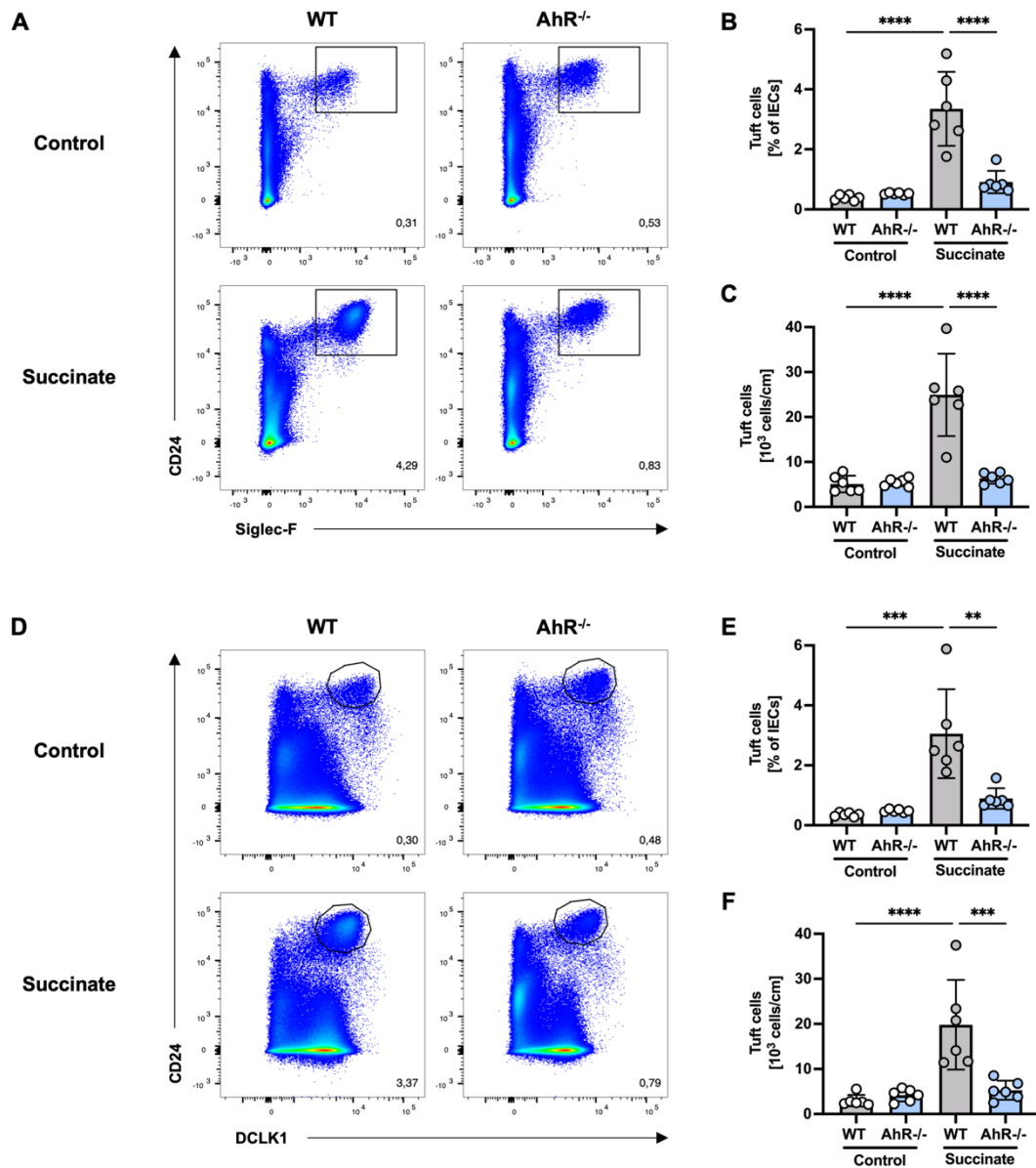


Figure 4.24 | AhR^{-/-} mice show impaired succinate-induced tuft cell expansion

Representative flow cytometry plots (A), frequencies (B), and absolute cell counts (C) of CD24⁺ Siglec-F⁺ tuft cells from the small intestine of WT and AhR^{-/-} mice upon succinate treatment. Representative flow cytometry plots (D), frequencies (E), and absolute cell counts (F) of CD24⁺DCLK1⁺ tuft cells. Data are represented as mean $\pm$ SD (n = 6). Statistical significance was tested using two-way ANOVA with Tukey multiple-comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

In summary, the activation of the intestinal tuft cell-ILC2 immune circuit after succinate treatment was significantly restrained by the deletion of AhR in mice seen by a lack of tuft cell hyperplasia and ILC2 proliferation. This indicates that AhR signaling might be of great importance for a proper type 2 immune response to intestinal helminth infections *in vivo*.

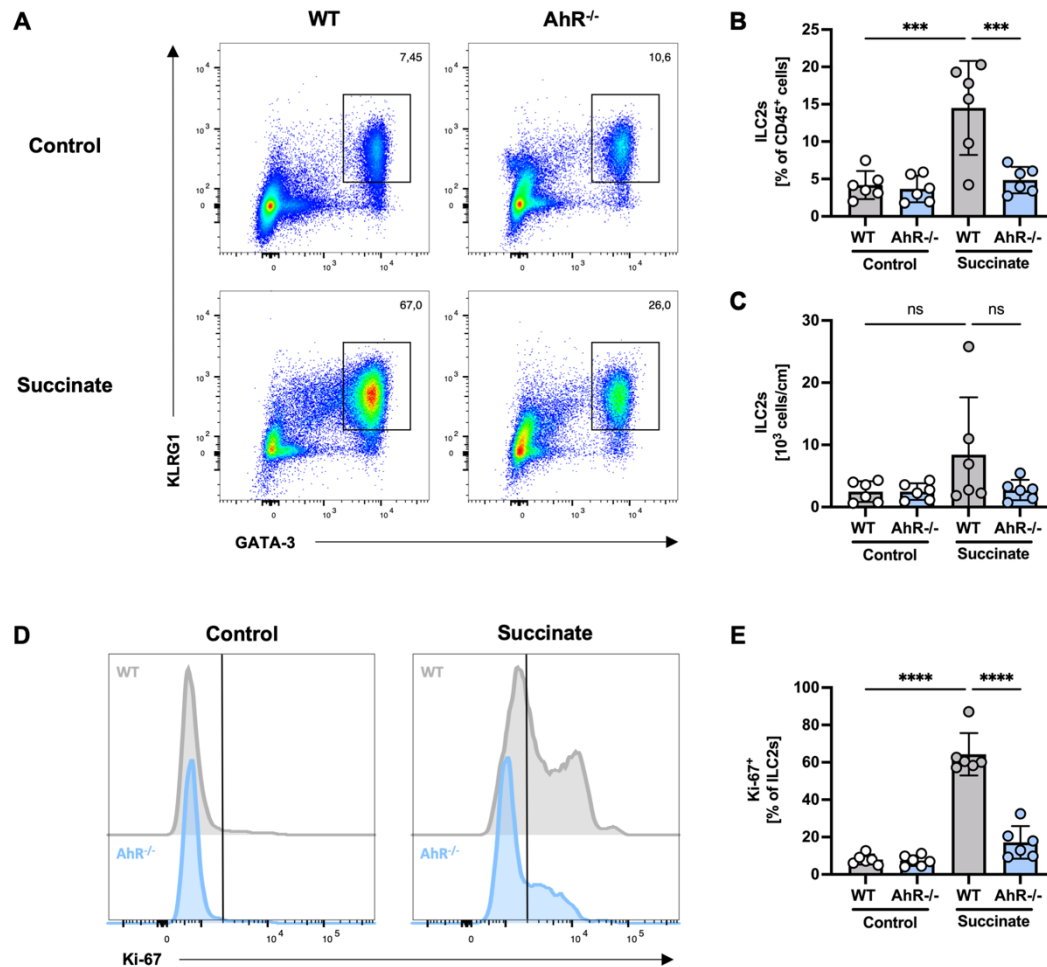


Figure 4.25 | Reduced ILC2 proliferation in AhR^{-/-} mice upon succinate treatment

Representative flow cytometry plots (A), frequencies (B), and absolute cell counts (C) of KLRG1⁺ GATA-3⁺ ILC2s from the small intestine of WT and AhR^{-/-} mice upon succinate treatment. Representative histograms of Ki-67 expression by ILC2s (D) and frequencies of Ki-67⁺ ILC2s. Data are represented as mean ± SD (n = 6). Statistical significance was tested using two-way ANOVA with Tukey multiple-comparison test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

5. Discussion

After being extensively studied as an environmental sensor for xenobiotics, the role of the AhR as a receptor for dietary compounds and endogenous ligands derived from host or microbial metabolism has been refined in the past years. As a result, more and more physiological functions of AhR in a variety of cellular processes such as differentiation, cell signaling, metabolism, inflammation, and immune regulation have moved into focus (Tripathi & Lee, 2020). For instance, the AhR has been shown to influence the development of immune cells and to shape the host response to infections with certain microbial pathogens (Stockinger *et al*, 2014; Gutiérrez-Vázquez & Quintana, 2018). Hence, a reduced resistance of AhR^{-/-} mice to acute bacterial infections with *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, and *Listeria monocytogenes* was demonstrated (Moura-Alves *et al*, 2014; Shi *et al*, 2007; Kimura *et al*, 2014). Similarly, AhR deficiency in mice increased the susceptibility to parasitic infection with *Cryptosporidium tyzzeri*, displayed by an elevated fecal parasite burden compared to WT mice (Maradana *et al*, 2023). In contrast, AhR deficiency in mice improved the host response to *Heligmosomoides polygerus bakeri* infection, a common gastrointestinal helminth of rodents (Li *et al*, 2018). Hence, the impact of the AhR on the disease outcome strongly depends on the infection model that is examined.

Therefore, further research is necessary to gain new insights into the role of the AhR in type 1 and type 2 immunity to infections. This thesis investigated how modulation of AhR signaling in mice affected on the one hand the host response to chronic *Salmonella* Typhimurium infection and on the other hand the tuft cell-mediated intestinal immunity to helminths. Moreover, the selective expression pattern of its repressor AhRR was studied in the small intestine *in vivo* in comparison to mouse intestinal organoids as *in vitro* model to further understand the regulation of AhR signaling in intestinal homeostasis.

5.1. AhR deficiency in mice results in unrestrained EME and elevated susceptibility to STM infection

The following chapter is adapted from Mayer et al., 2024 (Mayer M, Cengiz-Dartenne SC,(...), Förster I, Weighardt H. Dysregulation of stress erythropoiesis and enhanced susceptibility to Salmonella Typhimurium infection in aryl hydrocarbon receptor-deficient mice. The Journal of Infectious Diseases. 2024 Jun 6:jiae304.)

Here, we demonstrated that AhR^{-/-} mice are highly susceptible in a model of chronic systemic infection with the attenuated STM strain TAS2010, a model for typhoid fever in mice. Whereas WT mice were able to clear the infection after 5 weeks, we observed reduced bacterial clearance in spleen and liver in line with increased lethality in AhR^{-/-} mice. In addition, AhR^{-/-} mice showed an infection-induced anemia and, in turn, a significant increase in splenic EME with a major expansion of immature RBCs, possibly driven by increased serum levels of IL-6 and EPO. Furthermore, enhanced stress erythropoiesis was accompanied by a long-lasting remodeling of the splenic architecture, which could add to the diminished host defense in AhR^{-/-} mice.

Systemic STM infections affect the structure and function of the spleen and are characterized by splenomegaly and dynamic changes in the splenic architecture with a reduced area of white pulp and poorly defined T and B cell zones (Jackson *et al*, 2010; Rosche *et al*, 2015; Marcial-Juárez *et al*, 2023; Hyland *et al*, 2005). Especially in AhR^{-/-} mice, the white pulp areas were smaller and displayed altered B and T cell compartments. Also, the marginal zone appeared to be disrupted with a decrease in MZMΦs and MMMΦs, which remained stronger and prolonged in AhR^{-/-} mice. Disruption of splenic niches might be due to infection-induced recruitment of monocytes or expansion of remaining tissue-resident macrophages (Rosche *et al*, 2015; Marcial-Juárez *et al*, 2023; Chen *et al*, 2020). Furthermore, STM infection affects the splenic network of follicular dendritic cells and T follicular helper cells, which contribute to the destruction of splenic structure (Marcial-Juárez *et al*, 2023; Lee *et al*, 2011). This effect, however, is reversible, and the splenic organization reestablishes with resolution of the infection (Rosche *et al*, 2015; Marcial-Juárez *et al*, 2023). Whereas this occurred in WT mice 5 weeks after infection, the absence of the AhR did not allow a restoration of the splenic structure, which might contribute to a long-lasting disturbance of the immune response after STM infection.

Moreover, AhR^{-/-} mice developed a more profound macrocytic anemia upon TAS2010 infection when compared with WT mice, accompanied by a reduced bone marrow erythropoiesis, indicative of an anemia of infection (Gomes & Gomes, 2016). As a consequence, more immature CD71⁺ RBC precursors populate the spleen particularly in AhR^{-/-} mice, which is known to occur in infection-induced EME (Jackson *et al*, 2010; Rosche *et al*, 2015; Hyland *et al*, 2005). This expansion of RBC precursors largely contributed to the splenomegaly and could be involved in the long-term deterioration

of the splenic architecture. Naive AhR-deficient mice already showed signs of splenomegaly, altered hematologic features such as reduced RBC numbers and prolonged extramedullary hematopoiesis (Schmidt *et al*, 1996; Singh *et al*, 2011), as well as enhanced hematopoietic stem cell numbers and proliferation (Singh *et al*, 2011; Vaughan *et al*, 2021), indicating that AhR-deficient mice might be more prone to infection-induced splenomegaly, anemia, and EME. EME in peripheral organs such as spleen or liver is initiated by SEPs, which originate from bone marrow hematopoietic stem cells and populate peripheral organs already under steady state conditions (Paulson *et al*, 2020). In the spleen, these SEP1s get committed to the erythroid lineage by bone morphogenetic protein 4 and hedgehog signals and expand to SEP2s stem cell factor dependently to provide a pool of progenitors (Paulson *et al*, 2020; Perry *et al*, 2007). Following infection, hypoxia and EPO levels rise and induce terminal differentiation of SEPs to erythrocytes. Of note, we found enhanced numbers of immature SEP1s already in uninfected AhR^{-/-} mice as compared with WT mice. Also, SEP2s were enhanced after TAS2010 infection with a higher frequency in AhR^{-/-} mice, which led to an increase of all other erythroid precursor populations. In addition, the production of EPO, which induces the terminal differentiation of erythroid precursors via EPO receptor signaling in macrophages (Paulson *et al*, 2020; Chen *et al*, 2020; Millot *et al*, 2010), was significantly enhanced in STM-infected AhR^{-/-} mice. In line, numbers of splenic macrophages were increased in AhR^{-/-} mice after TAS2010 infection.

EPO production is further driven by HIF1- α , a transcription factor induced by hypoxia, which leads to the development of EME in an ARNT-dependent manner (Haase, 2013). ARNT is the shared cofactor of AhR and HIF1- α , enabling them to bind to their respective response elements. Thus, AhR and HIF1- α signaling is interconnected such that absence of AhR leads to enhanced HIF1- α signaling (Vorrink & Domann, 2014), which could explain the increased EPO levels in AhR^{-/-} mice. Since it was demonstrated that neutralization of EPO reduced the expansion of immature RBCs and bacterial burden in the spleen upon systemic STM infection (Jackson *et al*, 2010), elevated EPO levels probably contributed to increased susceptibility of AhR^{-/-} mice. Additionally, IL-6 directly mediates infection-derived anemia by induction of hepcidin, which triggers the degradation of the iron-exporter ferroportin, thus reducing iron levels for hemoglobin synthesis (Gardenghi *et al*, 2014). IL-6 is additionally able to block the development of late-stage erythroid precursors (Prince *et al*, 2012; McCranor *et al*,

2014). Thus, enhanced IL-6 serum levels in AhR^{-/-} mice could further decrease the formation of mature erythrocytes. Moreover, IFN- γ was shown to reduce the erythrocyte life span (Libregts *et al*, 2011). Therefore, elevated IL-6 and IFN- γ serum levels might promote infection-induced anemia and are likely to affect the AhR-dependent EME response and, in turn, splenic remodeling upon STM infection.

Taken together, AhR deficiency renders mice highly susceptible to infection with attenuated STM, resulting in increased bacterial burden and lethality. AhR^{-/-} mice showed enhanced macrocytic anemia after infection, which was counteracted with elevated EME, leading to a stronger and prolonged production of RBC precursors in the spleen. This may contribute to a prolonged infection-induced disruption of the splenic architecture, which might be responsible for the disturbed host defense.

5.2. Modulation of AhR signaling affects tuft cell-mediated immunity in the mouse small intestine

Numerous studies in recent years unveiled that tuft cells in the intestinal epithelium are sentinels, which monitor the intestinal content by using succinate as well as bitter and sweet taste receptors to initiate a mucosal type 2 immunity against parasites (Feng *et al*, 2024b). Since AhR signaling has been shown to be important for the maintenance of intestinal homeostasis and protection against intestinal infections (Lamas *et al*, 2018; Stockinger *et al*, 2021; Wiggins *et al*, 2023), this thesis investigated the role of the AhR in tuft-induced intestinal immune responses.

5.2.1. Influence of AhR signaling on tuft cell differentiation under homeostatic conditions

This study revealed that modulation of AhR signaling in mice has an impact on the differentiation of ISCs into intestinal epithelial tuft cells under steady state conditions (Figure 4.17 and 4.20). Here, the lack of AhR elevated the frequency of tuft cells in the small intestinal epithelium. In contrast, the absence of AhRR had no effect, whereas an increase of AhR activity by the additional loss of all three AhR-dependent CYP1 enzymes reduced intestinal tuft cell numbers in TripleCyp^{-/-}AhRR^{E/E} mice. As tuft cell-derived IL-25 activates ILC2s in the LP, in line with increased tuft cell numbers also the frequency of ILC2s tended to be raised in AhR^{-/-} mice (Figure 4.21 A-C).

The cell fate determination of stem cells in the intestine is tightly controlled by Notch signaling, which regulates the balance of *Hes1* and *Atoh1* expression (Noah & Shroyer, 2013). Whereas low Notch activity in progenitor cells favors the ATOH1-dependent differentiation into secretory lineages, high Notch activity indirectly represses ATOH1 by its negative regulator HES1 to direct progenitor cell fate towards absorptive enterocytes. Previous studies have identified *Atoh1* as a direct target gene of AhR, regulating *Atoh1* expression in a XRE-dependent manner (Yagishita *et al*, 2023; Zhou *et al*, 2023). Thus, the genetic ablation of AhR in mice restricts the commitment into secretory goblet cells and Paneth cells (Zhou *et al*, 2023). In contrast, dietary AhR ligands such as I3C and ICZ induce *Atoh1* expression but reduce *Hes1* expression by suppressing Notch signaling (Park *et al*, 2018; Zhou *et al*, 2023). Hence, the exposure to AhR ligands promotes the differentiation of goblet and Paneth cells, thereby increasing the production of mucins and lysozyme 1. Similarly, overexpression of IDO1 in the intestinal epithelium leads to an expansion of secretory cells (Alvarado *et al*, 2019). Of note, this effect is independent of the kynurenine pathway but mainly caused by non-enzymatic interaction of IDO1 with AhR to inhibit Notch signaling. As a result, a high epithelial IDO1 expression expands the intestinal mucus layer, modulates the mucus-associated microbiota and reduces the disease severity in mouse models of colitis and enteropathogenic *Escherichia coli* infection. In line with the literature, we found elevated frequencies of secretory Paneth cells and EECs in intestinal organoids of WT and AhRR^{E/E} mice upon stimulation with the AhR ligand 3MC (Figure 4.12).

Tuft cells have been defined as the fourth secretory cell type in the intestinal epithelium and can also be expanded by Notch blockage (Gerbe *et al*, 2011; VanDussen *et al*, 2012). Besides the influence of Notch signaling, our results indicated an inhibitory effect of AhR activation on the differentiation of tuft cells contrary to the other secretory lineages. Moreover, neither AhR activation nor AhR inhibition impacted *Atoh1* expression by tuft cells in intestinal organoids derived from *Atoh1* reporter mice (Figure 4.14 C, D). Two conflicting studies have investigated the dependence of *Atoh1* during tuft cell specification: Gerbe *et al*. demonstrated that ATOH1 is essential for the differentiation of tuft cells, because tuft cells are absent in *Atoh1*-deficient mice (Gerbe *et al*, 2011). Moreover, other key transcription factors for EECs, goblet cell, and Paneth cell formation such as *Neurog3*, *Gfi1*, *Spdef*, and *Sox9* are dispensable for tuft cell specification. In contrast, Bjerknes *et al*. reported that tuft cells can differentiate independently of ATOH1 and are even increased in the intestinal epithelium upon

Atoh1 deletion (Bjerknes *et al*, 2012). However, they used different tamoxifen-inducible Cre mouse lines to induce *Atoh1* deletion as well as variant tamoxifen doses and administration routes. Thus, divergent results of these two studies might be explained by artifacts caused via Cre-induced intestinal toxicity through high tamoxifen concentrations (Bjerknes *et al*, 2012). Recently, more and more studies have illustrated that tuft cells in the small intestine can emerge from an *Atoh1*-independent origin. Single-cell RNAseq analysis demonstrated that tuft cells strongly differ from the *Atoh1*-dependent secretory lineage including goblet cells and Paneth cells and even display a common trajectory with enterocytes (Herring *et al*, 2018).

Furthermore, the discrepancies in earlier studies can possibly be explained by secondary effects of the microbiome. Here, Paneth cell depletion due to the absence of *Atoh1* or *Sox9* results in a dysbiosis of the gut microbiota with an expansion of succinate-producing species, thereby driving an *Atoh1*-independent tuft cell formation via IL-13/IL-4R α signaling (Banerjee *et al*, 2020; Coutry *et al*, 2023). In the study by Coutry *et al*. SUCNR1-mediated tuft cell activation in response to endogenous microbiota does not depend on TRPM5 signaling. This observation is in contrast to previous publications, elucidating that TRPM5 is indispensable for tuft cell signaling via the SUCNR1 upon intestinal parasite infection or succinate treatment (Schneider *et al*, 2018; Lei *et al*, 2018; Nadsombati *et al*, 2018). Interestingly, commensal bacteria of the order *Bacteroidales*, which are described as succinate producers, are enriched by dysfunctional Paneth cells (Coutry *et al*, 2023). *Bacteroidales* belong to the phylum *Bacteroidetes*, which is shown to be slightly increased in the cecal microbiota of AhR^{-/-} mice (Murray *et al*, 2016). Another study demonstrated that different *Tritrachomonas* species as part of the commensal protists favor distinct nutritional niches and differ in the production of succinate, thereby shaping type 2 immunity and the competition with commensal bacteria. This illustrates how diet could directly influence the microbial composition and in turn the intestinal immunity (Gerrick *et al*, 2024). In summary, elevated frequencies of tuft cells and ILC2s in naive AhR^{-/-} mice might indicate a mild succinate-induced activation of the tuft cell-ILC2 circuit due to an altered microbiome caused by Paneth cell alteration upon AhR deficiency.

Although the IL-25 secretion by tuft cells strongly depends on the Ca²⁺-triggered TRPM5 activation, two studies recently described that tuft cells express constant *Il25* mRNA levels and already secrete low amounts of IL-25 without proper stimulation

(McGinty *et al*, 2020; Feng *et al*, 2024a). This homeostatic IL-25 release promotes a basal IL-13 secretion by ILC2s via IL-17RB signaling, potentially keeping ILC2s in a primed state. According to a preprint, the expression of IL-17RB on tuft cells attenuates the own tonic IL-25 signal to enable a dynamic regulation of the tuft cell-ILC2 circuit and prevent hypoproliferation of ILC2s (Feng *et al*, 2024a). Since the AhR is further reported to inhibit intestinal ILC2 functions (Li *et al*, 2018), increased tuft cell numbers in AhR^{-/-} mice could also be traced back to slightly enhanced ILC2 numbers and thereby possibly elevated IL-13 secretion. Therefore, the analysis of Paneth cells, microbiota-derived succinate, and intestinal IL-13 levels could be essential to further understand how AhR signaling impacts the differentiation of tuft cells from epithelial progenitors under homeostatic conditions.

Of note, we observed different results in AhRR^{E/E} and TripleCyp^{-/-}AhRR^{E/E} mice (Figure 4.17 and 4.20). Whereas an enhanced AhR activity in TripleCyp^{-/-}AhRR^{E/E} mice seems to impair the development of tuft cells, AhRR^{E/E} mice did not show any phenotype compared to WT mice. This indicated that AhR signaling in the intestinal epithelium is more strongly regulated by negative feedback control via the three AhR-controlled CYP1 enzymes than the AhRR. Other than the AhR itself, which is ubiquitously expressed in various cell types throughout the body, the expression of AhRR is mainly restricted to immune cells, especially at barrier sites like the gut. Hence, previous data of our group demonstrated that IECs barely express the AhRR (Brandstätter *et al*, 2016), which might explain why the ablation of AhRR had no effect on the development of epithelial tuft cells. On the other hand, by using a *Cyp1a1* fate-reporter mouse that indicates AhR activity via an eYFP reporter signal, Schiering *et al*. reported that the genetic deletion of CYP1 enzymes retarded AhR ligand degradation, leading to an enhanced eYFP expression primarily in IECs. Conversely, constitutive *Cyp1a1* expression globally in the body as well as specifically in IECs decreases ligand availability and phenocopy AhR^{-/-} mice with regard to intestinal immunity. This implies that IECs serve as gatekeepers for the natural AhR ligand supply in the intestine by negative feedback regulation mainly via CYP1 enzyme activity, thereby also influencing the epithelial tuft cell differentiation.

5.2.2. Role of AhR signaling in tuft cell-ILC2 circuit activation

Once the intestinal tuft cell-ILC2 circuit is activated by colonization with protists or helminth infection, the expansion of tuft cells is induced via IL-13/IL-4R α /STAT6 signaling. Therefore, in a next step, we investigated the differentiation of tuft cells by IL-13 treatment in small intestinal organoids. In line with the *ex vivo* results under steady state, AhR activation by stimulation with AhR ligands dampened IL-13-mediated tuft cell expansion in organoids derived from WT, AhRR^{E/E}, and TripleCyp^{-/-} AhRR^{E/E} mice (Figure 4.7 and 4.9). Since IL-13-induced tuft cell formation was not impaired in AhR^{-/-} organoids, the observed effect upon stimulation with 3MC or FICZ is definitely AhR-dependent. In addition, we figured out that also in intestinal organoids the differentiation of tuft cells via IL-13/IL-4R α signaling does not depend on ATOH1, because *Atoh1* expression was significantly decreased after IL-13 treatment in tuft cells of *Atoh1* reporter organoids (Figure 4.14).

Gracz *et al.* demonstrated that SOX4 expression by progenitor cells is required to drive tuft cell hyperplasia independently of *Atoh1* in type 2 immune responses (Gracz *et al.*, 2018). IL-13 stimulation in control organoids induces both *Sox4* and *Dclk1* upregulation, whereas *Sox4*-deficient organoids fail to expand tuft cells. Similarly, mice with an IEC-specific deletion of *Sox4* show a limited expansion of tuft cells following helminth infection, accompanied by a reduced parasite clearance. In addition, the enzyme O-linked-N-acetylglucosaminylation (O-GlcNAc) transferase modifies STAT6 by O-GlcNAcylation to further promote the expression of *Pou2f3*, a key transcription factor for tuft cell specification (Zhao *et al.*, 2022). Interestingly, another study reported that IL-13 activates BMP signaling in progenitor cells in order to restrict tuft cell hyperplasia following helminth infection via an intrinsic negative feedback loop (Lindholm *et al.*, 2022). A blockage of the BMP type I receptor ALK2 by its inhibitor dorsomorphin homolog 1 (DMH1) disturbs this feedback loop and leads to an increased tuft cell expansion both *in vivo* after *N. brasiliensis* infection and *in vitro* after IL-13 treatment of intestinal organoids. After a rapid induction by IL-13, *Sox4* expression returns back to initial levels after 24 hours. However, this downregulation after 24 hours is absent with the addition of DMH1, leading to unrestrained tuft cell expansion. Of note, in another system AhR activation suppresses SOX4, hindering breast cancer metastasis (Hanieh, 2015). Thus, further experiments are required to

investigate if AhR activation impairs IL-13-mediated tuft cell hyperplasia in intestinal organoids by modulating SOX4 signaling.

Additionally, this thesis revealed that the *in vivo* tuft cell-ILC2 circuit activation by succinate is significantly suppressed in mice upon AhR deficiency. This was demonstrated by a complete lack of tuft cell expansion and ILC2 proliferation in the small intestine of AhR^{-/-} mice compared to WT mice after administration of succinate-supplemented drinking water for 7 days (Figure 4.23, 4.24 and 4.25). These results were in contrast to our *in vitro* data, where AhR activation had an inhibitory effect on tuft cell differentiation in intestinal organoids.

Multiple studies in the last decade have shown that tuft cells generate distinct effector molecules via specific intrinsic signaling pathways depending on the activating input signals (Feng *et al*, 2024b). One of the best-studied tuft cell agonists is luminal succinate, which is sensed by tuft cells upon colonization with *Tritrichomonas* protists. It is confirmed that succinate is detected by the SUCNR1 and triggers the release of IL-25 and ACh through the canonical taste signaling pathway via GNAT3, PLCβ2, and TRPM5 (Nadsombati *et al*, 2018; Howitt *et al*, 2020; Lei *et al*, 2018; Schneider *et al*, 2018). However, the production of CysLTs is not reported after tuft cell activation via the SUCNR1 (McGinty *et al*, 2020). Helminths, such as *N. brasiliensis*, *H. polygyrus*, and *T. spiralis*, activate tuft cells in a SUCNR1-independent manner by so far unknown receptors, resulting in the secretion of IL-25, ACh, and CysLTs (Feng *et al*, 2024b). Similar to the succinate-induced cascade, TRPM5 is indispensable for initiating type 2 immunity to helminths (Howitt *et al*, 2016; Nadsombati *et al*, 2018; Luo *et al*, 2019). In contrast, GNAT3 is not required for the tuft cell response to *N. brasiliensis* infection (Nadsombati *et al*, 2018).

Even though the role of the AhR in tuft cell functions has not yet been well studied, AhR signaling is known to control the intestinal ILC2/ILC3 balance to mount an adequate host response to various pathogens (Li *et al*, 2018). Since the AhR intrinsically suppresses ILC2 functions such as IL-13 secretion, AhR^{-/-} mice show an enhanced protection against *H. polygyrus* infection. This study by Li *et al*. seems to be in clear contrast to our results after succinate treatment, where we observed an impaired tuft cell hyperplasia and ILC2 proliferation in AhR^{-/-} mice. However, as mentioned above, our experimental model for tuft cell activation by succinate might differ from the tuft cell response to helminth infections. Nevertheless, it would be

essential to investigate whether the restricted activation of the tuft cell-ILC2 circuit in our model is also associated with a reduced resistance to parasite infections in *AhR*^{-/-} mice. Moreover, mouse lines with a conditional deletion of *AhR* in hematopoietic cells or IECs could clarify if the impaired circuit activation is caused by *AhR*-dependent alterations in the taste signal transduction of tuft cells or in the function of ILC2s. In addition, these experiments might potentially provide explanations for the contrasting results obtained in *in vitro* and *in vivo* experiments, because intestinal organoids are only composed of epithelial cells, thereby lacking the crosstalk of tuft cells with ILC2s. Thus, a co-culture of organoids with immune cells, especially ILC2s, is necessary to better model the tuft cell-mediated intestinal immunity *in vitro*.

Overall, this thesis in context with the literature illustrates how complex tuft cells respond to various stimuli via several signaling cascades. Otherwise, multiple intracellular protein interactions are still unknown to this day. Understanding how the interplay of tuft cells with ILC2s and the gut microbiome is affected by *AhR* signaling is central to further unravel the role of *AhR* in the regulation of immune responses to intestinal protists and helminths. Here, the use of organoids as an *in vitro* model side by side with animal models might give further valuable insights into the influence of *AhR* signaling in the tuft cell-mediated type 2 immunity in the small intestine.

5.2.3. Differences in *AhRR* expression by IECs *in vitro* compared to *in vivo*

In line with previous data of our group, this study indicated that the *AhRR* is barely expressed by IECs but highly expressed by immune cells located in the LP of the small intestine (Figure 4.16, 4.17 and 4.22) (Brandstätter *et al*, 2016). Even, *in vivo* *AhR* ligand stimulation with 3MC could not increase *AhRR* expression in IECs (Iriady, 2024). Similarly, IECs in untreated *AhRR*-deficient/EGFP organoids derived from *AhRR*^{E/E} and *TripleCyp*^{-/-}*AhRR*^{E/E} mice showed no *AhRR* expression (Figure 4.7 and 4.10). However, in contrast to the *in vivo* results, *AhRR* expression by IECs including tuft cells was significantly induced by 3MC or FICZ stimulation in both *AhRR* reporter organoids with a stronger increase upon the additional loss of the CYP1 enzymes. Moreover, the treatment with IL-13 seemed to further promote the increase of *AhRR* expression upon *AhR* stimulation. As LP cells are missing in the organoid model, we performed direct and indirect co-culture experiments of freshly isolated intestinal crypts with LP cells of matching genotype. Interestingly, the direct addition of LP cells but not the CM from DMSO- or 3MC-treated LP cells inhibited the 3MC-induced *AhRR* expression by IECs

in intestinal crypts (Figure 4.15). Hence, the lack of LP cells in intestinal organoids could explain the discrepancies between the *in vivo* and *in vitro* results. But it remains unsolved how and why LP cells might suppress *AhRR* expression upon AhR activation *in vivo* and which of the different cell types contained in LP cells mediates this effect.

On one hand, these results possibly point out that a direct interaction with LP cells is necessary to inhibit *AhRR* expression by IECs. But we could not observe that LP cells attached to the isolated intestinal crypts. On the other hand, we cannot rule out that soluble factors such as cytokines secreted by LP cells mediated the observed effect, although the culture of crypts in LP-CM was not able to restrain the induction of *AhRR* expression by IECs. Perhaps, the concentration of soluble mediators was much lower in the LP-CM compared to the direct co-culture of crypts and IECs, where both cell types were embedded in a dome of Matrigel matrix. Thus, in further experiments it would be interesting to examine whether a 1:1 mixture of Matrigel with LP-CM instead of normal culture medium could inhibit the 3MC-induced *AhRR* expression by IECs.

Based on our results, some cell types and soluble mediators may be excluded as possible candidates for the inhibition of *AhRR* expression by IECs *in vivo*. As isolated intestinal crypts still contain IELs also after one day in culture, they probably do not cause the observed effect. Also, the cytokine IL-13, which is among others secreted by ILC2s, can be excluded to suppress *AhRR* expression, because it rather enhances the 3MC-induced *AhRR* expression in intestinal organoids. For example, another soluble mediator secreted by LP cells, which directly acts on IECs, is IL-22. AhR activation has been shown to induce IL-22 production by ILC3s under steady state conditions, which is crucial for the maintenance of the intestinal epithelial homeostasis and therefore might also modulate *AhRR* expression by IECs (Liang *et al*, 2006; Kiss *et al*, 2011; Monteleone *et al*, 2011). In addition, TGF- β , which is produced by IECs and selective intestinal immune cell populations such as DCs and T_{reg} cells, plays an essential role in regulating intestinal barrier integrity (Ihara *et al*, 2017; Xiao *et al*, 2017). Interestingly, TGF- β signaling has been also associated with AhR signaling, as TGF- β stabilizes HIF-1 α to drive the dissociation of the AhR/ARNT complex (Nakano *et al*, 2020). Further potential candidates could be identified by secretome screening of LP cells upon AhR stimulation. To validate if IL-22, TGF- β or other potential candidates can inhibit *AhRR* expression in IECs, these specific cytokines could be added to the culture medium of intestinal crypts upon stimulation with AhR ligands.

Moreover, RNAseq analysis of *ex vivo* isolated IECs in comparison to IECs from intestinal organoids could give new insights into the regulation of AhR signaling by AhRR in the small intestine and the crosstalk between IECs and LP cells.

5.2.4. Use of small intestinal organoids as *in vitro* model

In 2009, Sato *et al.* described the first long-term mouse small intestinal organoid culture from isolated adult ISCs with a physiological 3D structure containing crypt-villus domains (Sato *et al.*, 2009). Only two years later, human small intestinal organoids have been successfully grown (Sato *et al.*, 2011b). Both, mouse and human organoids display a cell composition similar to that of the respective adult small intestinal epithelium. Tuft cells are one of the rarest IEC types, but in mice are shown to rise in frequency from 0.3 % to about 3 % in response to type 2 immune stimuli. The enrichment of specific cell types in organoids by changing the media composition facilitates the research on these rare epithelial cells (Boonekamp *et al.*, 2020). Thus, tuft cell-enriched mouse intestinal organoids as used in this thesis serve as valuable tools to unravel tuft cell functions *in vitro*. Further studies indicated a spatial heterogeneity of tuft cells along the crypt-villus axis through single-cell RNAseq analysis of the mouse small intestine (Haber *et al.*, 2017; Manco *et al.*, 2021). Whereas tuft-1 cells express neuronal-like genes and are localized toward the villus bottom, tuft-2 cells zonated toward the villus tip are characterized by an immune-like signature. Interestingly, in 2024, Huang *et al.* reported similar tuft cell subtypes in human intestinal organoid cultures (Huang *et al.*, 2024). As organoid models lack microbiome and LP cells, these results might indicate the existence of an epithelial intrinsic-induced tuft cell maturation program.

On the other hand, the missing crosstalk of epithelial cells with microbiota or other cell types, such as immune cells, enteric neurons, vascular cells, and stromal cells, is a main downside of these epithelial-only intestinal organoids. As emphasized by our contradictory *in vitro* versus *in vivo* results with regard to tuft cell differentiation and AhRR expression by IECs, organoid systems with an increased complexity are crucial to assess cellular and molecular interaction networks within the gut. Over the past years, more and more co-culture systems have been developed, which selectively include non-epithelial cellular components to examine their direct influence on the intestinal barrier (Hentschel *et al.*, 2021; Zheng *et al.*, 2024). For example, single bacterial species or fecal-derived microbial communities can be integrated for several

days via microinjections into the organoid lumen to further understand host-microbe interactions (Williamson *et al*, 2018). To elucidate the interplay between the intestinal epithelium and the immune system, novel techniques combine immune cell population with organoids. For instance, Recaldin *et al*. described a human intestinal immuno-organoid model with tissue-resident memory T cells, which partially integrate within the epithelial barrier and resemble features of IELs (Recaldin *et al*, 2024). Moreover, the co-culture of mouse small intestinal organoids with LP-derived ILC3s illustrates a bi-directional Notch-mediated signaling between the epithelium and ILC3s (Read *et al*, 2024). Whereas ILC3s drive the differentiation of goblet cells in organoids, goblet cell-enriched organoids promote the IL-22 production by ILC3s, in turn inducing the upregulation of IL-22 target genes in IECs. In particular, the integration of ILC2s in intestinal organoid models is of great importance to analyze the tuft cell-ILC2 circuit *in vitro*. Recently, Jowett *et al*. demonstrated that the epithelial niche in mouse as well as human small intestinal organoids supports the tissue-specific maturation of ILC precursors into all ILC subsets (Jowett *et al*, 2022). Furthermore, organoid-derived ILC2s are able to proliferate upon succinate-induced IL-25 secretion by tuft cells. Hence, this co-culture system could allow to investigate the role of tuft cells in intestinal type 2 immunity to infections.

Overall, mouse and human intestinal organoids represent a very useful *in vitro* model to study the intestinal barrier in health and disease. However, further development of multicellular organoid technologies is necessary to accurately simulate the complex *in vivo* microenvironment of the small intestine.

6. References

- Agbor LN, Elased KM & Walker MK (2011) Endothelial cell-specific aryl hydrocarbon receptor knockout mice exhibit hypotension mediated, in part, by an attenuated angiotensin II responsiveness. *Biochem Pharmacol* 82: 514–523
- Akchurin O, Patino E, Dalal V, Meza K, Bhatia D, Brovender S, Zhu Y-S, Cunningham-Rundles S, Perelstein E, Kumar J, *et al* (2019) Interleukin-6 Contributes to the Development of Anemia in Juvenile CKD. *Kidney Int reports* 4: 470–483
- Alvarado DM, Chen B, Iticovici M, Thaker AI, Dai N, VanDussen KL, Shaikh N, Lim CK, Guillemin GJ, Tarr PI, *et al* (2019) Epithelial Indoleamine 2,3-Dioxygenase 1 Modulates Aryl Hydrocarbon Receptor and Notch Signaling to Increase Differentiation of Secretory Cells and Alter Mucus-Associated Microbiota. *Gastroenterology* 157: 1093-1108.e11
- Apetoh L, Quintana FJ, Pot C, Joller N, Xiao S, Kumar D, Burns EJ, Sherr DH, Weiner HL & Kuchroo VK (2010) The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. *Nat Immunol* 11: 854–861
- Arike L, Seiman A, van der Post S, Rodriguez Piñeiro AM, Ermund A, Schütte A, Bäckhed F, Johansson ME V & Hansson GC (2020) Protein Turnover in Epithelial Cells and Mucus along the Gastrointestinal Tract Is Coordinated by the Spatial Location and Microbiota. *Cell Rep* 30: 1077-1087.e3
- Avraham R (2023) Untangling Cellular Host-Pathogen Encounters at Infection Bottlenecks. *Infect Immun* 91: e0043822
- Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME & Ouellette AJ (2000) Secretion of microbicidal α -defensins by intestinal Paneth cells in response to bacteria. *Nat Immunol* 1: 113–118
- Banerjee A, Herring CA, Chen B, Kim H, Simmons AJ, Southard-Smith AN, Allaman MM, White JR, Macedonia MC, McKinley ET, *et al* (2020) Succinate Produced by Intestinal Microbes Promotes Specification of Tuft Cells to Suppress Ileal Inflammation. *Gastroenterology* 159: 2101-2115.e5
- Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, *et al* (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 449: 1003–1007
- Barouki R, Aggerbeck M, Aggerbeck L & Coumoul X (2012) The aryl hydrocarbon receptor system. *Drug Metabol Drug Interact* 27: 3–8
- Basu R, O’Quinn DB, Silberberger DJ, Schoeb TR, Fouser L, Ouyang W, Hatton RD & Weaver CT (2012) Th22 cells are an important source of IL-22 for host protection against enteropathogenic bacteria. *Immunity* 37: 1061–1075

- Benoun JM, Peres NG, Wang N, Pham OH, Rudisill VL, Fogassy ZN, Whitney PG, Fernandez-Ruiz D, Gebhardt T, Pham Q-M, *et al* (2018) Optimal protection against Salmonella infection requires noncirculating memory. *Proc Natl Acad Sci U S A* 115: 10416–10421
- Bhandari J, Thada PK & DeVos E Typhoid Fever. In *StatPearls. Treasure Island (FL): StatPearls Publishing* pp 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1088888/>.
- Bharadwaj S, Anim JT, Ebrahim F & Aldahham A (2009) Granulomatous inflammatory response in a case of typhoid fever. *Med Princ Pract Int J Kuwait Univ Heal Sci Cent* 18: 239–241
- Billipp TE, Fung C, Webeck LM, Sargent DB, Gologorsky MB, Chen Z, McDaniel MM, Kasal DN, McGinty JW, Barrow KA, *et al* (2024) Tuft cell-derived acetylcholine promotes epithelial chloride secretion and intestinal helminth clearance. *Immunity* 57: 1243-1259.e8
- Bjeldanes LF, Kim JY, Grose KR, Bartholomew JC & Bradfield CA (1991) Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc Natl Acad Sci U S A* 88: 9543–9547
- Bjerknes M, Khandanpour C, Möröy T, Fujiyama T, Hoshino M, Klisch TJ, Ding Q, Gan L, Wang J, Martín MG, *et al* (2012) Origin of the brush cell lineage in the mouse intestinal epithelium. *Dev Biol* 362: 194–218
- Boonekamp KE, Dayton TL & Clevers H (2020) Intestinal organoids as tools for enriching and studying specific and rare cell types: advances and future directions. *J Mol Cell Biol* 12: 562–568
- Brandstätter O, Schanz O, Vorac J, König J, Mori T, Maruyama T, Korkowski M, Haarmann-Stemann T, von Smolinski D, Schultze JL, *et al* (2016) Balancing intestinal and systemic inflammation through cell type-specific expression of the aryl hydrocarbon receptor repressor. *Sci Rep* 6: 26091
- Brestoff JR & Artis D (2013) Commensal bacteria at the interface of host metabolism and the immune system. *Nat Immunol* 14: 676–684
- Brown DE, McCoy MW, Pilonieta MC, Nix RN & Detweiler CS (2010) Chronic murine typhoid fever is a natural model of secondary hemophagocytic lymphohistiocytosis. *PLoS One* 5: e9441
- Campbell SJ, Henderson CJ, Anthony DC, Davidson D, Clark AJ & Wolf CR (2005) The murine Cyp1a1 gene is expressed in a restricted spatial and temporal pattern during embryonic development. *J Biol Chem* 280: 5828–5835
- Carver LA & Bradfield CA (1997) Ligand-dependent interaction of the aryl hydrocarbon receptor with a novel immunophilin homolog in vivo. *J Biol Chem* 272: 11452–11456

- Cengiz SC (2020) Functions of the Aryl hydrocarbon Receptor (AhR) and the AhR-Repressor (AhRR) in defense against *Salmonella Typhimurium* and *Toxoplasma gondii* infections.
- Chalkidi N, Paraskeva C & Koliarakis V (2022) Fibroblasts in intestinal homeostasis, damage, and repair. *Front Immunol* 13: 924866
- Chen Y, Xiang J, Qian F, Diwakar BT, Ruan B, Hao S, Prabhu KS & Paulson RF (2020) Epo receptor signaling in macrophages alters the splenic niche to promote erythroid differentiation. *Blood* 136: 235–246
- Coutry N, Nguyen J, Soualhi S, Gerbe F, Meslier V, Dardalhon V, Almeida M, Quinquis B, Thirion F, Herbert F, *et al* (2023) Cross talk between Paneth and tuft cells drives dysbiosis and inflammation in the gut mucosa. *Proc Natl Acad Sci U S A* 120: e2219431120
- Denison MS & Heath-Pagliuso S (1998) The Ah receptor: a regulator of the biochemical and toxicological actions of structurally diverse chemicals. *Bull Environ Contam Toxicol* 61: 557–568
- Denison MS & Nagy SR (2003) Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu Rev Pharmacol Toxicol* 43: 309–334
- Diny NL, Schonfeldova B, Shapiro M, Winder ML, Varsani-Brown S & Stockinger B (2022) The aryl hydrocarbon receptor contributes to tissue adaptation of intestinal eosinophils in mice. *J Exp Med* 219
- Dong B, Cheng W, Li W, Zheng J, Wu D, Matsumura F & Vogel CFA (2011) FRET analysis of protein tyrosine kinase c-Src activation mediated via aryl hydrocarbon receptor. *Biochim Biophys Acta* 1810: 427–431
- Durrin LK, Jones PB, Fisher JM, Galeazzi DR & Whitlock JPJ (1987) 2,3,7,8-Tetrachlorodibenzo-p-dioxin receptors regulate transcription of the cytochrome P1-450 gene. *J Cell Biochem* 35: 153–160
- Farquhar MG & Palade GE (1963) Junctional complexes in various epithelia. *J Cell Biol* 17: 375–412
- Feng X, Andersson T, Gschwend J, Flüchter P, Berest I, Muff JL, Carchidi D, Lechner A, de Tenorio JC, Brander N, *et al* (2024a) Tuft cell IL-17RB restrains IL-25 bioavailability and reveals context-dependent ILC2 hypoproliferation. *bioRxiv Prepr Serv Biol* doi:10.1101/2024.03.04.583299 [PREPRINT]
- Feng X, Flüchter P, De Tenorio JC & Schneider C (2024b) Tuft cells in the intestine, immunity and beyond. *Nat Rev Gastroenterol Hepatol*
- Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, Nebert DW, Rudikoff S, Ward JM & Gonzalez FJ (1995) Immune system impairment and

- hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science* 268: 722–726
- Frericks M, Meissner M & Esser C (2007) Microarray analysis of the AHR system: tissue-specific flexibility in signal and target genes. *Toxicol Appl Pharmacol* 220: 320–332
- Fueldner C, Riemschneider S, Haupt J, Jungnickel H, Schulze F, Zoldan K, Esser C, Hauschildt S, Knauer J, Luch A, *et al* (2022) Aryl Hydrocarbon Receptor Activation by Benzo[a]pyrene Prevents Development of Septic Shock and Fatal Outcome in a Mouse Model of Systemic *Salmonella enterica* Infection. *Cells* 11
- Fukunaga BN, Probst MR, Reisz-Porszasz S & Hankinson O (1995) Identification of functional domains of the aryl hydrocarbon receptor. *J Biol Chem* 270: 29270–29278
- Gagliani N, Vesely MCA, Iseppon A, Brockmann L, Xu H, Palm NW, de Zoete MR, Licona-Limón P, Paiva RS, Ching T, *et al* (2015) Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature* 523: 221–225
- Gandhi R, Kumar D, Burns EJ, Nadeau M, Dake B, Laroni A, Kozoriz D, Weiner HL & Quintana FJ (2010) Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3(+) regulatory T cells. *Nat Immunol* 11: 846–853
- Gardenghi S, Renaud TM, Meloni A, Casu C, Crielgaard BJ, Bystrom LM, Greenberg-Kushnir N, Sasu BJ, Cooke KS & Rivella S (2014) Distinct roles for hepcidin and interleukin-6 in the recovery from anemia in mice injected with heat-killed *Brucella abortus*. *Blood* 123: 1137–1145
- Gerbe F, van Es JH, Makrini L, Brulin B, Mellitzer G, Robine S, Romagnolo B, Shroyer NF, Bourgaux J-F, Pignodel C, *et al* (2011) Distinct ATOH1 and Neurog3 requirements define tuft cells as a new secretory cell type in the intestinal epithelium. *J Cell Biol* 192: 767–780
- Gerbe F, Sidot E, Smyth DJ, Ohmoto M, Matsumoto I, Dardalhon V, Cesses P, Garnier L, Pouzolles M, Brulin B, *et al* (2016) Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature* 529: 226–230
- Gerrick ER, Zlitni S, West PT, Carter MM, Mechler CM, Olm MR, Caffrey EB, Li JA, Higginbottom SK, Severyn CJ, *et al* (2024) Metabolic diversity in commensal protists regulates intestinal immunity and trans-kingdom competition. *Cell* 187: 62-78.e20
- Di Giandomenico S, Kermani P, Mollé N, Yabut MM, Abu-Zeinah G, Stephens T, Messali N, Schreiner R, Brenet F, Rafii S, *et al* (2020) Megakaryocyte TGFβ1 partitions erythropoiesis into immature progenitor/stem cells and maturing precursors. *Blood* 136: 1044–1054

- Gomes AC & Gomes MS (2016) Hematopoietic niches, erythropoiesis and anemia of chronic infection. *Exp Hematol* 44: 85–91
- Gracz AD, Samsa LA, Fordham MJ, Trotier DC, Zwarycz B, Lo Y-H, Bao K, Starmer J, Raab JR, Shroyer NF, *et al* (2018) Sox4 Promotes Atoh1-Independent Intestinal Secretory Differentiation Toward Tuft and Enteroendocrine Fates. *Gastroenterology* 155: 1508-1523.e10
- Gu Y-Z, Hogenesch JB & Bradfield CA (2000) The PAS Superfamily: Sensors of Environmental and Developmental Signals. *Annu Rev Pharmacol Toxicol* 40: 519–561
- Gutiérrez-Vázquez C & Quintana FJ (2018) Regulation of the Immune Response by the Aryl Hydrocarbon Receptor. *Immunity* 48: 19–33
- Haase VH (2013) Regulation of erythropoiesis by hypoxia-inducible factors. *Blood Rev* 27: 41–53
- Haber AL, Biton M, Rogel N, Herbst RH, Shekhar K, Smillie C, Burgin G, Delorey TM, Howitt MR, Katz Y, *et al* (2017) A single-cell survey of the small intestinal epithelium. *Nature* 551: 333–339
- Hahn ME, Allan LL & Sherr DH (2009) Regulation of constitutive and inducible AHR signaling: complex interactions involving the AHR repressor. *Biochem Pharmacol* 77: 485–497
- Hanieh H (2015) Aryl hydrocarbon receptor-microRNA-212/132 axis in human breast cancer suppresses metastasis by targeting SOX4. *Mol Cancer* 14: 172
- Hankinson O (2005) Role of coactivators in transcriptional activation by the aryl hydrocarbon receptor. *Arch Biochem Biophys* 433: 379–386
- Hentschel V, Seufferlein T & Armacki M (2021) Intestinal organoids in coculture: redefining the boundaries of gut mucosa ex vivo modeling. *Am J Physiol Gastrointest Liver Physiol* 321: G693–G704
- Herring CA, Banerjee A, McKinley ET, Simmons AJ, Ping J, Roland JT, Franklin JL, Liu Q, Gerdes MJ, Coffey RJ, *et al* (2018) Unsupervised Trajectory Analysis of Single-Cell RNA-Seq and Imaging Data Reveals Alternative Tuft Cell Origins in the Gut. *Cell Syst* 6: 37-51.e9
- Howitt MR, Cao YG, Gologorsky MB, Li JA, Haber AL, Biton M, Lang J, Michaud M, Regev A & Garrett WS (2020) The Taste Receptor TAS1R3 Regulates Small Intestinal Tuft Cell Homeostasis. *ImmunoHorizons* 4: 23–32
- Howitt MR, Lavoie S, Michaud M, Blum AM, Tran S V, Weinstock J V, Gallini CA, Redding K, Margolskee RF, Osborne LC, *et al* (2016) Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* 351: 1329–1333

- Huang L, Bernink JH, Giladi A, Krueger D, van Son GJF, Geurts MH, Busslinger G, Lin L, Begthel H, Zandvliet M, *et al* (2024) Tuft cells act as regenerative stem cells in the human intestine. *Nature* 634: 929–935
- Hubbard TD, Murray IA, Bisson WH, Lahoti TS, Gowda K, Amin SG, Patterson AD & Perdew GH (2015) Adaptation of the human aryl hydrocarbon receptor to sense microbiota-derived indoles. *Sci Rep* 5: 12689
- Hyland L, Villarreal-Ramos B, Clarke B, Baaten B & Hou S (2005) Bone marrow immunosuppression in Salmonella-infected mice is prolonged following influenza virus infection. *Exp Hematol* 33: 1477–1485
- Ihara S, Hirata Y & Koike K (2017) TGF- β in inflammatory bowel disease: a key regulator of immune cells, epithelium, and the intestinal microbiota. *J Gastroenterol* 52: 777–787
- Ikuta T, Eguchi H, Tachibana T, Yoneda Y & Kawajiri K (1998) Nuclear localization and export signals of the human aryl hydrocarbon receptor. *J Biol Chem* 273: 2895–2904
- Inra CN, Zhou BO, Acar M, Murphy MM, Richardson J, Zhao Z & Morrison SJ (2015) A perisinusoidal niche for extramedullary haematopoiesis in the spleen. *Nature* 527: 466–471
- Iriady I (2024) Role of the AhR-Repressor and AhR-dependent CYP1 Monooxygenases in Immune Cells Regulation.
- Isomaki AM (1973) A new cell type (tuft cell) in the gastrointestinal mucosa of the rat. A transmission and scanning electron microscopic study. *Acta Pathol Microbiol Scand A: Suppl* 240, 241–235
- Jackson A, Nanton MR, O'Donnell H, Akue AD & McSorley SJ (2010) Innate immune activation during Salmonella infection initiates extramedullary erythropoiesis and splenomegaly. *J Immunol* 185: 6198–6204
- Jarvi O & Keyrilainen O (1956) On the cellular structures of the epithelial invasions in the glandular stomach of mice caused by intramural application of 20-methylcholantren. *Acta Pathol Microbiol Scand Suppl* 39: 72–73
- Jin U-H, Lee S-O, Sridharan G, Lee K, Davidson LA, Jayaraman A, Chapkin RS, Alaniz R & Safe S (2014) Microbiome-derived tryptophan metabolites and their aryl hydrocarbon receptor-dependent agonist and antagonist activities. *Mol Pharmacol* 85: 777–788
- Jowett GM, Read E, Roberts LB, Coman D, Vilà González M, Zabinski T, Niazi U, Reis R, Trieu T-J, Danovi D, *et al* (2022) Organoids capture tissue-specific innate lymphoid cell development in mice and humans. *Cell Rep* 40: 111281
- Kazlauskas A, Poellinger L & Pongratz I (1999) Evidence that the co-chaperone p23

- regulates ligand responsiveness of the dioxin (Aryl hydrocarbon) receptor. *J Biol Chem* 274: 13519–13524
- Kim S-H, Henry EC, Kim D-K, Kim Y-H, Shin KJ, Han MS, Lee TG, Kang J-K, Gasiewicz TA, Ryu SH, *et al* (2006) Novel compound 2-methyl-2H-pyrazole-3-carboxylic acid (2-methyl-4-o-tolylazo-phenyl)-amide (CH-223191) prevents 2,3,7,8-TCDD-induced toxicity by antagonizing the aryl hydrocarbon receptor. *Mol Pharmacol* 69: 1871–1878
- Kimura A, Abe H, Tsuruta S, Chiba S, Fujii-Kuriyama Y, Sekiya T, Morita R & Yoshimura A (2014) Aryl hydrocarbon receptor protects against bacterial infection by promoting macrophage survival and reactive oxygen species production. *Int Immunol* 26: 209–220
- Kimura A, Naka T, Nakahama T, Chinen I, Masuda K, Nohara K, Fujii-Kuriyama Y & Kishimoto T (2009) Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. *J Exp Med* 206: 2027–2035
- Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y & Kishimoto T (2008) Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. *Proc Natl Acad Sci U S A* 105: 9721–9726
- Kiss EA, Vonarbourg C, Kopfmann S, Hobeika E, Finke D, Esser C & Diefenbach A (2011) Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. *Science* 334: 1561–1565
- Krdija D, El Marjou F, Guirao B, Richon S, Leroy O, Bellaiche Y, Hannezo E & Matic Vignjevic D (2019) Active cell migration is critical for steady-state epithelial turnover in the gut. *Science* 365: 705–710
- Kudo I, Hosaka M, Haga A, Tsuji N, Nagata Y, Okada H, Fukuda K, Kakizaki Y, Okamoto T, Grave E, *et al* (2018) The regulation mechanisms of AhR by molecular chaperone complex. *J Biochem* 163: 223–232
- Kugler P, Höfer D, Mayer B & Drenckhahn D (1994) Nitric oxide synthase and NADP-linked glucose-6-phosphate dehydrogenase are co-localized in brush cells of rat stomach and pancreas. *J Histochem Cytochem Off J Histochem Soc* 42: 1317–1321
- Kumar MB, Ramadoss P, Reen RK, Vanden Heuvel JP & Perdew GH (2001) The Q-rich Subdomain of the Human AhReceptor Transactivation Domain Is Required for Dioxin-mediated Transcriptional Activity*. *J Biol Chem* 276: 42302–42310
- Kupz A, Bedoui S & Strugnell RA (2014) Cellular requirements for systemic control of *Salmonella enterica* serovar Typhimurium infections in mice. *Infect Immun* 82: 4997–5004
- Kupz A, Scott TA, Belz GT, Andrews DM, Greyer M, Lew AM, Brooks AG, Smyth MJ, Curtiss R 3rd, Bedoui S, *et al* (2013) Contribution of Thy1+ NK cells to protective

- IFN- γ production during *Salmonella typhimurium* infections. *Proc Natl Acad Sci U S A* 110: 2252–2257
- Lamas B, Natividad JM & Sokol H (2018) Aryl hydrocarbon receptor and intestinal immunity. *Mucosal Immunol* 11: 1024–1038
- Lee S & Zhan H (2024) Deciphering the differential impact of thrombopoietin/MPL signaling on hematopoietic stem/progenitor cell function in bone marrow and spleen. *Stem Cell Reports* 19: 211–223
- Lee SK, Rigby RJ, Zotos D, Tsai LM, Kawamoto S, Marshall JL, Ramiscal RR, Chan TD, Gatto D, Brink R, *et al* (2011) B cell priming for extrafollicular antibody responses requires Bcl-6 expression by T cells. *J Exp Med* 208: 1377–1388
- Lei W, Ren W, Ohmoto M, Urban JFJ, Matsumoto I, Margolskee RF & Jiang P (2018) Activation of intestinal tuft cell-expressed *Sucnr1* triggers type 2 immunity in the mouse small intestine. *Proc Natl Acad Sci U S A* 115: 5552–5557
- Li L-X, Benoun JM, Weiskopf K, Garcia KC & McSorley SJ (2016) *Salmonella* Infection Enhances Erythropoietin Production by the Kidney and Liver, Which Correlates with Elevated Bacterial Burdens. *Infect Immun* 84: 2833–2841
- Li S, Bostick JW, Ye J, Qiu J, Zhang B, Urban JFJ, Avram D & Zhou L (2018) Aryl Hydrocarbon Receptor Signaling Cell Intrinsically Inhibits Intestinal Group 2 Innate Lymphoid Cell Function. *Immunity* 49: 915-928.e5
- Li Y, Innocentin S, Withers DR, Roberts NA, Gallagher AR, Grigorieva EF, Wilhelm C & Veldhoen M (2011) Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. *Cell* 147: 629–640
- Liang SC, Tan X-Y, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M & Fouser LA (2006) Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 203: 2271–2279
- Libregts SF, Gutiérrez L, de Bruin AM, Wensveen FM, Papadopoulos P, van Ijcken W, Özgür Z, Philipsen S & Nolte MA (2011) Chronic IFN- γ production in mice induces anemia by reducing erythrocyte life span and inhibiting erythropoiesis through an IRF-1/PU.1 axis. *Blood* 118: 2578–2588
- Lindholm HT, Parmar N, Drurey C, Campillo Poveda M, Vornwald PM, Ostrop J, Díez-Sánchez A, Maizels RM & Oudhoff MJ (2022) BMP signaling in the intestinal epithelium drives a critical feedback loop to restrain IL-13-driven tuft cell hyperplasia. *Sci Immunol* 7: eabl6543
- Lkhagva-Yondon E, Seo MS, Oh Y, Jung J, Jeon E, Na K, Yoo HS, Kim WC, Esser C, Song SU, *et al* (2023) The aryl hydrocarbon receptor controls mesenchymal stromal cell-mediated immunomodulation via ubiquitination of eukaryotic elongation factor-2 kinase. *Cell Death Dis* 14: 812

- Loub WD, Wattenberg LW & Davis DW (1975) Aryl hydrocarbon hydroxylase induction in rat tissues by naturally occurring indoles of cruciferous plants. *J Natl Cancer Inst* 54: 985–988
- Luciano, L. Reale E (1969) A new cell type (“brush cell”) in the gall bladder epithelium of the mouse. *J Submicrosc Cytol* 1: 43–52
- Luo X-C, Chen Z-H, Xue J-B, Zhao D-X, Lu C, Li Y-H, Li S-M, Du Y-W, Liu Q, Wang P, *et al* (2019) Infection by the parasitic helminth *Trichinella spiralis* activates a Tas2r-mediated signaling pathway in intestinal tuft cells. *Proc Natl Acad Sci U S A* 116: 5564–5569
- MacPherson L, Ahmed S, Tamblyn L, Krutmann J, Förster I, Weighardt H & Matthews J (2014) Aryl hydrocarbon receptor repressor and TiPARP (ARTD14) use similar, but also distinct mechanisms to repress aryl hydrocarbon receptor signaling. *Int J Mol Sci* 15: 7939–7957
- MacPherson L, Tamblyn L, Rajendra S, Bralha F, McPherson JP & Matthews J (2013) 2,3,7,8-Tetrachlorodibenzo-p-dioxin poly(ADP-ribose) polymerase (TiPARP, ARTD14) is a mono-ADP-ribosyltransferase and repressor of aryl hydrocarbon receptor transactivation. *Nucleic Acids Res* 41: 1604–1621
- Manco R, Averbukh I, Porat Z, Bahar Halpern K, Amit I & Itzkovitz S (2021) Clump sequencing exposes the spatial expression programs of intestinal secretory cells. *Nat Commun* 12: 3074
- Maradana MR, Marzook NB, Diaz OE, Mkandawire T, Diny NL, Li Y, Liebert A, Shah K, Tolaini M, Kváč M, *et al* (2023) Dietary environmental factors shape the immune defense against *Cryptosporidium* infection. *Cell Host Microbe* 31: 2038-2050.e4
- Marcial-Juárez E, Pérez-Toledo M, Nayar S, Pipi E, Alshayea A, Persaud R, Jossi SE, Lamerton R, Barone F, Henderson IR, *et al* (2023) Salmonella infection induces the reorganization of follicular dendritic cell networks concomitant with the failure to generate germinal centers. *iScience* 26: 106310
- Martin B, Hirota K, Cua DJ, Stockinger B & Veldhoen M (2009) Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. *Immunity* 31: 321–330
- Mascanfroni ID, Takenaka MC, Yeste A, Patel B, Wu Y, Kenison JE, Siddiqui S, Basso AS, Otterbein LE, Pardoll DM, *et al* (2015) Metabolic control of type 1 regulatory T cell differentiation by AHR and HIF1- α . *Nat Med* 21: 638–646
- Mathias A & Corthésy B (2011) N-Glycans on secretory component: mediators of the interaction between secretory IgA and gram-positive commensals sustaining intestinal homeostasis. *Gut Microbes* 2: 287–293
- Mayer M, Cengiz-Dartenne SC, Thiem M, Hatzfeld P, Semeniuk A, Wang N, Strugnell RA, Förster I & Weighardt H (2024) Dysregulation of stress erythropoiesis and

- enhanced susceptibility to *Salmonella* Typhimurium infection in AhR-deficient mice. *J Infect Dis*
- McCranor BJ, Kim MJ, Cruz NM, Xue Q-L, Berger AE, Walston JD, Civin CI & Roy CN (2014) Interleukin-6 directly impairs the erythroid development of human TF-1 erythroleukemic cells. *Blood Cells Mol Dis* 52: 126–133
- McGinty JW, Ting H-A, Billipp TE, Nadsombati MS, Khan DM, Barrett NA, Liang H-E, Matsumoto I & von Moltke J (2020) Tuft-Cell-Derived Leukotrienes Drive Rapid Anti-helminth Immunity in the Small Intestine but Are Dispensable for Anti-protist Immunity. *Immunity* 52: 528-541.e7
- Metidji A, Omenetti S, Crotta S, Li Y, Nye E, Ross E, Li V, Maradana MR, Schiering C & Stockinger B (2018) The Environmental Sensor AHR Protects from Inflammatory Damage by Maintaining Intestinal Stem Cell Homeostasis and Barrier Integrity. *Immunity* 49: 353-362.e5
- Meyer BK & Perdew GH (1999) Characterization of the AhR-hsp90-XAP2 core complex and the role of the immunophilin-related protein XAP2 in AhR stabilization. *Biochemistry* 38: 8907–8917
- Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ & Bradfield CA (2010) An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol* 185: 3190–3198
- Millot S, Andrieu V, Letteron P, Lyoumi S, Hurtado-Nedelec M, Karim Z, Thibaudeau O, Bennada S, Charrier J-L, Lasocki S, *et al* (2010) Erythropoietin stimulates spleen BMP4-dependent stress erythropoiesis and partially corrects anemia in a mouse model of generalized inflammation. *Blood* 116: 6072–6081
- Mimura J, Ema M, Sogawa K & Fujii-Kuriyama Y (1999) Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. *Genes Dev* 13: 20–25
- von Moltke J, Ji M, Liang H-E & Locksley RM (2016) Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* 529: 221–225
- Monteleone I, Rizzo A, Sarra M, Sica G, Sileri P, Biancone L, MacDonald TT, Pallone F & Monteleone G (2011) Aryl hydrocarbon receptor-induced signals up-regulate IL-22 production and inhibit inflammation in the gastrointestinal tract. *Gastroenterology* 141: 237–48, 248.e1
- Moura-Alves P, Faé K, Houthuys E, Dorhoi A, Kreuchwig A, Furkert J, Barison N, Diehl A, Munder A, Constant P, *et al* (2014) AhR sensing of bacterial pigments regulates antibacterial defence. *Nature* 512: 387–392
- Mowat AM & Agace WW (2014) Regional specialization within the intestinal immune system. *Nat Rev Immunol* 14: 667–685
- Murphy KM & Weaver C (2017) Janeway's immunobiology 9th ed. New York : Garland

Science

- Murray IA, Nichols RG, Zhang L, Patterson AD & Perdew GH (2016) Expression of the aryl hydrocarbon receptor contributes to the establishment of intestinal microbial community structure in mice. *Sci Rep* 6: 33969
- Nadjsombati MS, McGinty JW, Lyons-Cohen MR, Jaffe JB, DiPeso L, Schneider C, Miller CN, Pollack JL, Nagana Gowda GA, Fontana MF, *et al* (2018) Detection of Succinate by Intestinal Tuft Cells Triggers a Type 2 Innate Immune Circuit. *Immunity* 49: 33-41.e7
- Nakano N, Sakata N, Katsu Y, Nochise D, Sato E, Takahashi Y, Yamaguchi S, Haga Y, Ikeno S, Motizuki M, *et al* (2020) Dissociation of the AhR/ARNT complex by TGF- β /Smad signaling represses CYP1A1 gene expression and inhibits benze[a]pyrene-mediated cytotoxicity. *J Biol Chem* 295: 9033–9051
- Naruse M, Ishihara Y, Miyagawa-Tomita S, Koyama A & Hagiwara H (2002) 3-Methylcholanthrene, which binds to the arylhydrocarbon receptor, inhibits proliferation and differentiation of osteoblasts in vitro and ossification in vivo. *Endocrinology* 143: 3575–3581
- Ndako JA, Dojumo VT, Akinwumi JA, Fajobi VO, Owolabi AO & Olatinsu O (2020) Changes in some haematological parameters in typhoid fever patients attending Landmark University Medical Center, Omuaran-Nigeria. *Heliyon* 6: e04002
- Ndjim M, Gasmi I, Herbert F, Joséphine C, Bas J, Lamrani A, Coutry N, Henry S, Zimmermann VS, Dardalhon V, *et al* (2024) Tuft cell acetylcholine is released into the gut lumen to promote anti-helminth immunity. *Immunity* 57: 1260-1273.e7
- Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, Joosten LAB, van der Meer JWM, Mhlanga MM, Mulder WJM, *et al* (2020) Defining trained immunity and its role in health and disease. *Nat Rev Immunol* 20: 375–388
- Neutra MR, Frey A & Kraehenbuhl JP (1996) Epithelial M cells: gateways for mucosal infection and immunization. *Cell* 86: 345–348
- Noah TK & Shroyer NF (2013) Notch in the intestine: regulation of homeostasis and pathogenesis. *Annu Rev Physiol* 75: 263–288
- O'Leary CE, Feng X, Cortez VS, Locksley RM & Schneider C (2021) Interrogating the Small Intestine Tuft Cell-ILC2 Circuit Using In Vivo Manipulations. *Curr Protoc* 1: e77
- O'Leary CE, Schneider C & Locksley RM (2019) Tuft Cells-Systemically Dispersed Sensory Epithelia Integrating Immune and Neural Circuitry. *Annu Rev Immunol* 37: 47–72
- Okumura R & Takeda K (2016) Maintenance of gut homeostasis by the mucosal

- immune system. *Proc Jpn Acad Ser B Phys Biol Sci* 92: 423–435
- Olivares-Villagómez D & Van Kaer L (2018) Intestinal Intraepithelial Lymphocytes: Sentinels of the Mucosal Barrier. *Trends Immunol* 39: 264–275
- Opitz CA, Litzenburger UM, Sahm F, Ott M, Tritschler I, Trump S, Schumacher T, Jestaedt L, Schrenk D, Weller M, *et al* (2011) An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* 478: 197–203
- Oyesola OO, Shanahan MT, Kanke M, Mooney BM, Webb LM, Smita S, Matheson MK, Campioli P, Pham D, Früh SP, *et al* (2021) PGD2 and CTRH2 counteract Type 2 cytokine-elicited intestinal epithelial responses during helminth infection. *J Exp Med* 218
- Park J-H, Lee J-M, Lee E-J, Hwang W-B & Kim D-J (2018) Indole-3-Carbinol Promotes Goblet-Cell Differentiation Regulating Wnt and Notch Signaling Pathways AhR-Dependently. *Mol Cells* 41: 290–300
- Paulson RF, Hariharan S & Little JA (2020) Stress erythropoiesis: definitions and models for its study. *Exp Hematol* 89: 43-54.e2
- Pelaseyed T, Bergström JH, Gustafsson JK, Ermund A, Birchenough GMH, Schütte A, van der Post S, Svensson F, Rodríguez-Piñeiro AM, Nyström EEL, *et al* (2014) The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Immunol Rev* 260: 8–20
- Perdew GH (1988) Association of the Ah receptor with the 90-kDa heat shock protein. *J Biol Chem* 263: 13802–13805
- Peres NG, Wang N, Whitney P, Engel S, Shreenivas MM, Comerford I, Hocking DM, Erazo AB, Förster I, Kupz A, *et al* (2021) CD4+ T cell immunity to Salmonella is transient in the circulation. *PLoS Pathog* 17: e1010004
- Perry JM, Harandi OF & Paulson RF (2007) BMP4, SCF, and hypoxia cooperatively regulate the expansion of murine stress erythroid progenitors. *Blood* 109: 4494–4502
- Prince OD, Langdon JM, Layman AJ, Prince IC, Sabogal M, Mak HH, Berger AE, Cheadle C, Chrest FJ, Yu Q, *et al* (2012) Late stage erythroid precursor production is impaired in mice with chronic inflammation. *Haematologica* 97: 1648–1656
- Probst MR, Reisz-Porszasz S, Agbunag R V, Ong MS & Hankinson O (1993) Role of the aryl hydrocarbon receptor nuclear translocator protein in aryl hydrocarbon (dioxin) receptor action. *Mol Pharmacol* 44: 511 LP – 518
- Progatky F & Pachnis V (2022) The role of enteric glia in intestinal immunity. *Curr Opin Immunol* 77: 102183

- Qiu J, Guo X, Chen Z-ME, He L, Sonnenberg GF, Artis D, Fu Y-X & Zhou L (2013) Group 3 innate lymphoid cells inhibit T-cell-mediated intestinal inflammation through aryl hydrocarbon receptor signaling and regulation of microflora. *Immunity* 39: 386–399
- Qiu J, Heller JJ, Guo X, Chen ZE, Fish K, Fu Y-X & Zhou L (2012) The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells. *Immunity* 36: 92–104
- Rangan KJ & Hang HC (2017) Biochemical Mechanisms of Pathogen Restriction by Intestinal Bacteria. *Trends Biochem Sci* 42: 887–898
- Rannug A, Rannug U, Rosenkranz HS, Winqvist L, Westerholm R, Agurell E & Grafström AK (1987) Certain photooxidized derivatives of tryptophan bind with very high affinity to the Ah receptor and are likely to be endogenous signal substances. *J Biol Chem* 262: 15422–15427
- Read E, Peña-Cearra A, Coman D, Jowett GM, Chung MWH, Coales I, Syntaka S, Finlay RE, Tachó-Piñot R, van Der Post S, *et al* (2024) Bi-directional signaling between the intestinal epithelium and type-3 innate lymphoid cells regulates secretory dynamics and interleukin-22. *Mucosal Immunol* 17: 1–12
- Recaldin T, Steinacher L, Gjeta B, Harter MF, Adam L, Kromer K, Mendes MP, Bellavista M, Nikolaev M, Lazzaroni G, *et al* (2024) Human organoids with an autologous tissue-resident immune compartment. *Nature* 633: 165–173
- Rhodin J & Dalhamn T (1956) Electron microscopy of the tracheal ciliated mucosa in rat. *Z Zellforsch Mikrosk Anat* 44: 345–412
- Rodríguez-Piñeiro AM, Bergström JH, Ermund A, Gustafsson JK, Schütte A, Johansson ME V & Hansson GC (2013) Studies of mucus in mouse stomach, small intestine, and colon. II. Gastrointestinal mucus proteome reveals Muc2 and Muc5ac accompanied by a set of core proteins. *Am J Physiol Gastrointest Liver Physiol* 305: G348-56
- Rojas R & Apodaca G (2002) Immunoglobulin transport across polarized epithelial cells. *Nat Rev Mol Cell Biol* 3: 944–956
- Rosche KL, Aljasham AT, Kipfer JN, Piatkowski BT & Konjufca V (2015) Infection with *Salmonella enterica* Serovar Typhimurium Leads to Increased Proportions of F4/80+ Red Pulp Macrophages and Decreased Proportions of B and T Lymphocytes in the Spleen. *PLoS One* 10: 1–20
- Rothhammer V & Quintana FJ (2019) The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. *Nat Rev Immunol* 19: 184–197
- Di Sabatino A, Santacroce G, Rossi CM, Broglio G & Lenti MV (2023) Role of mucosal immunity and epithelial–vascular barrier in modulating gut homeostasis. *Intern*

Emerg Med 18: 1635–1646

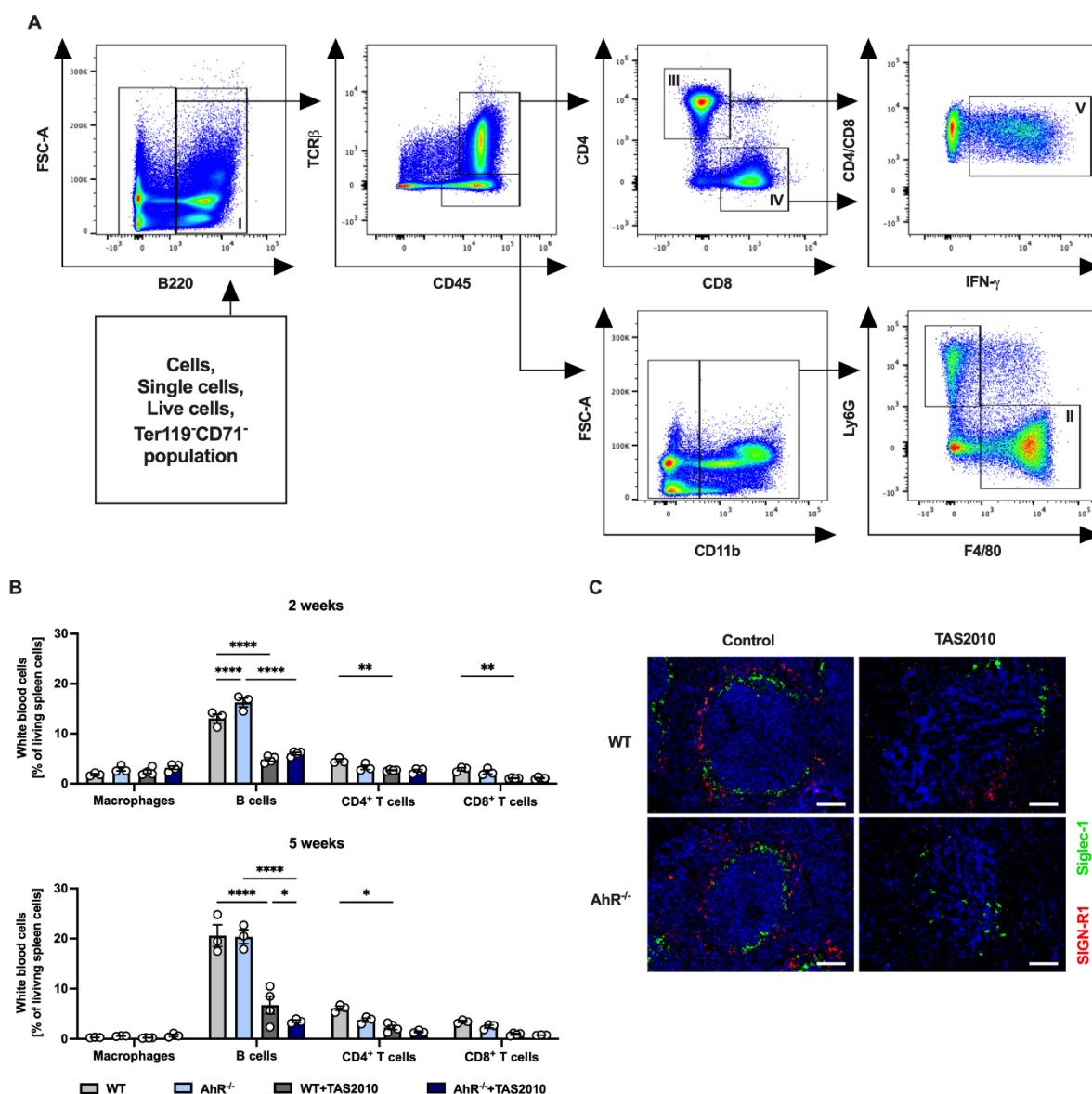
- Sahu N, Rath S & Padhy R (2016) Role of Abdominal Ultrasound in the Diagnosis of Typhoid Fever in Pediatric Patients. *J Med Ultrasound* 24
- Santos RL, Zhang S, Tsois RM, Kingsley RA, Adams LG & Bäumler AJ (2001) Animal models of Salmonella infections: enteritis versus typhoid fever. *Microbes Infect* 3: 1335–1344
- Sato T & Clevers H (2013) Primary mouse small intestinal epithelial cell cultures. *Methods Mol Biol* 945: 319–328
- Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M & Clevers H (2011a) Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* 469: 415–418
- Sato T, Stange DE, Ferrante M, Vries RGJ, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, *et al* (2011b) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 141: 1762–1772
- Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, *et al* (2009) Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 459: 262–265
- Schiering C, Wincent E, Metidji A, Iseppon A, Li Y, Potocnik AJ, Omenetti S, Henderson CJ, Wolf CR, Nebert DW, *et al* (2017) Feedback control of AHR signalling regulates intestinal immunity. *Nature* 542: 242–245
- Schmidt J V, Su GH, Reddy JK, Simon MC & Bradfield CA (1996) Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. *Proc Natl Acad Sci U S A* 93: 6731–6736
- Schneider C, O'Leary CE & Locksley RM (2019) Regulation of immune responses by tuft cells. *Nat Rev Immunol* 19: 584–593
- Schneider C, O'Leary CE, von Moltke J, Liang H-E, Ang QY, Turnbaugh PJ, Radhakrishnan S, Pellizzon M, Ma A & Locksley RM (2018) A Metabolite-Triggered Tuft Cell-ILC2 Circuit Drives Small Intestinal Remodeling. *Cell* 174: 271-284.e14
- Sherr DH & Monti S (2013) The role of the aryl hydrocarbon receptor in normal and malignant B cell development. *Semin Immunopathol* 35: 705–716
- Shi LZ, Faith NG, Nakayama Y, Suresh M, Steinberg H & Czuprynski CJ (2007) The aryl hydrocarbon receptor is required for optimal resistance to *Listeria monocytogenes* infection in mice. *J Immunol* 179: 6952–6962
- Singh KP, Garrett RW, Casado FL & Gasiewicz TA (2011) Aryl hydrocarbon receptor-

- null allele mice have hematopoietic stem/progenitor cells with abnormal characteristics and functions. *Stem Cells Dev* 20: 769–784
- Socolovsky M, Nam H, Fleming MD, Haase VH, Brugnara C & Lodish HF (2001) Ineffective erythropoiesis in Stat5a-/-5b-/- mice due to decreased survival of early erythroblasts. *Blood* 98: 3261–3273
- Stockinger B, Meglio P Di, Gialitakis M & Duarte JH (2014) The Aryl Hydrocarbon Receptor: Multitasking in the Immune System. *Annu Rev Immunol* 32: 403–432
- Stockinger B, Shah K & Wincent E (2021) AHR in the intestinal microenvironment: safeguarding barrier function. *Nat Rev Gastroenterol Hepatol* 18: 559–570
- Tian K, Jing D, Lan J, Lv M & Wang T (2024) Commensal microbiome and gastrointestinal mucosal immunity: Harmony and conflict with our closest neighbor. *Immunity, Inflamm Dis* 12: e1316
- Tremaroli V & Bäckhed F (2012) Functional interactions between the gut microbiota and host metabolism. *Nature* 489: 242–249
- Trikha P & Lee DA (2020) The role of AhR in transcriptional regulation of immune cell development and function. *Biochim Biophys Acta - Rev Cancer* 1873: 188335
- Vancamelbeke M & Vermeire S (2017) The intestinal barrier: a fundamental role in health and disease. *Expert Rev Gastroenterol Hepatol* 11: 821–834
- VanDussen KL, Carulli AJ, Keeley TM, Patel SR, Puthoff BJ, Magness ST, Tran IT, Maillard I, Siebel C, Kolterud Å, et al (2012) Notch signaling modulates proliferation and differentiation of intestinal crypt base columnar stem cells. *Development* 139: 488–497
- VanDussen KL & Samuelson LC (2010) Mouse atonal homolog 1 directs intestinal progenitors to secretory cell rather than absorptive cell fate. *Dev Biol* 346: 215–223
- Vaughan KL, Franchini AM, Kern HG & Lawrence BP (2021) The Aryl Hydrocarbon Receptor Modulates Murine Hematopoietic Stem Cell Homeostasis and Influences Lineage-Biased Stem and Progenitor Cells. *Stem Cells Dev* 30: 970–980
- Veldhoen M, Hirota K, Christensen J, O'Garra A & Stockinger B (2009) Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells. *J Exp Med* 206: 43–49
- Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld J-C & Stockinger B (2008) The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* 453: 106–109
- Vorderstrasse BA & Lawrence BP (2006) Protection against lethal challenge with

- Streptococcus pneumoniae* is conferred by aryl hydrocarbon receptor activation but is not associated with an enhanced inflammatory response. *Infect Immun* 74: 5679–5686
- Vorrink SU & Domann FE (2014) Regulatory crosstalk and interference between the xenobiotic and hypoxia sensing pathways at the AhR-ARNT-HIF1 α signaling node. *Chem Biol Interact* 218: 82–88
- Wang GL, Jiang BH, Rue EA & Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci U S A* 92: 5510–5514
- Wang H, Foong JPP, Harris NL & Bornstein JC (2022a) Enteric neuroimmune interactions coordinate intestinal responses in health and disease. *Mucosal Immunol* 15: 27–39
- Wang J, Zhao Y, Zhang X, Tu W, Wan R, Shen Y, Zhang Y, Trivedi R & Gao P (2022b) Type II alveolar epithelial cell aryl hydrocarbon receptor protects against allergic airway inflammation through controlling cell autophagy. *Front Immunol* 13: 964575
- Wang N, Scott TA, Kupz A, Shreenivas MM, Peres NG, Hocking DM, Yang C, Jebeli L, Beattie L, Groom JR, *et al* (2023) Vaccine-induced inflammation and inflammatory monocytes promote CD4⁺ T cell-dependent immunity against murine salmonellosis. *PLoS Pathog* 19: e1011666
- Watson JH & Brinkman GL (1964) Electron Microscopy of the Epithelial Cells of Normal and Bronchitic Human Bronchus. *Am Rev Respir Dis* 90: 851–866
- Wiggins BG, Wang Y-F, Burke A, Grunberg N, Vlachaki Walker JM, Dore M, Chahrour C, Pennycook BR, Sanchez-Garrido J, Vernia S, *et al* (2023) Endothelial sensing of AHR ligands regulates intestinal homeostasis. *Nature* 621: 821–829
- Williamson IA, Arnold JW, Samsa LA, Gaynor L, DiSalvo M, Cocchiari JL, Carroll I, Azcarate-Peril MA, Rawls JF, Allbritton NL, *et al* (2018) A High-Throughput Organoid Microinjection Platform to Study Gastrointestinal Microbiota and Luminal Physiology. *Cell Mol Gastroenterol Hepatol* 6: 301–319
- Xiao K, Cao S, Jiao L, Song Z, Lu J & Hu C (2017) TGF- β 1 protects intestinal integrity and influences Smads and MAPK signal pathways in IPEC-J2 after TNF- α challenge. *Innate Immun* 23: 276–284
- Yagishita Y, Joshi T, Kensler TW & Wakabayashi N (2023) Transcriptional Regulation of Math1 by Aryl Hydrocarbon Receptor: Effect on Math1(+) Progenitor Cells in Mouse Small Intestine. *Mol Cell Biol* 43: 43–63
- Yang Q, Bermingham NA, Finegold MJ & Zoghbi HY (2001) Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. *Science* 294: 2155–2158

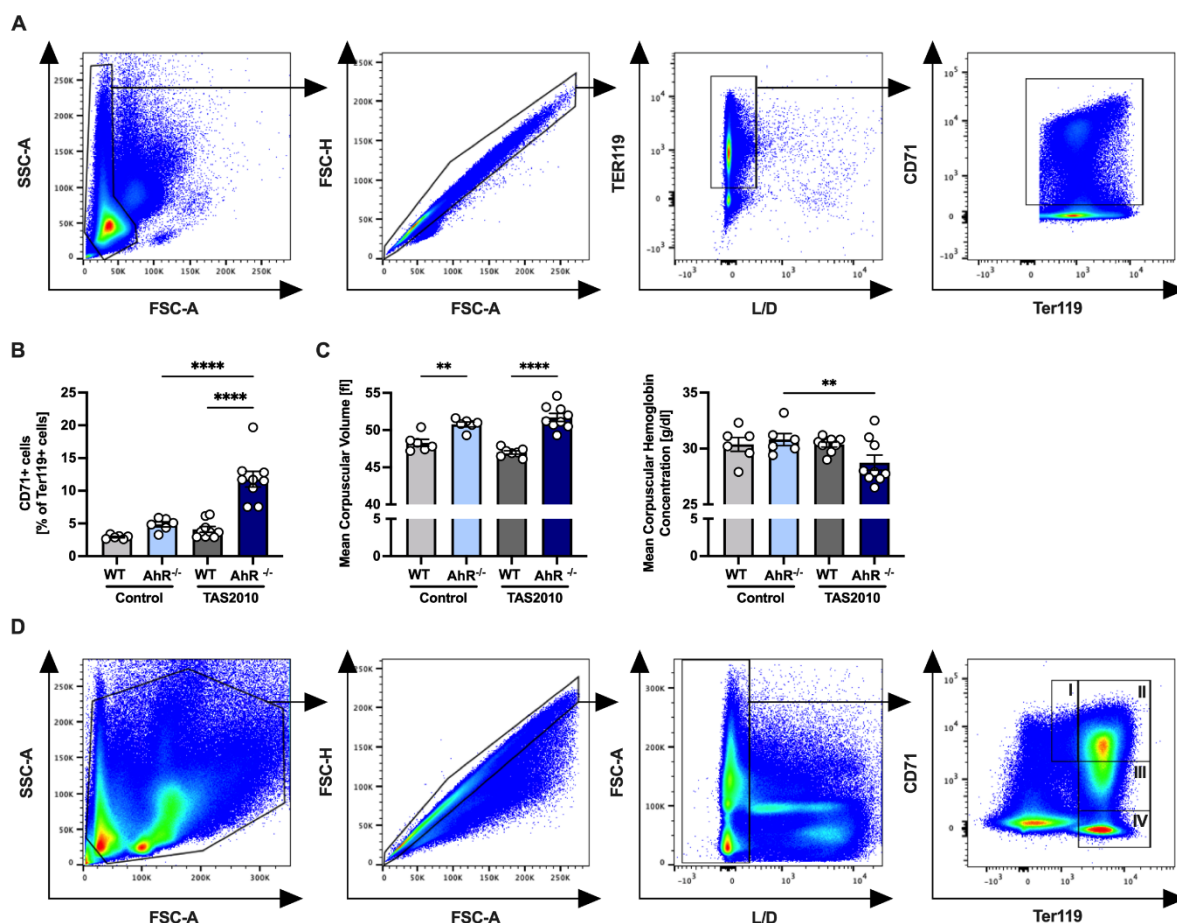
- Yu M, Wang Q, Ma Y, Li L, Yu K, Zhang Z, Chen G, Li X, Xiao W, Xu P, *et al* (2018) Aryl Hydrocarbon Receptor Activation Modulates Intestinal Epithelial Barrier Function by Maintaining Tight Junction Integrity. *Int J Biol Sci* 14: 69–77
- Zhang M, Hu Y, Yang F, Zhang J, Zhang J, Yu W, Wang M, Lv X, Li J, Bai T, *et al* (2022) Interaction between AhR and HIF-1 signaling pathways mediated by ARNT/HIF-1 β . *BMC Pharmacol Toxicol* 23: 26
- Zhao M, Ren K, Xiong X, Xin Y, Zou Y, Maynard JC, Kim A, Battist AP, Koneripalli N, Wang Y, *et al* (2022) Epithelial STAT6 O-GlcNAcylation drives a concerted anti-helminth alarmin response dependent on tuft cell hyperplasia and Gasdermin C. *Immunity* 55: 623-638.e5
- Zheng L, Zhan Y, Wang C, Fan Q, Sun D, Li Y & Xiong Y (2024) Technological advances and challenges in constructing complex gut organoid systems. *Front cell Dev Biol* 12: 1432744
- Zhou X, Chakraborty D, Murray IA, Coslo D, Kehs Z, Vijay A, Ton C, Desai D, Amin SG, Patterson AD, *et al* (2023) Aryl Hydrocarbon Receptor Activation Coordinates Mouse Small Intestinal Epithelial Cell Programming. *Lab Invest* 103: 100012

7. Appendix



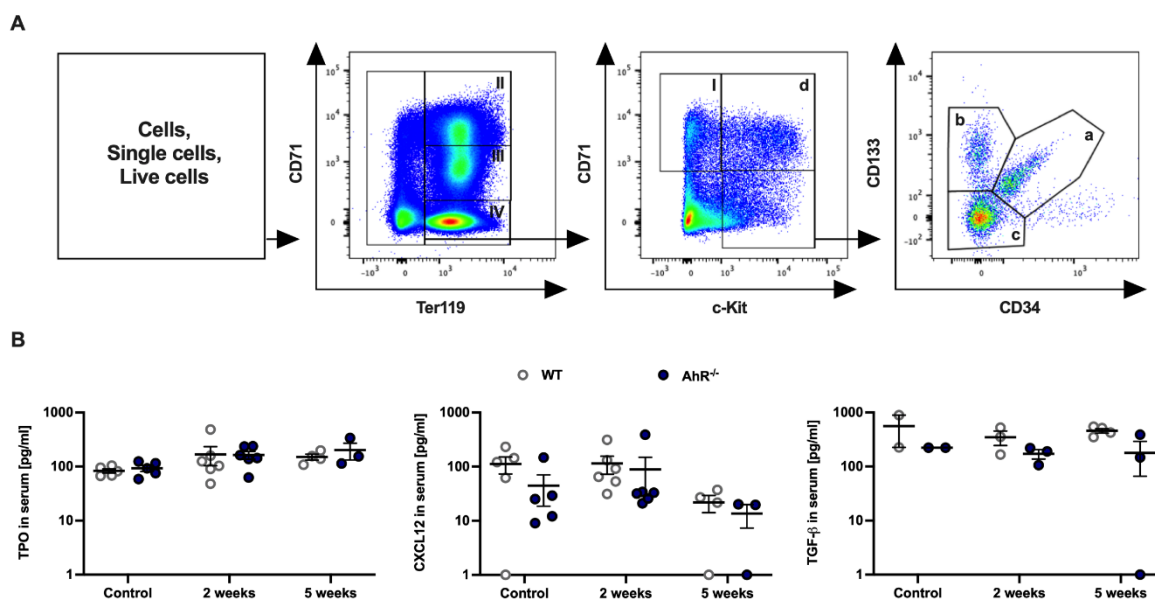
Appendix Figure 7.1 | Characterization of splenic immune cell subsets

(A) Representative gating strategy for flow cytometry analysis of B cells (I), macrophages (II), CD4⁺ (III) and CD8⁺ (IV) T cells and IFN- γ ⁺ T cells (V) in spleen of uninfected and infected WT and AhR^{-/-} mice. (B) Frequencies of macrophages, B and T cells in spleen of uninfected and infected WT and AhR^{-/-} mice analyzed by flow cytometry; control: n = 3 (WT/AhR^{-/-}, 2wk/5wk), TAS2010: n = 4 (WT/AhR^{-/-}, 2wk), n = 4 (WT, 5wk), n = 3 (AhR^{-/-}, 5wk). (C) Representative images of immunofluorescence analysis of SIGN-R⁺ marginal zone macrophages (MZMΦs) and Siglec-1⁺ metallophilic macrophages (MMMΦ) in spleen of uninfected and infected WT and AhR^{-/-} mice; Scale bar: 100 μ m. Data are represented as mean $\pm$ SEM and significance was calculated by two-way ANOVA with Tukey multiple comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).



Appendix Figure 7.2 | Analysis of erythroid cells in blood and bone marrow

(A) Representative gating strategy for flow cytometry analysis of CD71⁺ reticulocytes in blood of uninfected and infected WT and AhR^{-/-} mice. (B) Frequency of CD71⁺ reticulocytes in blood of uninfected and infected WT and AhR^{-/-} mice analyzed by flow cytometry; control: n = 6 (WT/AhR^{-/-}), TAS2010: n = 9 (WT/AhR^{-/-}). (C) Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) of RBCs in blood of uninfected and infected WT and AhR^{-/-} mice by hematology analysis; MCV, control: n = 6 (WT/AhR^{-/-}), TAS2010: n = 7 (WT), n = 9 (AhR^{-/-}), MCHC, control: n = 6 (WT/AhR^{-/-}), TAS2010: n = 9 (WT/AhR^{-/-}). (D) Representative gating strategy for flow cytometry analysis of uninfected and infected WT and AhR^{-/-} bone marrow erythroid cell populations: I: Ter119^{med}CD71^{hi} proerythroblasts (ProE), II: Ter119^{hi}CD71^{hi} basophilic and polychromatic erythroblasts (BasoE + PolyE), III: Ter119^{hi}CD71^{med} orthochromatic erythroblasts and reticulocytes (OrthoE + Retic) and IV: Ter119^{hi}CD71^{low} mature red blood cells (mRBC). Data are represented as mean ± SEM and significance was calculated by two-way ANOVA with Tukey multiple comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).



Appendix Figure 7.3 | Analysis of erythroid cells in spleen and niche factors in serum regulating erythropoiesis

(A) Representative gating strategy for flow cytometry analysis of erythroid cell populations in spleen of uninfected and infected WT and $AhR^{-/-}$ mice: a: $c\text{-Kit}^+CD133^+CD34^+$ stress erythroid progenitors (SEP1), b: $c\text{-Kit}^+CD133^+CD34^-$ SEP2, c: $c\text{-Kit}^+CD133^-CD34^-$ burst-forming unit-erythroid (BFU-E), d: $c\text{-Kit}^+CD71^+$ colony-forming unit-erythroid (CFU-E), I: $Ter119^{med}CD71^{hi}$ proerythroblasts (ProE), II: $Ter119^{hi}CD71^{hi}$ basophilic and polychromatic erythroblasts (BasoE + PolyE), III: $Ter119^{hi}CD71^{med}$ orthochromatic erythroblasts and reticulocytes (OrthoE + Retic) and IV: $Ter119^{hi}CD71^{low}$ mature red blood cells (mRBC). (B) Levels of TPO, CXCL12 and TGF- β in serum of uninfected and infected WT and $AhR^{-/-}$ mice; TPO/CXCL12 - control: $n = 5$ (WT/ $AhR^{-/-}$), TAS2010: $n = 6$ (WT/ $AhR^{-/-}$, 2wk), $n = 4$ (WT, 5wk), $n = 3$ ($AhR^{-/-}$, 5wk), TGF- β - control: $n = 2$ (WT/ $AhR^{-/-}$), TAS2010: $n = 3$ (WT/ $AhR^{-/-}$, 2wk), $n = 4$ (WT, 5wk), $n=3$ ($AhR^{-/-}$, 5wk). Data are represented as mean $\pm$ SEM and significance was calculated by two-way ANOVA with Tukey multiple comparison test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Appendix Table 7.1 | Contributions of Michelle Mayer to the publication of Mayer et al., 2024

Mayer M*, Cengiz-Dartenne SC*, Thiem M, Hatzfeld P, Semeniuk A, Wang N, Strugnell RA, Förster I, Weighardt H. Dysregulation of stress erythropoiesis and enhanced susceptibility to *Salmonella Typhimurium* infection in aryl hydrocarbon receptor-deficient mice. *The Journal of Infectious Diseases*. 2024 Jun 6:jiae304. doi: 10.1093/infdis/jiae304. Epub ahead of print. PMID: 38842164.

*contributed equally to the study

Methodology	Figure 4.1 C, Figure 4.2 D (Controls), Figure 4.3, Figure 4.4 C, Figure 4.5 A, Appendix Figure 7.1 C, Appendix Figure 7.2, Appendix Figure 7.3 A
Data analysis	Figure 4.1 C, Figure 4.2 D, Figure 4.3, Figure 4.4 C, Figure 4.5 A, Appendix Figure 7.1 C, Appendix Figure 7.2, Appendix Figure 7.3 A
Data curation and visualization	Figure 4.1, Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5, Appendix Figure 7.1, Appendix Figure 7.2, Appendix Figure 7.3
Writing	Major contribution in draft preparation and revision

Düsseldorf, den _____

Prof. Dr. Irmgard Förster

8. Curriculum Vitae

9. List of publications

Weighardt H, Shapiro M, **Mayer M**, Förster I, Stockinger B, Diny NL. The AHR repressor limits expression of antimicrobial genes but not AHR-dependent genes in intestinal eosinophils. *Journal of Leukocyte Biology*. 2024 Jul 25;116(2):369-378. doi: 10.1093/jleuko/qiae105. PMID: 38701199; PMCID: PMC11271977.

Mayer M*, Cengiz-Dartenne SC*, Thiem M, Hatzfeld P, Semeniuk A, Wang N, Strugnell RA, Förster I, Weighardt H. Dysregulation of stress erythropoiesis and enhanced susceptibility to *Salmonella* Typhimurium infection in aryl hydrocarbon receptor-deficient mice. *The Journal of Infectious Diseases*. 2024 Jun 6;jiae304. doi: 10.1093/infdis/jiae304. Epub ahead of print. PMID: 38842164.

Graelmann FJ*, Gondorf F*, Majlesain Y, Niemann B, Klepac K, Gosejacob D, Gottschalk M, **Mayer M**, Iriady I, Hatzfeld P, Lindenberg SK, Wunderling K, Thiele C, Abdullah Z, He W, Hiller K, Händler K, Beyer MD, Ulas T, Pfeifer A, Esser C, Weighardt H, Förster I*, Reverte-Salisa L*. Differential cell type-specific function of the aryl hydrocarbon receptor and its repressor in diet-induced obesity and fibrosis. *Molecular Metabolism*. 2024 Jul;85:101963. doi: 10.1016/j.molmet.2024.101963. Epub 2024 May 29. PMID: 38821174; PMCID: PMC11214421.

Schneider S*, Kovacevic A*, **Mayer M**, Dicke AK, Arévalo L, Koser SA, Hansen JN, Young S, Brenker C, Kliesch S, Wachten D, Kirfel G, Strünker T, Tüttelmann F, Schorle H. Cylicins are a structural component of the sperm calyx being indispensable for male fertility in mice and human. *eLife*. 2023 Nov 28;12:RP86100. doi: 10.7554/eLife.86100. PMID: 38013430; PMCID: PMC10684152.

*contributed equally to the study

10. Acknowledgements

Finally, it is time to say **THANK YOU** to all those who have supported me in the past years on the way to my doctorate and who have contributed to this work in many different ways:

First of all, I would like to express my sincere gratitude to my supervisors Prof. Dr. Irmgard Förster and PD Dr. Heike Weighardt for giving me the opportunity to conduct my PhD project on this interesting topic and for their continuous support during this time. Their immense expertise and encouragement, as well as their thought-provoking impulses have enabled me to develop into an independent scientist.

Furthermore, I want to thank my mentor Prof. Dr. Johannes Hegemann for being the second referee of my PhD thesis, for his scientific advice, and for his ability to view and question my results from a different perspective.

A big thank you goes to all the past and present members of the Förster and Weighardt laboratory for their incredible scientific and mental support during my PhD time. They have become more than colleagues to me and created a warm and familiar working atmosphere that is unique. I am very grateful for their endless help with experiments, for the amusing conversations during our coffee breaks, and for our great adventures outside of the lab. Special thanks to Sevgi for introducing me into the *Salmonella* project, which ultimately ended in our joint publication.

I also want to thank the Jürgen Manchot Foundation for my doctoral scholarship, and the Manchot Graduate School “Molecules of Infection” for all scientific and financial support. Many thanks to Stephanie for her organizational assistance.

I am also thankful to Prof. Dr. Christoph Schneider for giving me the possibility to perform my stay abroad in his laboratory in Zurich and for sharing his great knowledge in tuft cell biology, and to all collaboration partners for their expertise.

Last but not least, I would like to thank my family, especially my parents and my brother, as well as my friends who supported and motivated me during my whole studies, starting from the Bachelor in Molecular Biomedicine to my PhD now. A special thanks goes to Simon, not only for building me up during difficult phases, but above all for his love on every single day. Thank you for always being there for me.