

# **Mitochondrial signaling as gatekeeper of intestinal health**

Habilitationsschrift

vorgelegt von

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## Research Question

The basis of this habilitation project is the hypothesis that **mitochondrial function and in particular mitochondrial unfolded protein response (MT-UPR) reflects the metabolic environment and serves as a checkpoint critically contributing to disease outcomes by controlling cell fate and immune activation**. Cellular metabolism has long been regarded as consequence of a given functional state; however, it is now believed that the metabolism itself can determine cellular phenotypic transitions. Hence, mitochondria increasingly emerge as key players in cell fate decisions, translating challenging conditions into changes of the cellular metabolism<sup>1</sup>. The MT-UPR senses disturbances of mitochondrial proteostasis and aims at restoring organelle function. Alterations in mitochondrial function and MT-UPR-activation are integral aspects of several pathologies, including inflammatory bowel diseases, colorectal cancer and obesity<sup>2</sup>.

Consequently, the research presented here is guided by the following fundamental questions: ***“How do mitochondrial signaling and metabolism integrate into the cellular signaling network to determine cellular phenotype and properties?”*** and ***“How does regulation of mitochondrial function contribute to tissue homeostasis or vice versa, does impaired metabolic flexibility underlie pathological situations?”*** These questions were tackled within the frame of the intestinal epithelium, which beyond its key role in gastrointestinal processes and pathologies also serves as a prototypic model of stem cell regulation and differentiation events.



## Table of contents

Research Question .....	3
Table of contents .....	5
The intestinal epithelium .....	7
Inflammatory bowel diseases .....	9
Mitochondrial signaling and function in epithelial homeostasis .....	11
The mitochondrial unfolded protein response .....	11
Ckmt1—a mitochondrial protein reduced under inflammatory conditions .....	12
New mouse models of MT-UPR signaling.....	13
Metabolic injury and tissue healing in the intestine .....	13
Metabolic injury under germ free conditions .....	16
Metabolic injury and the microbiome .....	19
Mitochondrial dysfunction and inflammation impair the intestinal stem cell niche .....	19
Mitochondrial dysfunction and inflammation impair intestinal epithelial cell differentiation .....	20
Mitochondrial dysfunction and inflammation affect the enteroendocrine cell lineage .....	21
Diet, epithelial Metabolism and the intestinal stem cell niche .....	23
Dietary lipids affect the enteroendocrine cell lineage .....	24
Intestinal organoids to model epithelial biology .....	30
Conclusion and perspective .....	36
References .....	38
Featured publications .....	45
I. Mitochondrial function - gatekeeper of intestinal epithelial cell homeostasis, Rath E., Moschetta A., Haller D., <i>Nat Rev Gastroenterol Hepatol.</i> 2018 Aug;15(8):497-516.....	45
II. PKR activation in mitochondrial unfolded protein response-mitochondrial dsRNA might do the trick, Rath E., <i>Front Cell Dev Biol.</i> 2023 Aug 29;11:1270341.....	46
III. Mitochondrial function controls intestinal epithelial stemness and proliferation, Berger E., Rath E., et al., <i>Nat Commun.</i> 2016; 7, 13171. ....	47
IV. Mitochondrial impairment drives intestinal stem cell transition into dysfunctional Paneth cells predicting Crohn's disease recurrence, Khaloian S.* , Rath E.* et al., <i>Gut.</i> Published Online First: 28 February 2020. ....	48
V. Mitochondrial perturbation in the intestine causes microbiota-dependent injury and gene signatures discriminative of inflammatory disease, Urbauer E., [...], Rath E., and Haller D, <i>Cell Host Microbe.</i> 2024 Aug 14;32(8):1347-1364.e10. ....	49
VI. Intestinal epithelial cell metabolism at the interface of microbial dysbiosis and tissue injury, Rath E. and Haller D., <i>Mucosal Immunol.</i> 2022 Apr;15(4):595-604. ....	50
VII. Inflammation Meets Metabolic Disease: Gut Feeling Mediated by GLP-1, Zietek T.and Rath E., <i>Front Immunol.</i> 2016 Apr 22;7:154.....	51
VIII. Reduced intestinal GLP-1+ cell numbers are associated with an inflammation-related epithelial metabolic signature, Urbauer E., [...], and Rath E .....	52

## TABLE OF CONTENTS

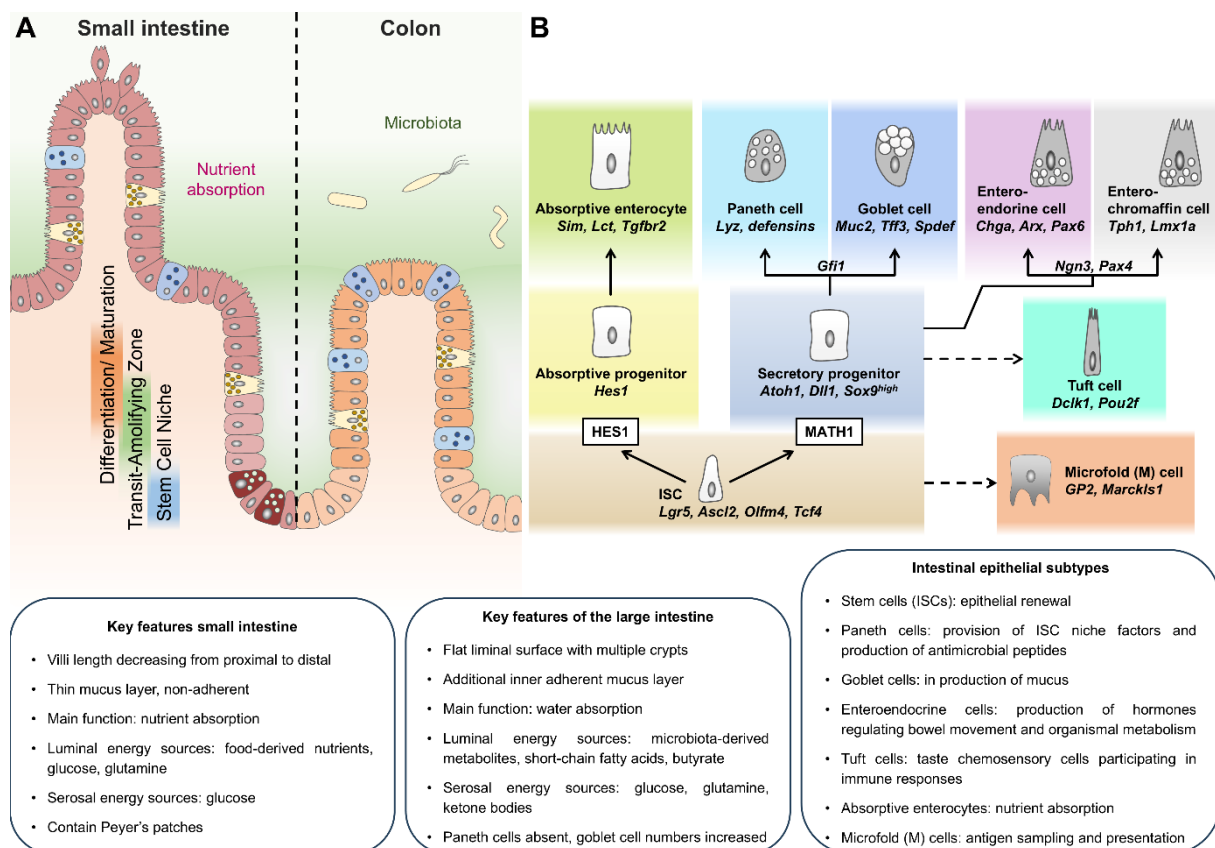
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IX. Drug screening, oral bioavailability and regulatory aspects: A need for human organoids, Zietek T., Boomgaarden WAD., and Rath E., <i>Pharmaceutics</i> . 2021 Aug 17;13(8):1280. ....	53
X. Intestinal organoids for assessing nutrient transport, sensing and incretin secretion, Zietek T.*, Rath E.*, et al., <i>Sci Rep</i> . 2015 Nov 19;5:16831. ....	54
XI. Organoids to Study Intestinal Nutrient Transport, Drug Uptake and Metabolism – Update to the Human Model and Expansion of Applications, Zietek T., [...] and Rath E., <i>Front. Bioeng. Biotechnol</i> . 2020 8:577656.....	55
XII. a Organoids—Mini Guts Help Answer Big Questions About Intestinal Nutrient Transport, Zietek T. and Rath E., <i>Front. Young Minds</i> . 2023 11:717455.....	56
XII. b Organoid—Mini Därme helfen große Fragen zum Nährstofftransport zu beantworten, Zietek T. and Rath E., <i>Front. Young Minds</i> . 2023 11:717455. (Deutsch) .....	57
Collaborator publications .....	58
(A) The gut microbiota drives the impact of bile acids and fat source in diet on mouse metabolism, Just S., [...], Rath E., et al., <i>Microbiome</i> . 2018 Aug 2;6(1):134. ....	58
(B) Intestinal sodium/glucose cotransporter 3 is downregulated in obese mice and humans, Soták M., Casselbrant A., Rath E., et al., <i>Life Sci</i> . 2021 Feb 15;267:118974.....	58
(C) A mitochondrial unfolded protein response inhibitor suppresses prostate cancer growth in mice via HSP60, Kumar R., [...], Rath E., et al., <i>J Clin Invest</i> . 2022 Jul 1;132(13):e149906.....	58
Featured Poster .....	59
I. Mitochondrial stress is associated with altered mtCK expression in the intestinal epithelium (4 <sup>th</sup> Seeon Conference, DGHM) .....	59
II. Featured Poster II – Dietary fatty acids increase intestinal L cell numbers independent of the development of obesity (18 <sup>th</sup> NuGOweek) .....	60
Complete publication list.....	61
Curriculum Vitae .....	65

## The intestinal epithelium

The intestinal epithelium is a multicellular interface intimately connected to a dense microbial milieu. By simultaneously secreting mucus, proteases, hormones, and immune mediators, intestinal epithelial cells (IECs) not only regulate digestive processes but also establish a physical and immune barrier to protect the host. Serving as a critical interface for nutritional signals, the complex microbial ecosystem, and the immune system, IECs are increasingly recognized as a key regulators of metabolic and immune homeostasis<sup>3</sup>.

The epithelial monolayer undergoes complete renewal every 3–5 days<sup>4</sup>, likely as a protective mechanism against injury and infection. With an estimated 1,400 cells shed from a single villus tip per day<sup>5</sup>, the intestinal epithelium exhibits the highest turnover rate of any fixed-cell population in the body. Maintaining tissue homeostasis and to mount regenerative responses to injury require precise regulation of cell proliferation and differentiation of stem-cell-derived progenitors. The intestinal epithelium comprises distinct IEC subtypes (**Figure 1**), including intestinal stem cells (ISCs), which drive the continuous renewal of the epithelial layer.



**Figure 1. The intestinal epithelium and epithelial subtypes**

(A) Tissue architecture of the intestinal epithelium and (B) differentiation hierarchy of intestinal epithelial cells giving subtypes and important transcription factors/ marker genes.

Self-renewal, proliferation, fate determination, differentiation, and apoptosis of IECs occur in distinct anatomical zones along the crypt-villus axis. The stem cell niche, located at the base of the crypts, contains both quiescent and actively dividing ISCs, along with early progenitor cells that enter the transit-amplifying zone. Here, IEC progenitors undergo multiple rounds of rapid proliferation before committing to either the secretory or absorptive lineage. As they migrate toward the villus tip, senescent IECs ultimately detach from the basement membrane and are exfoliated via anoikis<sup>6</sup>. This highly coordinated process ensures epithelial barrier integrity and is tightly regulated, yet it is profoundly affected or disrupted in the presence of inflammatory or neoplastic pathologies of the gastrointestinal tract such as inflammatory bowel diseases (IBD) or colorectal cancer (CRC)<sup>7, 8</sup>. The rapid phenotypic transitions during IEC differentiation are accompanied by dynamic changes in mitochondrial function and metabolism. Key differences between stem or progenitor cells and mature IECs include shifts in metabolic pathways such as oxidative phosphorylation (OXPHOS) and glycolysis. In line, growing evidence has positioned mitochondria at the core of diverse cellular functions, including cell cycle regulation, stemness, autophagy, pathogen recognition, and immune response modulation<sup>2, 9</sup>. Mitochondrial signaling is mediated by key molecules involved in energy metabolism, such as ATP and reactive oxygen species (ROS). In turn, these mediators are tightly linked to cellular stress response pathways, including mitochondrial unfolded protein response (MT-UPR), AMP-activated protein kinase (AMPK), and inflammasome activation (reviewed in **Featured Publication I**).

However, the molecular mechanisms linking mitochondrial dysfunction to pathological outcomes remain poorly understood. In particular, it is still unclear how mitochondrial signaling integrates into complex cellular stress pathways and whether mitochondrial impairment is a cause or consequence of diseases. Further elucidating mitochondrial signaling and function at the molecular level, along with characterizing their impact on IEC function and phenotype (**Featured Publication II, III, IV, VIII**), provides critical insights into disease-relevant mechanisms. Moreover, illustrating the role of epithelial mitochondrial fitness and metabolic dysfunction in IBD (**Featured Publication IV, V, VI, VIII**) helps identify key processes as potential targets for therapeutic intervention.

A major advancement in studying the intestinal epithelium is the development of intestinal organoid culture, which enables the generation of organ-like 3D structures directly from (induced) stem cells or crypts of murine and human origin. Adopting and refining this technique allows for tracking the differentiation of stem cells into all intestinal epithelial cell types while simultaneously monitoring metabolism at high (cellular) spatial resolution (**Featured Publication II, IV, VIII, X, XI**). Moreover, intestinal organoids provide a screening platform for identifying mitochondria-protective compounds that could serve as novel adjuvant therapies for intestinal pathologies (**Featured Publication IV, IX, XI**).

## Inflammatory bowel diseases

Crohn's disease (CD) and ulcerative colitis (UC) are the two main clinical phenotypes of IBD<sup>10</sup>, with dysbiotic changes in the intestinal microbiome emerging as a common link between host genetic susceptibility and external cues<sup>11</sup>. IBD exemplify the complex interplay between genetic and environmental factors in the development and progression of immune-mediated pathologies. Along with metabolic disorders, their incidence and prevalence are rising, following a pattern of industrialization<sup>12, 13</sup>. Crohn's disease is characterized by discontinuous and transmural inflammation, predominantly affecting the ileo-colonic region, whereas ulcerative colitis is restricted to the colonic mucosa. The intestinal epithelium represents the frontline of IBD pathogenesis, located between the two key drivers of intestinal inflammation: the luminal microbiome and the host immune system<sup>14</sup>. Consequently, a breach in this well-structured barrier is considered a cornerstone of chronic inflammation<sup>15</sup>. IECs are crucial for maintaining intestinal homeostasis, as they not only form a barrier, but also mediate interactions between the intestinal microbiota and the immune system. The loss of mucosal tolerance toward the commensal microbiota is a central paradigm in IBD pathogenesis<sup>16</sup>, highlighting the critical role of epithelial function in preventing or driving intestinal inflammation.

In the course of IBD, the intestinal epithelium is challenged by repetitive wounding due to recurrent inflammatory episodes triggered by numerous yet unidentified interacting factors. Accordingly, crypt hyperplasia, a hallmark of IBD<sup>17, 18</sup>, represents an excessive, chronically activated epithelial repair program, characterized by the expansion of transit-amplifying cells and a concomitant reduction in terminally differentiated epithelial cells<sup>19</sup>. Effective healing requires IEC dedifferentiation and phenotypic changes, as well as tightly regulated cell proliferation<sup>1</sup>. These processes are closely associated with alterations in mitochondrial function and metabolic shifts<sup>20, 21</sup>. Given the high metabolic demands of the epithelial interface, particularly under chronic inflammatory conditions, limited mitochondrial flexibility may thus ultimately tip the scale from homeostasis to pathology. Furthermore, the metabolic program meant to support regeneration could eventually contribute to epithelial dysfunction under persistent inflammatory stress.

Supporting the hypothesis that mitochondrial perturbations and limited metabolic flexibility sensitize the intestinal epithelium to additional insults<sup>22-24</sup> and represent an underlying cause of disease, approximately 5% of IBD susceptibility genes (>240) identified<sup>11, 25, 26</sup> are implicated in the direct regulation of mitochondrial homeostasis<sup>27</sup>, including key functions such as mitochondrial fatty acid oxidation<sup>28</sup>, mitochondrial fission<sup>29</sup>, and mitophagy<sup>16, 27, 30-34</sup>. Furthermore, mitochondrial-encoded DNA polymorphisms have been associated with ulcerative colitis<sup>35, 36</sup>, and studies using conplastic mouse models—harboring identical nuclear DNA but distinct mitochondrial DNA—suggest that enhanced mitochondrial OXPHOS activity and ATP levels confer protection against experimental colitis<sup>37</sup>.

At the same time, clear evidence underscores mitochondria as a central target of active inflammation in IBD. Altered expression patterns of mitochondrial genes and proteins, mitochondriopathy, disrupted mitochondrial dynamics, mitochondrial dysfunction, and activation of mitochondrial stress signaling have been observed in IBD patients<sup>1, 38, 39</sup>. Moreover, enterocytes from affected individuals exhibit swollen mitochondria with irregular cristae, indicative of impaired function<sup>40, 41</sup>. In line with these findings, reduced ATP levels have been reported in colonic tissue from a subset of Crohn's disease patients<sup>42</sup> (reviewed in **Featured Publication I**).

As previously mentioned, an altered microbial milieu is evident during both the initiation and progression of disease activity in IBD. Notably, the microbiome plays a crucial role in providing metabolic support to the epithelium, and disruptions in host-microbiome metabolic circuits — including butyrate<sup>43</sup>, carnitine<sup>44</sup>, purine<sup>45</sup>, and tryptophan metabolism<sup>46</sup> — have been implicated in the regulation of epithelial regenerative capacity and the development of chronic inflammation, making them particularly relevant in IBD<sup>39</sup>. Beyond the metabolic capacity of the epithelium itself, the availability of metabolic substrates may hence additionally determine disease outcomes or contribute to maladaptive host-microbiome metabolic interactions.

Consequently, metabolic fitness has emerged as a new frontier in intestinal epithelial homeostasis and disease pathogenesis, with multiple extrinsic factors, such as the microbiome and diet, converging with intrinsic factors at this critical junction. Building on this concept, “metabolic injury” has been introduced as a cell-autonomous mechanism of tissue wounding resulting from mitochondrial perturbation. In the context of IBD, metabolic injury arises from intrinsic defects in cellular metabolism, impairing the epithelium's regenerative capacity and contributing to unresolved injury. A combination of genetic predisposition and environmental triggers drive metabolic injury, leading to mitochondrial exhaustion, epithelial dysfunction, and recurrent inflammation (**Featured Publication VI**).

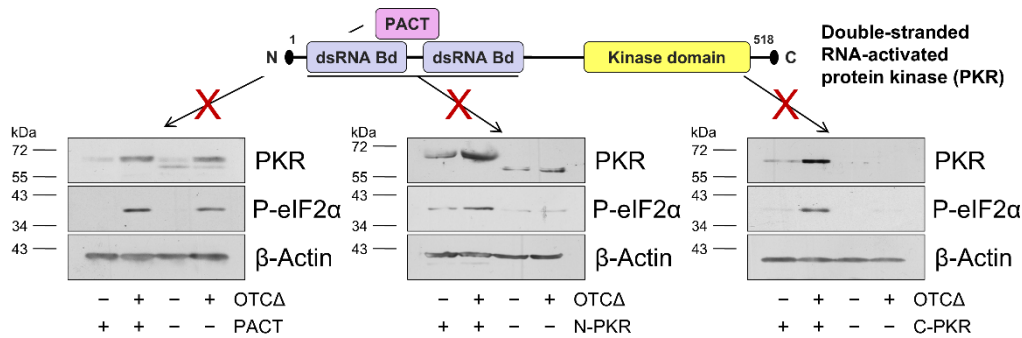
## Mitochondrial signaling and function in epithelial homeostasis

Comparing IECs derived from mouse models of inflammation<sup>47</sup> and IBD patients<sup>48</sup> with their non-inflamed counterparts by a gel-based proteome approach and subsequent microarray analyses<sup>47</sup> provided strong evidence for activated MT-UPR signaling and profound mitochondrial changes under inflammatory conditions. In particular, the mitochondrial chaperone heat shock protein (HSP) 60 was consistently increased, a hallmark of MT-UPR activation<sup>49, 50</sup>.

## The mitochondrial unfolded protein response

MT-UPR is a conserved signaling pathway activated in response to protein aggregation or misfolding within the mitochondrial matrix. Mitochondrial proteostasis is essential for cellular metabolism, including OXPHOS, the tricarboxylic acid (TCA) cycle, and fatty acid biosynthesis. Thus, disturbances in mitochondrial protein homeostasis trigger retrograde signaling, leading to the upregulation of chaperones and proteases within the mitochondrial matrix, such as Hsp60 and the caseinolytic mitochondrial matrix peptidase (ClpP). Together with direct regulation of mitochondrial RNA translation, MT-UPR thereby improves the mitochondrial folding environment and preserves mitochondrial integrity<sup>51</sup>. Cells must continuously adapt their metabolism to normal physiological fluctuations, and transient and cell-specific activation of MT-UPR represents a homeostatic response essential for processes such as proliferation and differentiation. However, sustained and uncontrolled activation of MT-UPR contributes to the structural and functional disruption of the epithelium and is associated with chronic, progressive pathologies such as IBD<sup>52</sup>.

Although a growing number of components involved in the mammalian MT-UPR have been identified in recent years, many fundamental questions remain unresolved. These include the nature of the initial signal as well as the unidentified molecular mediators responsible for sensing and transmitting the retrograde signal to the nucleus<sup>53</sup>. In 2011, we identified the double-stranded RNA (dsRNA)-activated protein kinase (PKR) as a signaling component of the mammalian MT-UPR and demonstrated its relevance to inflammatory bowel disease<sup>54</sup>, findings that have subsequently been corroborated<sup>55, 56</sup>. However, it remained unclear which specific signal triggers PKR activation in response to MT-UPR induced by the expression of a mutant form of ornithine transcarbamylase (OTCΔ), which accumulates in a misfolded state within the mitochondrial matrix<sup>50, 54</sup>. However, in 2018, it was demonstrated that PKR can be activated by mitochondrial RNA that exist as intermolecular dsRNA, in particular under stress conditions<sup>57</sup>. Interestingly, HSP60 has been implicated in the retention of mt-dsRNAs within mitochondria<sup>58</sup>. The identification of mitochondrial RNA as a signal for PKR activation aligns with findings showing that downstream MT-UPR signaling requires both the catalytic domain and the dsRNA-binding domain of PKR but is independent of cellular protein activator of PKR (PACT)<sup>47, 54</sup> (**Figure 2**).



**Figure 2. Mitochondrial UPR-associated Pkr signaling requires dsRNA binding ability but is independent of Pact**

MEF cells of different genotypes were transfected with OTCAΔ cDNA for 32h to induce MT-UPR and analyzed for PKR expression and eIF2α phosphorylation by Western blot.

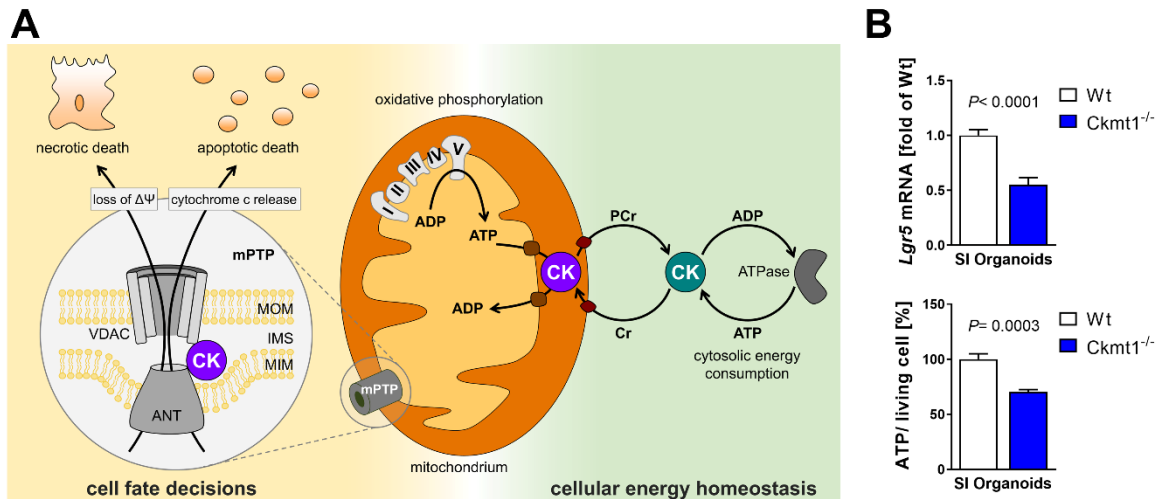
The hypothesis that also MT-UPR-associated PKR activation is mediated via mitochondrial dsRNA (**Featured Publication II**) was substantiated in 2024, when depletion of coenzyme Q, a component of OXPHOS, was shown to trigger cytosolic accumulation of mitochondrial RNA, leading to PKR activation and initiating MT-UPR signaling<sup>59</sup>. These findings contribute to a more comprehensive understanding of mitochondrial stress signaling and provide a framework for exploring novel therapeutic targets in pathology-associated mitochondrial dysfunction.

### Ckmt1—a mitochondrial protein reduced under inflammatory conditions

In the previously mentioned gel-based proteome analysis, mitochondrial proteins accounted for a large proportion of proteins regulated under inflammatory conditions<sup>47</sup>. A protein that, in contrast to Hsp60, showed markedly reduced expression, was ubiquitous mitochondrial creatine kinase (Ckmt1). Ckmt1 plays a crucial role in cellular energy shuffling between mitochondria and cytosol by catalyzing the reversible transfer of the phosphoryl group from phospho-creatine to ADP<sup>60</sup>. At the same time, Ckmt1 resides at the mitochondrial permeability transition pore (MTP) and is thought to maintain MTP closure. Being particularly susceptible to oxidative modifications, Ckmt1 may switch from an octameric to a dimeric conformation upon exposure to reactive oxygen species (ROS), leading to MTP opening and subsequent pro-apoptotic signaling<sup>60</sup>. Thus, Ckmt1 might be a crucial protein in IECs under inflammatory conditions, linking metabolism and mitochondrial live-or-death decisions.

Notably, reduced Ckmt1 levels in IECs were confirmed in various mouse models of chronic inflammation as well as IBD patients. In contrast, increased Ckmt1 expression was observed during the onset of inflammation *in vivo* and under mild mitochondrial stress conditions *in vitro*, suggesting a tissue-protective role for Ckmt1 (**Featured Poster I**). Utilizing Ckmt1-deficient mice to further investigate the role of Ckmt1 revealed that, despite a normal intestinal architecture, epithelial cells exhibit increased expression of stem cell marker genes. At the same time, intestinal organoids derived from these mice display diminished cellular ATP levels

(**Figure 3**), indicating disturbances in cellular energy metabolism. Further experiments indicated that *Ckmt1*<sup>-/-</sup> organoids might be more vulnerable to additional stressors, supporting the notion that cell intrinsic limitations in metabolic flexibility render the epithelium susceptible to additional triggers required for full-blown pathology. Moreover, these findings underscore the link between cellular metabolism and epithelial functions.



**Figure 3. Roles of *Ckmt1* in cellular metabolism and for epithelial homeostasis**

(A) Illustration of proposed functions of *Ckmt1* (B) Expression of the stem cell marker *Lgr5* and ATP levels in intestinal organoids derived from wild type (Wt) and *Ckmt1* knockout (*Ckmt1*<sup>-/-</sup>) mice.

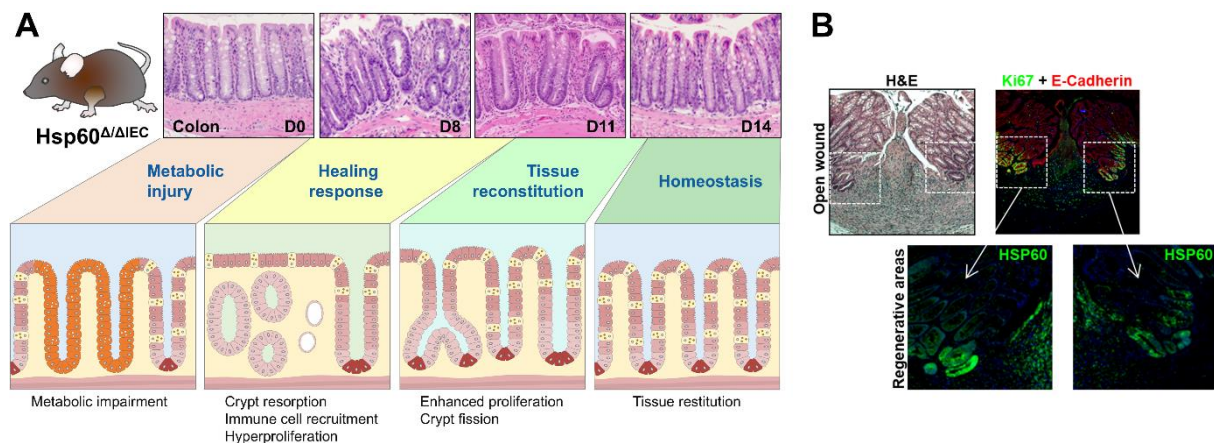
### New mouse models of MT-UPR signaling

Building on the findings of sustained HSP60 expression and activated MT-UPR signaling in IECs under chronic inflammation in both mice and humans<sup>61</sup>, novel mouse models bearing genetic modifications in MT-UPR-associated genes were developed. These comprised an inducible transgenic model (C/EBP homologous protein (Chop)) as well as inducible knockout models (Hsp60, ClpP). Using these MT-UPR models in combination with IEC-specific Cre lines (*Vil1Cre* and *Vil1CreER*<sup>T2-Tg</sup>) and an intestinal stem cell-specific Cre line (*Lgr5CreER*<sup>T2</sup>-IRES-*Egfp*<sup>Tg</sup>), the critical role of mitochondrial function and MT-UPR-signaling in epithelial homeostasis and regeneration was further scrutinized.

### Metabolic injury and tissue healing in the intestine

Tamoxifen-induced, IEC-specific loss of Hsp60 in the *Vil1CreER*<sup>T2-Tg</sup> model results in mitochondrial dysfunction and abolishes stemness and cell proliferation, likely due to the inability to meet the high metabolic and energetic demands of these cell types (**Featured Publication III**). In line, alterations in cell cycle progression and delayed wound closure are observed in mice overexpressing the MT-UPR-relevant transcription factor CHOP in IECs<sup>62</sup>. Notably, the complete loss of proliferative capacity in Hsp60-negative crypt IECs is

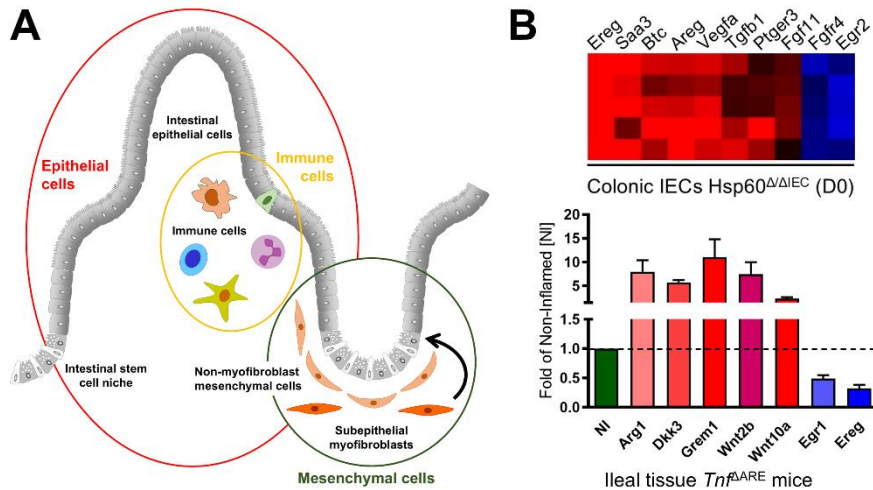
accompanied by a compensatory hyperproliferation of residual stem cells that escaped *Hsp60* deletion in this model (**Featured Publication III**). These highly proliferative tissue regions rapidly develop and are characterized by strong expression of Hsp60, initially forming crypt nodules and ultimately leading to complete tissue reconstitution. Most likely reflecting differences in proliferation rates and metabolic demands, a gradient is evident, with regenerative crypt architecture appearing and epithelial restitution occurring more rapidly in the proximal small intestine compared to the ileum and colon. This sequence of mucosal injury and healing mirrors physiological wounding and inflammatory flares (**Figure 4**).



#### Figure 4. Intestinal wound healing requires metabolic rewiring of epithelial cells

(A) Sequence of metabolic injury and mucosal healing in the epithelial-specific *Hsp60* knockout model. Representative H&E stainings of colonic tissue section, days (D) refer to day after end of tamoxifen treatment. (B) Colonic wound healing after injury induced by biopsy clams. Hyperproliferative, regenerative epithelial areas are strongly positive for Hsp60, indicating the requirement of a high mitochondrial capacity for healing processes.

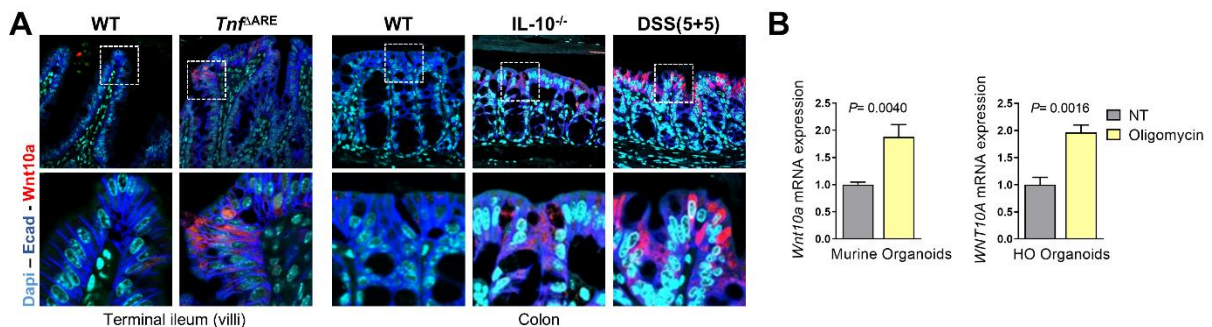
Interestingly, *Hsp60* deficiency restricted to intestinal stem cells (*Lgr5CreER*<sup>T2-Tg</sup> model) causes exclusively a transient loss of stem cells without affecting tissue morphology, highlighting metabolically impaired mature IECs as origin of regenerative signals. Indeed, HSP60-deficient IECs, including Paneth cells, have been shown to produce Wnt-related factors and express genes involved in the paracrine communication between IECs, immune cells, and mesenchymal cells<sup>63</sup> (**Featured publication III, Figure 5**), thereby creating an epithelial microenvironment that facilitates healing and orchestrates mucosal restitution. The Wnt signaling pathway, together with epidermal growth factor, Notch, and bone morphogenetic protein signaling, critically determines intestinal stemness and proliferation. Paneth cells, the surrounding mesenchymal stroma but also macrophages provide the respective ligands and soluble factors<sup>4</sup>. The induction of Wnt factors in genetic models of ileitis (*Tnf*<sup>ΔARE</sup> mice) and colitis (*Il-10*<sup>-/-</sup> mice) and chemically induced colitis (DSS colitis) underscores the physiological relevance of the observations made in the MT-UPR model (**Figure 6A**).



**Figure 5. Tissue communication coordinates mucosal healing processes**

(A) Signals from the microenvironment influence epithelial regeneration. (B) Hsp60-deficient metabolically impaired differentiated colonocytes are a source of growth factors and other mediators fostering epithelial turnover (upper panel). Wnt-related signaling changes under inflammatory conditions (lower panel, all genes given differ significantly from non-inflamed *Tn*<sup>ARE</sup> mice).

Furthermore, the upregulation of Wnt10a in murine and human intestinal organoids upon OXPPOS inhibition by oligomycin highlights the direct link between mitochondrial metabolism and Wnt factor expression (Figure 6B). However, tissue communication is likely mediated not only by Wnt factors and other secreted molecules such as cytokines<sup>63</sup> but also by metabolites themselves.



**Figure 6. Wnt10a is induced during inflammation, healing, and by OXPPOS inhibitor**

(A) Representative stainings of tissue sections from inflamed mice (*Tn*<sup>ARE</sup> mice, IL-10<sup>-/-</sup> mice) and mice exposed to DSS for 5 days (chemical injury) and a subsequent recovery phase of 5 days (healing) showing enhanced Wnt10a expression in mature cells in proximity to the lumen. (B) Wnt10a expression is induced in murine (left) and human (right) intestinal organoids treated with the OXPPOS inhibitor oligomycin for 6h.

Arginine and tryptophan metabolism are prominent examples for pathways giving rise to important tissue mediators, and genes involved in these pathways (*indolamine-2,3-dioxygenase (Ido)1*, *inducible nitric oxide synthase (Nos2)*, *arginase (Arg)1*) show pronounced

changes following epithelial Hsp60 loss (**Featured Publication V**). Additionally, apoptotic cells can be a source of metabolic mediators, as they release a wide range of molecules produced through metabolism that can act as tissue messengers promoting healing processes<sup>64, 65</sup>. Considering physiological IECs shedding at the villus tip but also pro-apoptotic processes induced by metabolic impairment and/or inflammatory conditions, dying cells and their metabolic signals might account for a significant proportion of tissue mediators in the intestine. In summary, IEC metabolism and metabolites might serve as critical signals to lamina propria cells, including immune cells, regulating wound healing-associated immune cell recruitment and phenotypes. Conversely, signals from the surrounding tissue might steer IEC proliferation to promote tissue repair, forming a tightly regulated circuit that restores mucosal homeostasis. These findings are particularly relevant to IBD and CRC, as a chronically stressed, metabolically compromised inflamed epithelium may foster a pro-proliferative microenvironment permissive to tumor development.

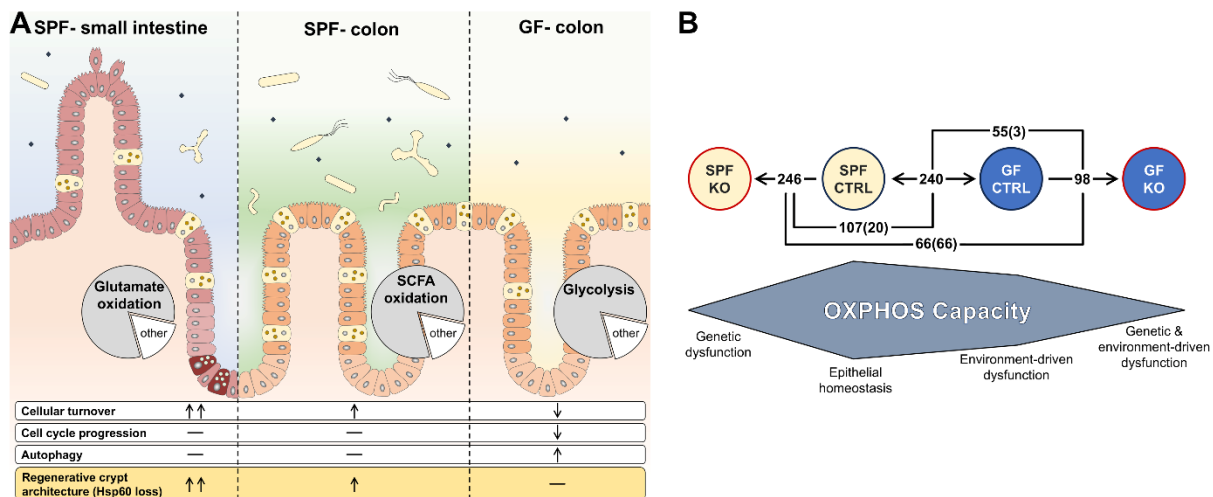
### **Metabolic injury under germ free conditions**

A remarkable finding is that regenerative crypt architecture fails to develop in the distal colon when *Vil1CreER<sup>T2-Tg</sup>*-mediated Hsp60 loss is induced in germ-free (GF) mice. Interestingly, this is not the case for the small intestine and the proximal colon, which retain an aberrant epithelial architecture similar to specific pathogen-free (SPF) mice. Nevertheless, IECs in the distal colon regain Hsp60 expression, although the increase in proliferation upon Hsp60 loss is attenuated compared to SPF mice (**Featured Publication V**). This pattern aligns with the previously mentioned temporal occurrence of regenerative crypt architecture in the SPF model and similarly, might reflect differences in epithelial proliferation rates and metabolism across different anatomical sides (**Figure 7A**).

Regarding their metabolic environment, IECs encounter several challenges: pH ranges from 7.3 in the ileum to 5.7 in the cecum<sup>66</sup>; a steep oxygen gradient across the epithelial layer from approximate 7% oxygen at the serosal side to 3% at the villus tip; and enormous blood and oxygen flow fluctuations after food intake<sup>67, 68</sup>. Both the small and large intestinal epithelium primarily depend on luminal rather than vascular energy supply and have a high energy demand due to extensive transport processes and rapid cell turnover - despite lower proliferation rates in the colon<sup>69</sup>. IECs are highly specialized according to their anatomical location, while distinct anatomical and functional regions of the intestinal tract, in turn, create spatially adapted microbial habitats<sup>70</sup>. As a result, small intestinal enterocytes predominantly utilize glucose and glutamine for energy generation, whereas microbiota-derived short-chain fatty acids (SCFAs) serve as the main energy source for colonocytes<sup>1</sup>.

Consistently, GF mice show reduced ATP levels in colonic, but not small intestinal tissues<sup>71</sup>, and additional studies underscore the profound impact of the microbiota on colonic IEC

maturation and differentiation<sup>72, 73</sup>. Colonocytes from GF mice show a metabolic shift from OXPHOS to glycolysis<sup>74</sup> accompanied by induction of autophagy and diminished cell cycle progression<sup>71</sup>. Of note, the described accumulation of GF colonocytes in the G1 phase resembles the reduced cell cycle progression observed in Chop-overexpressing mice, which has been associated with reduced wound healing in the colon *in vivo*<sup>62</sup>. *Vice versa*, SCFAs regulate genes involved in mitochondrial biogenesis and energy metabolism while promoting IEC growth<sup>75-77</sup>. Likewise, other microbiota-derived metabolites including lactate<sup>20</sup> and purines<sup>78</sup> can fuel epithelial metabolism and accelerate colonocyte turnover<sup>79</sup>. As such, they act as checkpoint metabolites, critically determining mucosal regenerative capacity<sup>78</sup>. This highlights the importance of microbial-metabolic circuits in the intestine, in particular as meeting the energy demand required for mucosal healing appears to be a bottle neck during acute injury and chronic inflammation<sup>80-82</sup>.

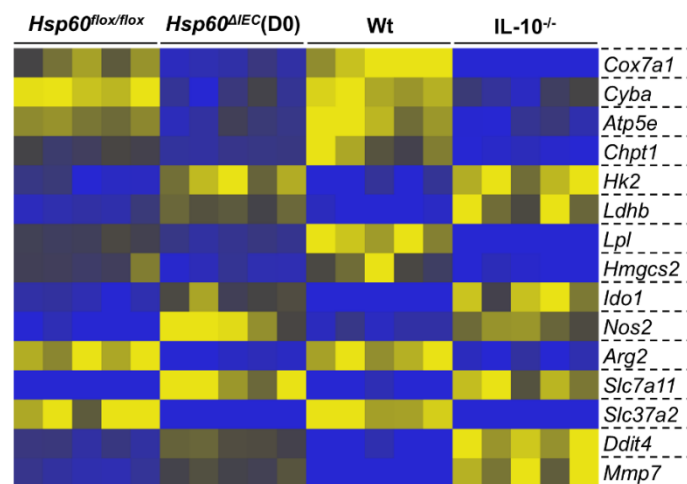


**Figure 7. Location and luminal environment determine intestinal epithelial metabolism and regenerative environment**

(A) Illustration of the main energy source and epithelial properties along the gastrointestinal tract and under germ-free conditions. (B) NanoString analysis of genes involved in metabolic processes and immunometabolism in Hsp60-deficient SPF and GF colonocytes and adequate controls. Numbers indicate significantly regulated genes, numbers in brackets indicate genes regulated in the same direction.

With regard to the absence of a regenerative crypt architecture in GF mice, the lack of metabolic support by the microbiota might have several implications: (I) The absence of mucosal aberrations could be due to the GF epithelium's inability to mount a proliferative response strong enough to cause overt crypt hyperplasia, owing to inadequate metabolic supply. Instead, metabolically impaired colonocytes are continuously replaced by Hsp60-positive cells in a steady-state-like turnover process. (II) Given that GF colonocytes exhibit diminished OXPHOS and compensatory upregulation of glycolysis, and that Hsp60 deficiency

similarly impairs OXPHOS while shifting metabolism towards glycolysis, the overall metabolic impact of Hsp60 loss in GF colonocytes might be attenuated. This hypothesis is supported by transcriptional profiling of genes involved in metabolic processes and immunometabolism in Hsp60-deficient SPF and GF colonocytes and adequate controls (**Featured Publication V**). Of note, the analysis comprises colonocytes early after induction of Hsp60 deletion (day 0), before onset of mucosal aberrations in SPF mice. Considering the mere number of differentially expressed genes (DEGs) between SPF Hsp60 knockout *versus* SPF control (246) and GF control *versus* SPF control (240) it becomes evident that the genetically induced metabolic dysfunction has an impact similar to that of the metabolically deprived environment (**Figure 7B**). In contrast, comparing GF Hsp60 knockout *versus* GF control revealed only 98 DEGs, 66 of which overlapped with those from the SPF Hsp60 knockout *versus* SPF control comparison. These genes showed the same direction of regulation and represent the core set of genes affected by Hsp60 loss. Accordingly, the transcriptional response to Hsp60 loss is diminished in the metabolically deprived GF environment. Supporting the hypothesis that GF colonocytes employ compensatory mechanisms to counteract inadequate luminal metabolic supply, 87 out of 107 DEGs shared between SPF Hsp60 knockout *versus* SPF control and GF Hsp60 knockout *versus* GF control comparisons were regulated in opposite directions (**Figure 7B**).



**Figure 8. Metabolically impaired and inflamed colonocytes show similar changes in metabolic gene expression patterns**

Heat map of significantly regulated metabolic genes comparing expression patterns of metabolically impaired Hsp60-deficient colonocytes with colonocytes derived from inflamed Il-10-deficient mice.

Highlighting the relevance of the findings from the Hsp60 model, the transcriptional fingerprint of metabolic genes in Hsp60-deficient colonocytes closely resembled that of colonocytes from inflamed Il-10-deficient mice (**Figure 8**). Most importantly, the metabolic

gene signature identified in the murine Hsp60-deficient mucosa successfully distinguished between inflamed and non-inflamed tissue sections of CD patients undergoing surgical therapy, underscoring the translational potential and clinical significance of metabolic alterations in IECs (**Featured Publication V**).

### **Metabolic injury and the microbiome**

Of note, the metabolic interaction of microbiome and host at the intestinal interface is bidirectional, and epithelial metabolism has been suggested to actively shape the microbiome<sup>83, 84</sup>. To this end, colonocytes primarily oxidize butyrate and other SCFAs via OXPHOS, with SCFA oxidation accounting for ~70% of epithelial oxygen consumption<sup>85</sup>. This high oxygen demand maintains a hypoxic epithelial surface, fostering a microbiota dominated by obligate anaerobes that convert fiber into fermentation products, making an otherwise inaccessible energy source available to the host<sup>86</sup>. Upon injury or inflammation, this mutualism is disrupted as colonocytes shift away from fatty acid oxidation, reducing epithelial oxygen consumption. The resulting increase in luminal oxygen, along with inflammatory by-products like nitrate, promotes the expansion of facultative anaerobes such as *Enterobacteriaceae*<sup>86, 87, 88</sup>. Consequently, *Vil1CreER<sup>T2-Tg</sup>*-mediated Hsp60 loss is accompanied by transient changes in the microbiome following the course of metabolic injury, healing and tissue restitution (**Featured Publication V**). This host-driven metabolic shaping of the microbiota may represent another piece of the puzzle in understanding how inflammation leads to dysbiosis, as the functional specificity and underlying causes of dysbiosis still remain poorly understood<sup>89, 90</sup> despite enormous efforts in cataloging microbiome alterations<sup>91</sup>.

### **Mitochondrial dysfunction and inflammation impair the intestinal stem cell niche**

However, microbiota alterations observed upon Hsp60 loss might not only result from changes in IEC metabolism but also from functional changes in epithelial subtypes. For instance, mucus-secreting goblet cells are reduced in the Hsp60-deficient colonic epithelium (**Featured Publication V**), and antimicrobial peptide (AMP)-producing Paneth cells acquire a dysfunctional phenotype following Hsp60 loss (**Featured Publication III, IV**).

Paneth cells (PCs) directly sense the microbial environment and release AMP-filled granules to shape the microbiota and prevent microbial invasion. In CD, PC defects are common<sup>92</sup> and are even discussed as sites of origin for CD<sup>93, 94</sup>. Consequently, PC dysfunction has been linked to CD-associated dysbiosis, characterized by decreased species richness and altered bacterial composition<sup>95, 96</sup>. Disturbances of AMP-packaging into granules and alterations in the granule exocytosis pathway manifest as disorganized and reduced numbers of cytoplasmic granules as well as a diffuse cytoplasmic lysozyme expression<sup>97</sup>. Notably, mitochondrial morphology and function closely correlate with PC phenotypes<sup>93, 97-99</sup>,

highlighting the critical role of metabolism for PC integrity and function. Consequently, phenotypic alterations of PCs are not exclusive to CD but are also observed in infections<sup>100</sup>, injury<sup>101</sup>, autophagy defects<sup>97, 99</sup>, and primary mitochondrial dysfunction<sup>94</sup>. Beyond their antimicrobial role, PCs are integral to the intestinal stem cell niche, providing Wnt signals to support stemness. However, the impact of PC dysfunction on intestinal stem cell homeostasis has been largely overlooked in the context of CD, despite evidence linking reduced PC defensin production to impaired Wnt signaling<sup>102, 103</sup>.

Building on the finding that metabolically impaired, Hsp60-negative PCs acquire Wnt10a expression in the *Vil1CreER<sup>T2-Tg</sup>* model to promote mucosal healing, the relation of PCs function and stem cells were further scrutinized in the intestinal stem cell-specific (*Lgr5CreER<sup>T2-Tg</sup>*) Hsp60 knockout model. Loss of stemness in this model is not a result of apoptotic cell death; rather, Hsp60 loss in intestinal stem cells drives their differentiation into Paneth cells. However, the inadequate metabolic supply resulting from Hsp60 deficiency-induced mitochondrial dysfunction maintains PCs in a dysfunctional state (**Featured Publication IV**).

Notably, these results and also the interrelation of PC dysfunction and intestinal stemness were reflected in *Tnfr<sup>AARE</sup>* mice, a model of CD-like ileitis in which inflammation depends on microbial composition<sup>104</sup>. In this model, inflammation severity correlated with the number of dysfunctional PCs as well as to reduced stemness. These findings were accompanied by MT-UPR induction and mitochondrial dysfunction in ileal crypts under inflammatory conditions. Most importantly, analysis of tissue sections from a cohort of CD patients confirmed the findings from the animal models regarding PC morphology and intestinal stem cells. Moreover, quantifying these alterations in the stem cell niche in non-affected tissue margins allowed for the prediction of early post-operative endoscopic recurrence (**Featured Publication IV**).

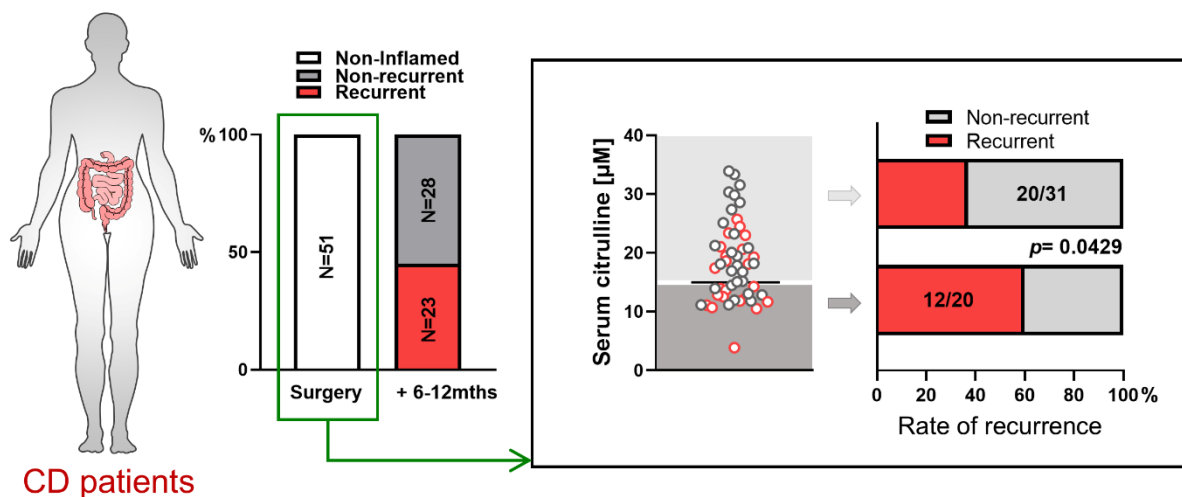
These data suggest that mitochondrial dysfunction and the associated aberrant phenotype of PCs and ISCs are early events in CD pathology. Importantly, several ileal mucosa-associated bacterial taxa have also been linked to endoscopic recurrence and can predict relapse in CD patients<sup>105</sup>, highlighting host-microbiome circuits before the onset of overt inflammation. Further research is needed to determine whether these alterations are initiating events or compensatory responses to maintain tissue homeostasis.

### **Mitochondrial dysfunction and inflammation impair intestinal epithelial cell differentiation**

Unsurprisingly, changes in IEC subtypes were not restricted to the stem cell niche upon loss of Hsp60 in the epithelium. In particular, a reduction in secretory cell types, including goblet, tuft, and enteroendocrine cells, along with the emergence of villi-localized Lyz-Muc2 double-positive cells, pointed to impairments in terminal differentiation<sup>106</sup>. Similar changes are observed in animal models of intestinal inflammation and IBD patients, raising the hypothesis

that altered epithelial cell composition under inflammatory conditions might be driven by compromised mitochondrial function (**Featured Publication VIII**).

Supporting the idea of diminished cell maturation, severely inflamed CD patients exhibit reduced plasma levels of citrulline<sup>107, 108</sup>, an amino acid produced in enterocytes of the small intestine in the mitochondrial matrix by the enzyme ornithine transcarbamylase (Otc). Reduced plasma citrulline levels might therefore indicate impaired mitochondrial function<sup>107, 109</sup> and consistently, mice show reduced citrulline level after IEC-specific loss of Hsp60 (**Featured Publication III**). Using the same CD patient cohort as for the assessment of the stem cell niche phenotype, a significantly higher probability of early post-operative endoscopic disease recurrence was observed for patients in remission with low levels of citrulline (**Figure 9**). This further underscores the strong link between epithelial mitochondrial function and inflammatory processes while also highlighting that mitochondrial metabolism is not only essential for the stem cell niche but also plays a key role in governing IEC differentiation and the functionality of terminally differentiated IECs.



**Figure 9. Serum citrulline level predict disease recurrence in CD patients**

Serum citrulline level in CD patients with non-active disease (non-inflamed) are lower in patients that experience recurrence 6-12 months later (REMIND-Cohort).

### Mitochondrial dysfunction and inflammation affect the enteroendocrine cell lineage

The reduction of goblet cells and Paneth cells have been extensively studied in the context of IBD and the associated dysbiosis<sup>97, 110-112</sup>. Yet, although data on their role in intestinal inflammation is scarce, enteroendocrine cells (EECs) might likewise contribute to pathogenesis and IBD symptoms like reduced appetite, anorexia and altered intestinal motility<sup>113-115</sup> (reviewed in **Featured Publication VII**).

Enteroendocrine cells (EECs) are traditionally known for their role in regulating gastrointestinal motility, digestive fluid secretion, and insulin levels by the release of peptide hormones. This, in turn, ensures optimal absorptive conditions after food intake and coordinates metabolism for efficient postprandial nutrient assimilation. However, beyond luminal nutrients, EECs can also sense microbiota-derived structures and mediate immune-related responses, establishing a complex paracrine and systemic signaling network. In particular, the L cell-derived incretin hormone glucagon-like peptide 1 (GLP-1) is increasingly recognized to exert direct effects on immune cells, orchestrating metabolic-inflammatory pathways. In IBD, a potential role for EECs in disease pathogenesis is suggested not only by the previously mentioned general disease symptoms, but also by a risk-associated SNP and autoantibodies targeting EEC function (reviewed in **Featured Publication VII**). The loss of GLP-1-expressing cells appears to be a common feature of both small and large intestinal inflammation, as it was consistently observed across four different mouse models of ileitis and colitis. Notably, this reduction was restricted to the site of active disease and correlated with the severity of inflammation, mirroring the decline in stem cell and Paneth cell function (**Featured Publication VIII**). Transcriptional analyses indicated skewed epithelial differentiation processes as underlying cause, linked to a metabolic shift characterized by enhanced glycolysis and attenuated mitochondrial metabolism. Most importantly, both the reduction in GLP-1<sup>+</sup> cells and associated metabolic alterations were recapitulated in a publicly available single-cell RNA sequencing dataset of ileal and colonic mucosal biopsies from healthy controls as well as inflamed and non-inflamed tissues of CD patients<sup>116</sup>. Supporting again a causative role for mitochondrial metabolism in steering epithelial differentiation, compromised mitochondrial function in mice with an IEC-specific knockout of ClpP, and OXPHOS inhibition in organoid cultures, was sufficient to impair EEC development. Remarkably, analysis of the single-cell RNA sequencing data set of CD patients revealed metabolic changes and signs of mitochondrial stress signaling as well as changes in epithelial subtype composition even in mucosal tissues that were considered non-inflamed by endoscopy (**Featured Publication VIII**). Given the chronic-remittent pathology of CD, these cellular metabolic changes might render the mucosa susceptible for additional inflammatory triggers and *vice versa*, could be an attractive therapeutic target to maintain remission.

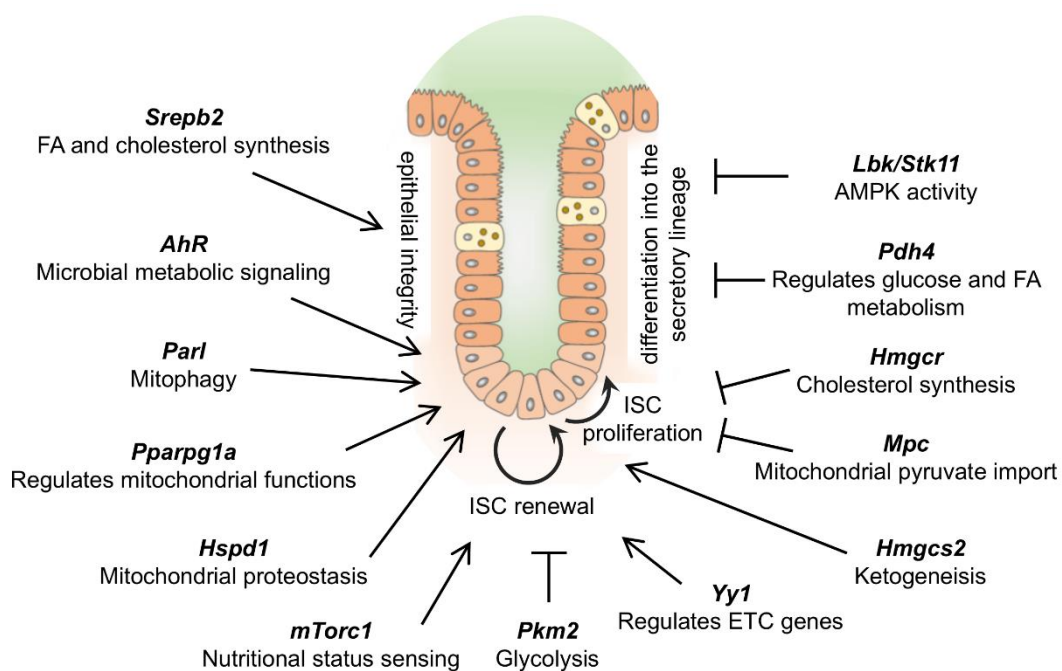
Regarding plasma GLP-1 levels, available data for IBD patients remain inconsistent<sup>117</sup>. This may be due to different methodological approaches<sup>118</sup>, but also biological challenges including that only about 10–15% of the secreted GLP-1 reaches the systemic circulation in the active form due to the rapid degradation of active GLP-1 by dipeptidyl peptidase (DPP)4 (resulting in a half-life of only 2 minutes)<sup>119, 120</sup>. Furthermore, overall changes in hormone secretion depend on the severity of inflammation and the extent of affected tissue, potentially making systemic detection challenging. Nevertheless, even if systemic changes in GLP-1 levels are not readily

detectable, (altered) paracrine GLP-1 signaling could still have profound effects on enterochromaffin cells<sup>121</sup>, intraepithelial lymphocytes<sup>122</sup>, and afferent neurons - ultimately impacting intestinal motility, mucosal immune responses, and feeding behavior under inflammatory conditions.

Consistently, a large-scale cohort study of IBD patients with concomitant type 2 diabetes indicated a therapeutic advantage of GLP-1 receptor agonists in managing IBD<sup>123</sup>. This underscores the potential of EECs and GLP-1-based therapies as independent, promising strategies for IBD while also highlighting that approaches aimed at enhancing cellular metabolic fitness to restore IEC functionality could yield beneficial outcomes by targeting IEC subtypes beyond the stem cell niche.

### Diet, epithelial Metabolism and the intestinal stem cell niche

The cell's metabolic pathways are tightly interrelated and mutually dependent. Thus, from a holistic perspective, it is intuitive that alterations in one pathway will affect the entire metabolic network, making the contribution of distinct pathways hard to illustrate<sup>124</sup>. Given the dynamic metabolic adaptations required along the crypt-villus axis, IECs rely on an overall functional cellular metabolism to maintain homeostasis. Accordingly, next to mitochondrial dysfunction<sup>94</sup> (**Featured Publication III, IV**), also mouse models deficient in genes impacting glucose metabolism<sup>125, 126</sup>, the pentose phosphate pathway<sup>127, 128</sup>, glutamine and fatty acid oxidation<sup>124, 129</sup>, cholesterol synthesis<sup>130, 131</sup> and the master regulator of the cellular metabolic state, mTOR<sup>132, 133</sup> exhibit profound changes in IEC proliferation, differentiation and function (**Figure 10**).



**Figure 10. Metabolic and mitochondrial genes impacting the intestinal stem cell niche<sup>134</sup>**

On the other hand, in a physiological context, dietary composition and nutrient availability represent key regulators of IEC metabolism and, consequently, shape the properties of the intestinal epithelium. In the context of obesity, type 2 diabetes and cancer, high-fat diets (HFD) have been extensively studied. In addition to promoting obesity, HFD feeding has been shown to accelerate the progression of gastrointestinal cancers<sup>135</sup>, and also promotes ileitis in *Tnf<sup>ΔARE</sup>* mice<sup>136</sup>. Specifically, high levels of dietary fat are associated with hyperproliferation of intestinal stem and progenitor cells and enhanced cell turnover<sup>135, 137</sup>, favoring malignant growth. However, the contribution of dietary lipids *versus* obesity is still under debate<sup>138</sup>. For instance, a pair-feeding study in mice concluded that obesity itself, rather than diet composition, drives intestinal epithelial stem cell proliferation<sup>139</sup>, and caloric restriction in leptin receptor-deficient *db/db* mice on a standard chow diet reduced body weight and reversed crypt cell hyperproliferation observed in *ad libitum* fed *db/db* mice<sup>140</sup>. In contrast, it was reported that *ex vivo* exposure of intestinal organoids to fatty acids including palmitic acid and oleic acid was sufficient to increase stem cell numbers recapitulate aspects of the *in vivo* HFD phenotype<sup>135</sup>.

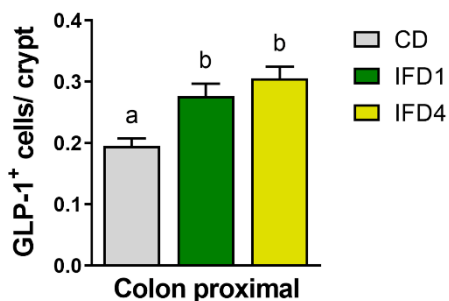
### **Dietary lipids affect the enteroendocrine cell lineage**

Nevertheless, HFD-induced changes in the stem cell niche and the transit amplifying zone also translate into altered differentiation patterns of IECs, including an increase in GLP-1-positive cells<sup>137</sup>. Similar observations have been reported for obese individuals consuming lipid-rich diets and higher numbers of L cells were linked to enhanced allocation of progenitors to the enteroendocrine lineage<sup>137, 141</sup>. Insulin signaling seems to play a key role in these mucosal changes<sup>137</sup>, as the loss of insulin receptor in IECs attenuates HFD-induced increases in EECs and GLP-1 expression<sup>142</sup>. Consistently, pathological conditions associated with insulin resistance, such as morbid obesity and type 2 diabetes, have been linked to dysregulation of transcription factors involved in EEC differentiation and reduced intestinal GLP-1 expression<sup>143, 144</sup>. L cell differentiation is facilitated through paracrine GLP-1 signaling<sup>145</sup>, and consequently, diets supplemented with fermentable fiber and SCFAs, acting as secretagogues on L cells, have been shown to increase colonic GLP-1-positive cell numbers<sup>146-148</sup>. HFDs could act in a similar way on EECs differentiation, as L cells are equipped with various fatty acid receptors to sense different types of lipids, in turn triggering hormone secretion (reviewed in **Featured Publication VII**).

To scrutinize the effect of dietary fat and obesity on L cell density in the intestinal epithelium, mouse strains with different susceptibilities to diet-induced obesity were fed various types of HFDs (**Featured Poster II**). Consistent with previous studies, BL/6J mice fed a palm oil-based HFD (P-HFD) providing 48kJ% from lipids, exhibited an increase in GLP-1<sup>+</sup> cell numbers in the ileum and colon. This effect was already significant after one week of feeding, became more pronounced after four weeks, and remained stable until week 12. After one week on P-HFD,

mice showed neither differences in body weight nor in basal blood glucose levels or area under the curve in an oral glucose tolerance test compared to control diet-fed mice, indicating a direct effect of dietary lipids on IEC differentiation. Further supporting a direct effect over an effect mediated by obesity or glucose metabolism, the increase in GLP-1<sup>+</sup> cell numbers was comparable across mouse strains with high (AKR/J), intermediate (BL/6J), and low (SWR/J) susceptibility to diet-induced obesity, despite their differences in body weight gain and glucose metabolism after four weeks on a 48kJ% P-HFD<sup>149</sup>. In line with this hypothesis, a study using leptin-deficient ob/ob mice, which develop obesity due to hyperphagia, did not find differences in jejunal or colonic GLP-1<sup>+</sup> cell numbers<sup>141</sup>.

Comparing BL/6J mice fed a 48kJ% P-HFD, a 60kJ% P-HFD, or an intermediate fat diet (IFD) providing 31kJ% from lipids based on soy oil and either borage oil or fish oil for four weeks revealed similar increases in colonic GLP-1<sup>+</sup> cells in the 48kJ% P-HFD and 60kJ% P-HFD groups, whereas the increase was diminished but still significant in the IFD groups. Notably, the different proportions of omega 3 and omega 6 fatty acids in the IFDs containing borage or fish oil did not affect the extent of colonic GLP-1<sup>+</sup> cell expansion (**Figure 11**). Together with the finding that GLP-1<sup>+</sup> cells did not further increase from week four to 12 of P-HFD, this suggests that the extent of GLP-1<sup>+</sup> cell expansion is saturable and rather depends on the total fat content than on the fatty acid composition. In contrast, BL/6J mice exposed to a lard-based HFD (L-HFD) providing 48kJ% from lipids for four weeks showed no increase in colonic GLP-1<sup>+</sup> cells and only a minor effect in the ileum, consistent with previous findings in bile-acid supplemented HFDs (**Collaborator Paper A**). Aside the total palmitic acid content, the main difference between P-HFD and L-HFD is the presence of cholesterol exclusively in L-HFD. Of note, in the fish oil-containing IFD, palmitic acid is approximately as abundant as in L-HFD<sup>150</sup>, while cholesterol content (5-10mg/kg) is comparable to that of the L-HFD-matched control diet. Hence, dietary cholesterol might interfere with fatty acid-induced expansion of GLP-1<sup>+</sup> cells.



**Figure 11. A borage or fish oil-based intermediate-fat diet increases colonic GLP-1<sup>+</sup> cells**

Mice were fed a borage (IFD1) or fish oil-based diet (IFD4) for 4 weeks *ad libitum*. GLP-1<sup>+</sup> cells were quantified in immunohistochemically stained colonic tissue sections.

Dietary fatty acids and sterols are predominantly absorbed in enterocytes of the proximal small intestine, whereas the abundance of GLP-1-producing cells gradually increases from

proximal to distal in the small intestine, reaching highest numbers in the ileum and colon. This raises the question of how dietary/luminal fatty acids and cholesterol might mediate their effects on EEC differentiation.

HFDs require the epithelium of the upper small intestine to absorb large quantities of dietary lipids, including triglycerides, phospholipids, and sterols. Following the uptake of lipid constituents such as free fatty acids and monoacylglycerols, triglycerides are resynthesized in enterocytes and, together with other lipophilic compounds, assembled into chylomicrons and released into lymphatic vessels. HFD feeding enhances intestinal lipoprotein secretion, and since cholesterol is essential for chylomicron assembly and secretion, HFDs consequently increase the epithelial demand for cholesterol. Accordingly, a study showed that, despite elevated dietary cholesterol intake on a high-fat Western diet, mice display reduced small intestinal cholesterol levels, enhanced *de novo* cholesterol synthesis, and activated cholesterol-saving mechanisms<sup>151</sup>. High levels of dietary lipids can furthermore exert lipotoxic effects on IECs, and thus, HFD-induced epithelial increases of fatty acid oxidation and lipoprotein secretion might also contribute to IECs protection. In response to lipid overload by HFD, the epithelium enhances its absorptive capacity by enlarging the epithelial surface area available for nutrient uptake<sup>151</sup>. Nevertheless, an overflow of dietary lipids into the distal small intestine and colon has been reported<sup>152, 153</sup>. The adaptations of the intestinal epithelium towards HFD exposure, including increased lipid absorption, fatty acid metabolism and IECs proliferation<sup>154</sup>, occur rapidly, with changes already detectable after one day<sup>154</sup>. Of note, beyond disturbing fatty acid oxidation<sup>155</sup>, mitochondrial dysfunction in enterocytes impairs chylomicron production<sup>156</sup> and more specifically, Hsp60 deficiency dysregulates cholesterol synthesis<sup>157</sup>, highlighting once again the essential role of mitochondria in IEC homeostasis.

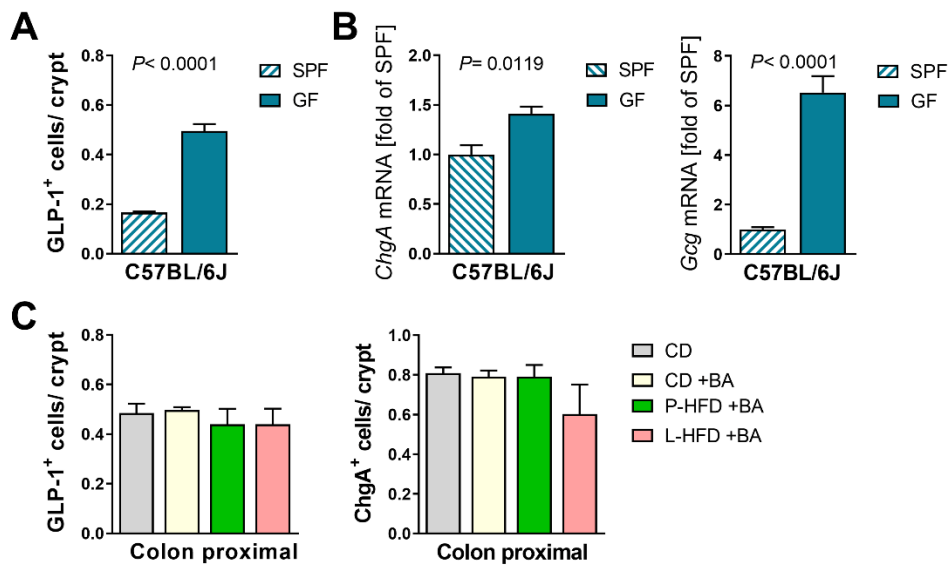
EECs are crucial for normal lipid absorption in mice<sup>158</sup>, and colonic GLP-1<sup>+</sup> cells have been suggested to sense luminal energy content and adjust intestinal transit time accordingly to ensure efficient nutrient absorption. Furthermore, GLP-1 and GLP-2 are thought to regulate entry of intestinal lipoproteins into the lymphatics and lymphatic flow<sup>159, 160</sup>. In line, mice lacking EECs exhibit lower plasma levels of triglycerides and cholesterol<sup>158</sup>. Additionally, EECs have been proposed to relay nutritional information to the intestinal stem cell niche, in turn regulating mitochondrial activity, ATP level, and proliferation in the crypt<sup>161</sup>. This is particularly relevant, as crypt-resident stem and progenitor cells are not directly exposed to the luminal environment, but are metabolically shielded by differentiated cells, for example through colonocytes consuming butyrate<sup>162</sup>.

Mechanistically, HFD-induced expansion of GLP-1<sup>+</sup> cells has been associated with increased fatty acid and cholesterol synthesis in stem and progenitor cells, activated Ppar and insulin receptor signaling<sup>137</sup>, and augmented expression of the scavenger receptor Scarb1<sup>142</sup>, mediating selective cholesterol uptake from the serosal side. In the intestinal stem cell niche,

high levels of *de novo* lipid and cholesterol synthesis are required to maintain membrane production for cell proliferation<sup>130, 131</sup>. Moreover, data from mice with an IEC-specific knockout of the transcription factor SREBP-2, abolishing endogenous cholesterol synthesis, indicate that neither luminal nor serosal cholesterol uptake can fully rescue epithelial cholesterol levels<sup>130</sup>. This, together with the finding that HFDs deplete epithelial cholesterol<sup>151</sup>., suggests that cholesterol is a rate-limiting metabolite for IEC proliferation and differentiation under challenging metabolic conditions.

Interestingly, dietary cholesterol has been proposed as a nutrient affecting the crosstalk between gut microbiota and host metabolism<sup>163</sup>. HFDs, independent of obesity<sup>164</sup> alter the gut microbiota structure, resulting in changes in microbial gene expression<sup>165</sup> and fecal metabolome, already after one week of feeding<sup>166</sup>. More specifically, the microbiome can metabolize dietary polyunsaturated fatty acids<sup>167</sup>, thereby altering luminal availability of different types of fatty acids. Regarding EECs, this is important as chain length and degree of fatty acid saturation determine the efficiency to stimulate GLP-1 secretion<sup>168</sup>. Different bacterial species might even have specific effects on EECs, for example abundance *Akkermansia muciniphila* is associated with L cell number<sup>169</sup> and a small *Staphylococcus epidermidis*-derived peptide, delta-toxin, is capable of directly stimulating GLP-1 secretion<sup>170</sup>. On the other hand, deficiency in colonic EECs leads to altered microbiota composition, most likely due to changes in colonic motility and luminal nutrient availability<sup>171</sup>.

In the previously mentioned mouse cohort characterized in **Collaborator Paper A**, which received bile-acid supplemented P-HFD or L-HFD for eight weeks, the dietary fat source influenced cecal microbiota structure. Metatranscriptomic analysis further highlighted 266 differentially regulated microbial genes between bile-acid supplemented P-HFD and L-HFD-fed mice. Yet, colonic concentrations of SCFAs did not significantly differ. This is noteworthy, as elevated SCFA levels have been suggested to mediate the dietary fiber-induced increase in colonic GLP-1<sup>+</sup> cells in colonized rats<sup>148</sup>, but also to decrease colonic L cell numbers in GF mice<sup>73</sup>. Interestingly, GF mice exhibit profoundly increased numbers of colonic GLP-1<sup>+</sup> cells (**Figure 12**) which decrease upon colonization<sup>73</sup>. *Vice versa*, colonized mice treated with antibiotics show reduced numbers of GLP-1<sup>+</sup> cells in the colon<sup>73</sup>. As differences in GLP-1-encoding *Gcg* gene expression between GF and colonized mice only begin to manifest after weaning, and weaning of GF mice to a HFD for one week prevented the rise in *Gcg* expression in the colon, the authors hypothesized that colonic energy availability determines *Gcg* expression<sup>73</sup>. However, in the GF mouse cohort included in **Collaborator Paper A**, neither the bile-acid supplemented P-HFD nor L-HFD led to a reduction in colonic GLP-1<sup>+</sup> cells after eight weeks of feeding (**Figure 12**). Of note, these mice were not weaned onto the HFDs but started receiving HFDs at the age of ten weeks.



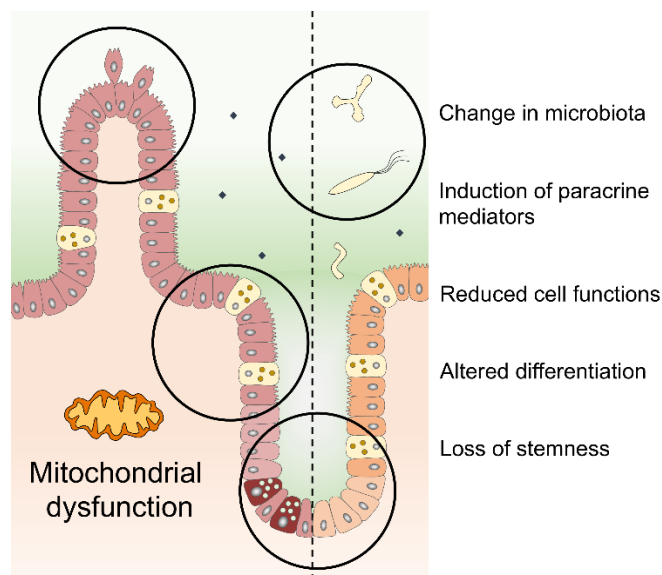
**Figure 12. GLP-1<sup>+</sup> cells are increased in germ-free mice independent of diet**

(A) Germ-free (GF) mice have increased numbers of colonic GLP-1<sup>+</sup> cells as well as increased mRNA expression of the enteroendocrine cell marker *Chga* and GLP-1 encoding *Gcg*. (B) GF mice were fed a lard or palm oil-based high-fat diet supplemented with bile acids from 10 to 18 weeks of age *ad libitum*. GLP-1<sup>+</sup> and Chga<sup>+</sup> cells were quantified in immunohistochemically stained colonic tissue sections.

This might indicate a critical time window directly after weaning for epithelial metabolic programming of EEC differentiation in GF. In contrast, the data discussed clearly demonstrate the high plasticity of the intestinal epithelium in response to dietary changes in adult mice with a normal microbiota. Most interestingly, albeit not increasing colonic GLP-1<sup>+</sup> cells, L-HFD increased colonic serotonin (5-HT)-producing enterochromaffin cells to a similar extent as the P-HFD (**Featured Poster II**). Enterochromaffin cells also interact with the microbiome and participate in lipid absorption<sup>172</sup>. Both, L cells and enterochromaffin cells affect each other *via* paracrine signaling and bidirectionally regulate expression and secretion of GLP-1 and 5-HT<sup>173</sup>.

Further research needs to clarify the exact mechanisms and specificity of fatty acid-mediated regulation of the enteroendocrine cell lineage and whether and how cholesterol metabolism is entangled in this process. However, these data already highlight the potential of precise nutritional interventions not only in the context of metabolic diseases. They also underline the necessity for careful interpretation of data from diet-induced obesity models due to distinct effects of the fat source<sup>174</sup>. Of note, this is not restricted to the lipid composition, but also includes different types carbohydrates, fibers, and proteins, and their abundance in the respective diet<sup>175, 176</sup>, and even the texture of diet has been shown to affect the expression of IEC subtype marker genes<sup>177</sup>.

In the context of IBD, diet and nutritional compounds are extensively discussed. Yet, there is neither a clear indication of the effectiveness of primary nutritional therapies in induction and maintenance of remission in adults nor a success in identifying nutrients/dietary compounds which contribute to the risk of developing IBD<sup>178</sup>. This lack of evidence might be attributed to the heterogeneous nature of these diseases or to the possibility that diet impacts IBD etiology only in combination with certain genetic or environmental factors. Considering the data showing that (I) IEC metabolism is changed even in the absence of overt tissue alterations, (II) skewed IECs differentiation is a hallmark of pathology, (III) distinct nutritional constituents exert specific effects on epithelial subtype composition, it is conceivable that a subset of IBD patients might benefit from nutritional or nutraceutical interventions.



**Summary Figure. Effects of mitochondrial dysfunction in the intestine.**

## Intestinal organoids to model epithelial biology

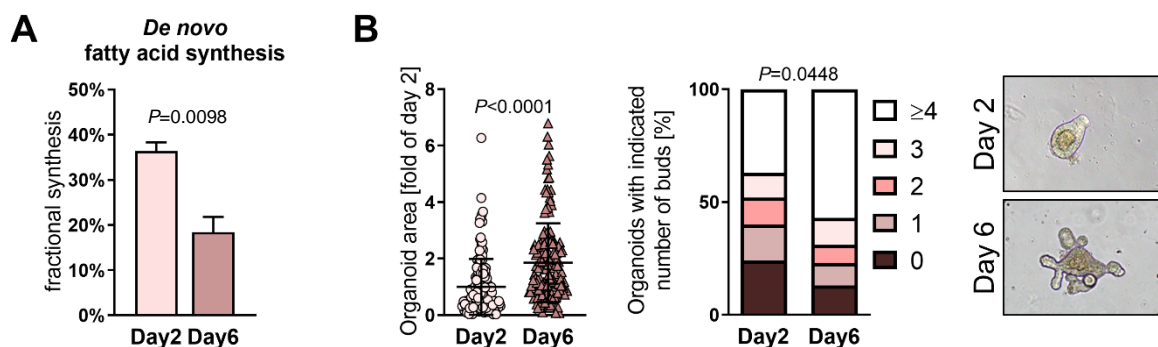
A key methodological foundation to tackle the research questions of this thesis was the refinement, advancement and application of intestinal organoid culture (**Featured Paper X, XI**).

Until the breakthrough of long-term *in vitro* cultivation of intestinal organoids by Sato *et al.* in 2009<sup>179</sup>, studying IECs *in vitro* remained highly challenging<sup>180</sup>. Primary IECs can only be cultivated for a limited time frame, as they rapidly undergo apoptosis. Moreover, numbers of certain IEC subtypes are low, and differentiation state is poor in primary cultures, restricting experimental read-outs. Immortalized IEC lines, on the other hand, come with their own limitations. Derived from genetically modified or tumorigenic tissue, they fail to recapitulate important physiological properties of the intestinal epithelium. For example, Caco-2 cells, even though known to possess a rather small intestinal phenotype<sup>181</sup>, were originally derived from a colon carcinoma, and phenotypic as well as functional characteristics highly differ from native human enterocytes<sup>182</sup>. In addition, most cell lines are restricted to only a single IEC subtype, further limiting their applicability in studying the complex intercellular interactions of epithelial cell biology. In contrast, intestinal organoids reflect key properties and functions of the native epithelium. Containing all IEC subtypes and preserving location-specific functions as well as epithelial architectural organization, organoids brought enormous benefits to molecular research on gastrointestinal processes, pathologies, and stem cell research (reviewed in **Featured Paper IX**).

Intestinal organoids can be grown from intestinal or induced pluripotent stem cells; however, the most straightforward approach to establishing organoid lines is to cultivate isolated primary crypts from the individual and/or location of interest. Only a limited amount of tissue is needed; for example, human intestinal organoid cultures can be established from crypts derived from biopsy samples. The availability of patient-derived organoid lines has revolutionized the possibilities of personalized medicine. As intestinal organoids recapitulate epithelial functions, genetic signatures, and, in the case of colorectal cancer, several properties of the original tumor, they allow for personalized drug response predictions<sup>183, 184</sup>. In combination with high-throughput drug screens, the detection of gene-drug associations is facilitated, and already today, treatment of cystic fibrosis patients based solely on patient-derived organoid readouts is possible in the Netherlands<sup>185</sup>.

Also in the field of basic research, intestinal organoids have substantially contributed to elucidating the complex regulation of the intestinal stem cell niche. In particular, the identification of Lgr4 and Lgr5 as receptors of R-Spondins resulted from organoid experiments<sup>186</sup>, and the metabolic interaction between Paneth and Lgr5<sup>+</sup> stem cells has also been characterized using this tool<sup>20</sup>. Yet, many functions of the native epithelium remain to be explored in detail, including intestinal transport processes, hormone secretion, and their

interconnection with epithelial and whole-body metabolism. These processes are highly relevant, as they contribute to intestinal pathologies such as malabsorption syndromes and, beyond the intestine, to diseases like type 2 diabetes and represent potential therapeutic targets. Intestinal nutrient transporters, for example, are not only involved in the absorption of nutrients from ingested food but also act as sensors that trigger enteroendocrine hormone secretion and mediate the transport of certain drugs<sup>187, 188</sup>. However, the expression, function, substrate specificity and, importantly, interaction of epithelial transporters are not yet fully understood (**Collaborator Paper B**), as illustrated by the fact that the underlying causes of fructose malabsorption are still unknown<sup>189</sup>. Furthermore, in addition to the epithelium forming the barrier between the host and its microbiota, IEC metabolism acts as a gatekeeper of nutrient and drug availability for the entire organism. For instance, IEC fatty acid oxidation has been implicated in the regulation of food intake<sup>190, 191</sup>, and a broad range of drugs is metabolized by intestinal cytochrome P450 enzymes<sup>192</sup>. Murine and human intestinal organoids are excellent model systems in this critical field of research, as they allow for the characterization of transport processes, downstream signaling, and hormone secretion (**Featured Paper X**). Additionally, metabolic readouts such as respiration, protein turnover, fatty acid oxidation, and synthesis have been successfully assessed in organoids with high resolution (**Featured Paper III, XI, Figure 13**). Of note, culture conditions, particularly media composition and organoid size, must be fine-tuned depending on the experimental question (e.g., nutrient uptake versus proliferative capacity) to ensure an optimal abundance of the desired IEC subtype(s) (**Featured Paper XI**).

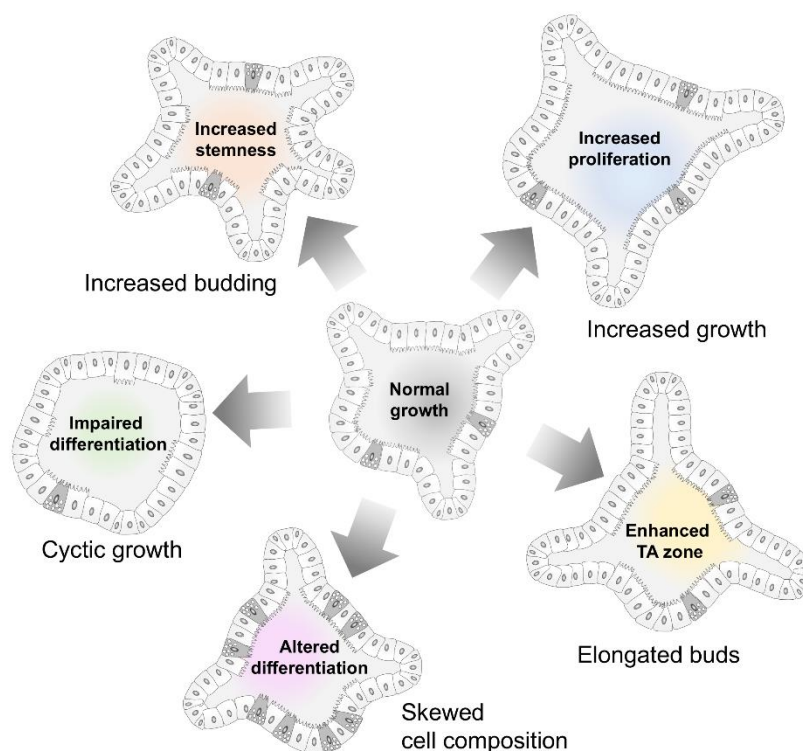


**Figure 13. De novo fatty acid synthesis decreases during intestinal organoid culture**

(A) Analysis of *de novo* fatty acid synthesis using  $^{13}\text{C}_2$ -acetate (Josef Eckers) indicates reduced rates of synthesis paralleling (B) organoid growth. Organoid growth results in a higher ratio of differentiated to proliferating cells, supporting an essential role for fatty acid synthesis for cell proliferation.

Accordingly, also for illustrating the key role of mitochondrial function in epithelial stemness on a molecular level, intestinal organoids served as an essential tool (**Featured Paper III, IV**). In the context of epithelial homeostasis, metabolism, and inflammatory pathologies, three

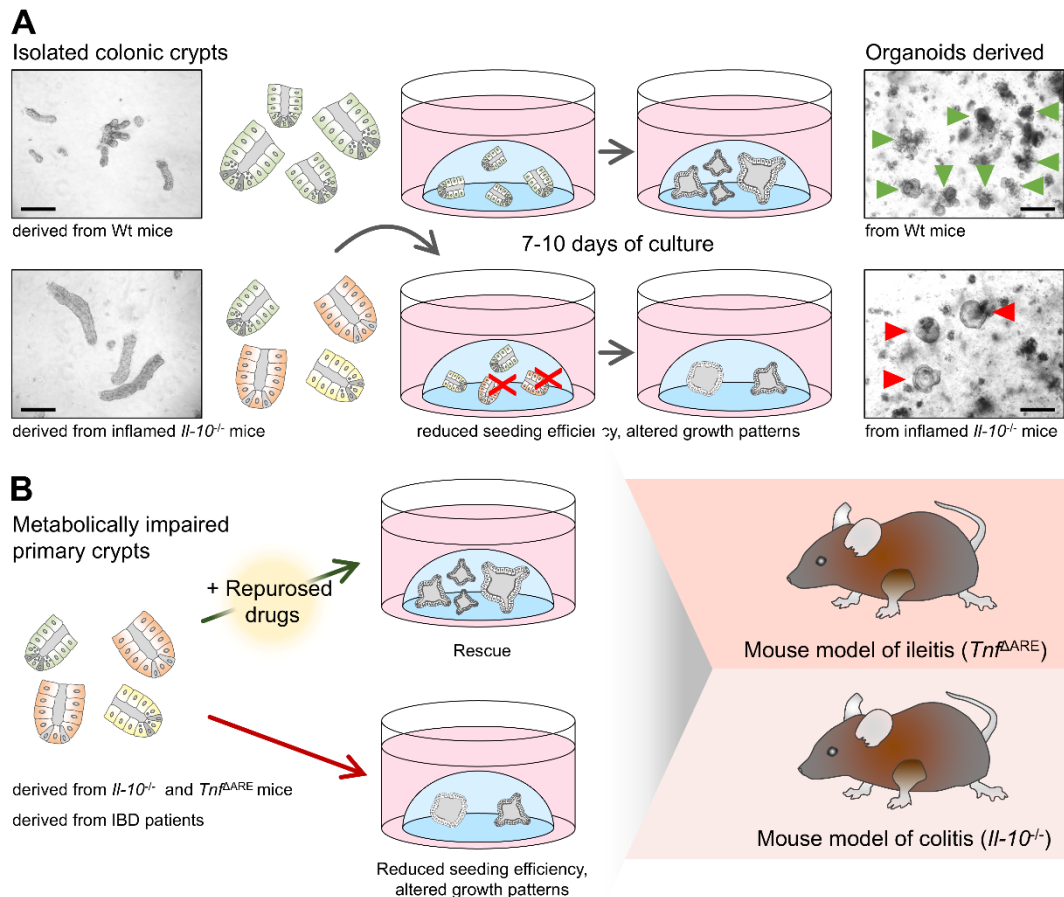
features of intestinal organoids greatly facilitate basic research: (I) using organoids derived from mice with floxed genes of interest and expressing an inducible Cre recombinase (e.g., *Vil1CreER<sup>T2</sup>*), deletion of floxed alleles is possible *ex vivo* simply by adding tamoxifen to the culture medium<sup>193</sup>; (II) primary crypts are intrinsically programmed by an inflamed environment and retain this imprint for at least first passages of cultivation<sup>194, 195</sup>; (III) organoid growth patterns can be linked to ISC niche properties and differentiation processes<sup>196</sup> which are particularly relevant under inflammatory conditions (**Figure 14**).



**Figure 14. Distinct intestinal organoid growth pattern are linked to changes cellular functions**

In a more applied setting, intestinal organoids thus allow for compound screening e.g., substances with known effects on mitochondrial function (such as nutritional compounds, microbiota-derived structures or drugs) to characterize their impact on the intestinal stem cell niche based on morphological readouts. Complementary, the potential of promising substances to rescue inflammation-imprinted metabolic dysfunction in the intestinal stem cell niche and associated growth defects can be evaluated in primary crypts isolated from inflamed murine and human tissues. Crypts derived from inflammatory environments show alterations in morphology, a reduced capability to give rise to organoid structures, and outgrowing organoids often show aberrant growth patterns, reflecting *in vivo* disturbances of cellular functions (**Figure 15**). Proof of concept for this strategy has been demonstrated for dichloroacetate (DCA), an FDA-approved drug that shifts cellular metabolism from glycolysis

to mitochondrial respiration. Ileal crypts derived from  $Tnfr^{ARE}$  mice fail to develop into organoid structures, yet the addition of DCA to the culture medium rescues the phenotype (**Featured Paper IV**). Importantly, subsequent withdrawal of DCA did not affect organoid growth, providing evidence that metabolic reprogramming might be a therapeutic target in IBD.

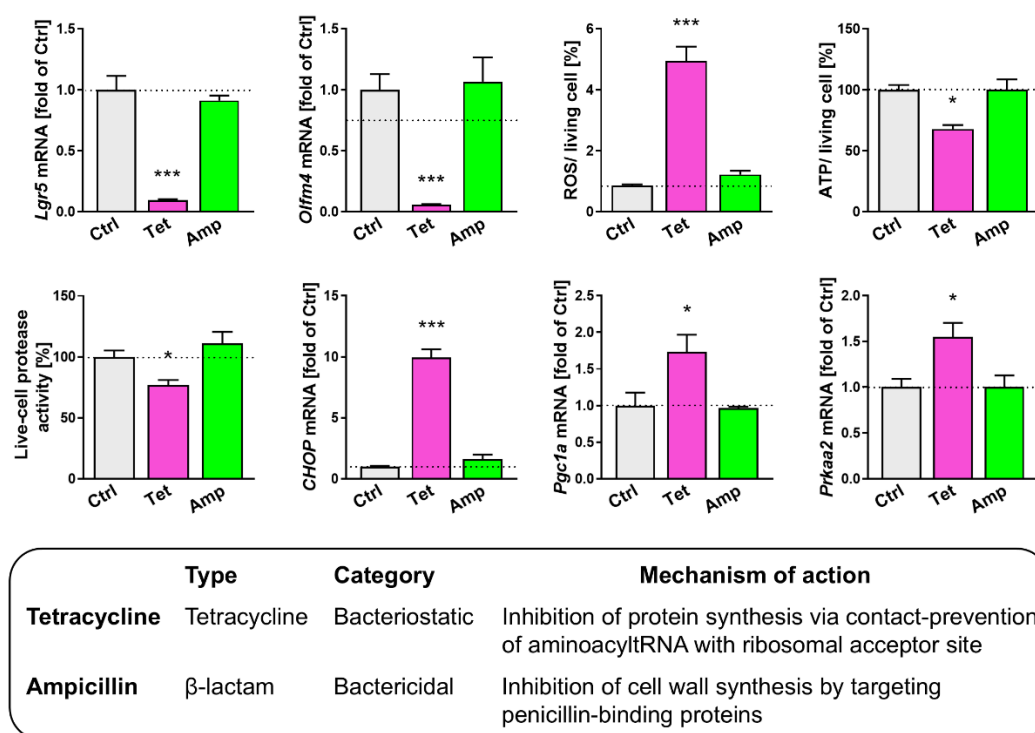


**Figure 15. Schematic illustrations of drug testing approaches in intestinal organoids**

(A) Crypts derived from inflamed intestinal tissues show reduced seeding efficiency and altered growth patterns. (B) Repurposed drugs can be tested for their efficiency to rescue organoid growth *ex vivo* and subsequently, promising candidates can be tested *in vivo*.

Notably, 5-amino salicylic acid (5-ASA), often the first treatment option for newly diagnosed IBD patients, has been shown to reverse mitochondrial and metabolic dysfunction evoked by exposure of mice to pre-IBD risk factors<sup>197</sup>. Initial attempts at mitochondria-targeted therapies, including P110, a small peptide inhibitor of mitochondrial fission, have yielded promising results<sup>198</sup>. Additionally, dietary compounds such as L-carnitine, which plays a key role in fatty acid oxidation, and also possesses mitochondrion-specific antioxidant properties<sup>199, 200</sup>, represent promising candidates when administered in pharmacologically relevant doses. *Vice versa*, antibiotics might foster IBD relapses by targeting IEC mitochondria<sup>134</sup>. Repeated antibiotic exposure, especially during childhood, has been associated with an increased risk

of developing IBD<sup>201, 202</sup>. The prevailing hypothesis suggests that microbiota disturbances may have long-term detrimental effects on microbiota composition or that early inflammatory episodes might indicate a predisposition to later disease development<sup>203</sup>. However, antibiotics can also directly target the host. Due to their bacterial origin, mitochondria are effectively targeted by several classes of antibiotics, and clinically relevant doses have been shown to impair mitochondrial functions in human cell lines<sup>204-207</sup>. In particular, tetracyclines disrupt mitochondrial protein balance by inhibiting mitochondrial translation, triggering the mitochondrial unfolded protein response and reducing OXPHOS capacity<sup>208</sup>. Thus, antibiotics might directly affect the ISC niche *via* mitochondrial impairment, causing disturbances in tissue homeostasis and tipping the balance toward inflammation. These largely overlooked effects of antibiotics can also be investigated using organoid cultures (**Figure 16**).



**Figure 16. Antibiotics can effect mitochondria and intestinal stem cells**

Intestinal organoids were exposed to antibiotics (Tet = 0.21mM; Amp = 0.29mM) for 24h. *Lgr5* and *Olfm4* = intestinal stem cell markers; *Chop* = marker for mitochondrial stress signaling, *Pgc1 $\alpha$*  = mitochondrial biogenesis; *Pkkaa2* = AMPK subunit.

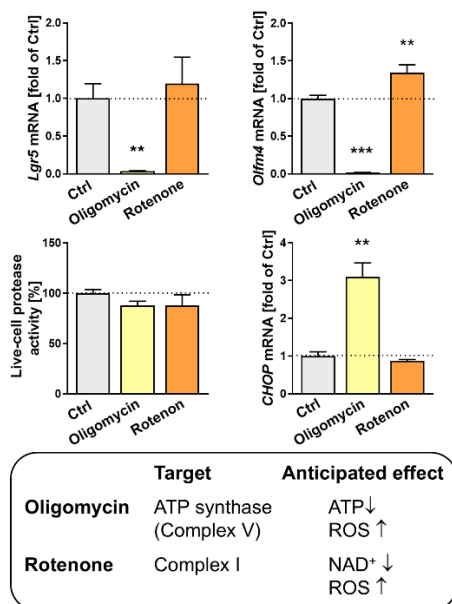
To develop clinically relevant therapeutic interventions focusing on metabolism and the ISC niche, a deeper molecular understanding of the signals and mediators involved in regenerative tissue responses and the resolution of metabolic injuries is crucial. The establishment of organoid culture and co-culture systems, including organoids bearing different genotypes or organoids combined with other cell types (e.g., fibroblasts, macrophages)<sup>63</sup>, has become a

cornerstone of research to unravel the complex regulation of mucosal healing. In the long term, these models may contribute to the development of novel strategies for combating intestinal diseases.

## Conclusion and perspective

Without doubt, the research summarized in this thesis supports the hypothesis that mitochondrial function is indeed a gatekeeper of epithelial homeostasis.

Alterations in intestinal cell metabolism and mitochondria-associated genes and proteins observed under full-blown inflammation can be attributed, at least in part, to changes in epithelial cell composition. For instance, the metabolism of transit-amplifying cells, which expand under inflammatory conditions, is characterized by lower oxygen consumption compared to terminally differentiated epithelial cells<sup>209</sup>. Yet, the data presented here strongly support that mitochondrial disturbances play a causative role in gastrointestinal pathologies, and are not merely consequences of pathological changes. However, many aspects still need to be scrutinized: What are the specific molecular mechanisms by which mitochondrial activity translates into epithelial cell functions? Which signals arise from metabolically impaired cells that orchestrate tissue responses and healing? How are specific microbial signals integrated? For example, targeting different complexes of the respiratory chain yielded different outcomes regarding intestinal stemness, and relating different metabolic states to distinct epithelial properties will be crucial to gaining a more comprehensive understanding of mitochondrial-epithelial steering circuits.

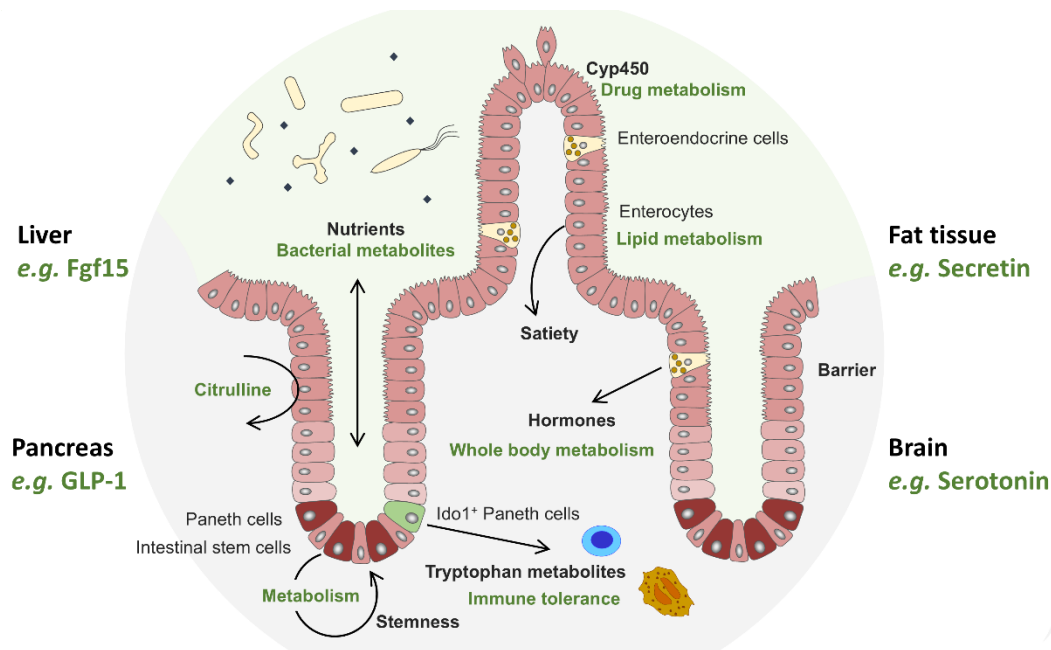


**Figure 17. Targeting complex I or V of the respiratory chain yields distinct outcomes on intestinal stem cell function.**

Intestinal organoids were treated with oligomycin or rotenone for 24h. *Lgr5* and *Olfm4* = intestinal stem cell markers; *Chop* = marker for mitochondrial stress signaling

The findings obtained in the epithelium also provide a foundation for transferring insights into mitochondrial signaling to other tissues. Expanding readouts beyond the intestinal epithelium will help to identify fundamental principles of mitochondrial function in cellular homeostasis. In line with the concept that cell-autonomous mitochondrial perturbations trigger release of paracrine mediators, liver-specific Hsp60 loss was shown to induce oxidative stress, mitophagy, and an environment favoring cholangiocellular hyperproliferation and neoplastic

transformation<sup>210</sup>. Additionally, MT-UPR signaling has been explored in the context of prostate cancer (**Collaborator Paper C**), and currently, the relevance of mitochondrial proteostasis in adipocyte physiology is in the focus of research. This project also aims at illustrating how signals arising from mitochondrial dysfunction in a specific organ are propagated and affect systemic homeostasis.



**Figure 18. Illustration of intestinal epithelial functions that are likely affected by mitochondrial function including possible mediators involved in signal propagation to other organs.**

These ongoing and future research avenues highlight the vast and exciting questions that remain to be explored. Yet, generating knowledge is only one part of scientific progress - effectively communicating scientific insights is equally important. In times of increasing misinformation and the prevalence of "alternative facts," it is essential to foster curiosity, encourage critical thinking, and inspire the next generation of researchers. Science communication plays a pivotal role not only in engaging students and young scientists but also in ensuring that scientific findings remain accessible and meaningful to society (**Featured Paper XII**). By making science tangible and relevant, we empower individuals to ask informed questions and make evidence-based decisions. In this spirit, the pursuit of knowledge should not remain confined to academic circles but should actively contribute to shaping an informed and responsible society.

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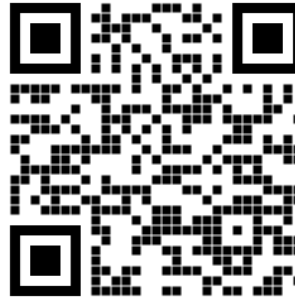
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## Featured publications

- I. Mitochondrial function - gatekeeper of intestinal epithelial cell homeostasis, Rath E., Moschetta A., Haller D., Nat Rev Gastroenterol Hepatol. 2018 Aug;15(8):497-516.



<https://doi.org/10.1038/s41575-018-0021-x>

- II. PKR activation in mitochondrial unfolded protein response-mitochondrial dsRNA might do the trick, Rath E., *Front Cell Dev Biol.* 2023 Aug 29;11:1270341.



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- III. Mitochondrial function controls intestinal epithelial stemness and proliferation, Berger E., Rath E., et al., *Nat Commun.* 2016; 7, 13171.



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- IV. Mitochondrial impairment drives intestinal stem cell transition into dysfunctional Paneth cells predicting Crohn's disease recurrence, Khaloian S.\*, Rath E.\* et al., *Gut*. Published Online First: 28 February 2020.



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- V. Mitochondrial perturbation in the intestine causes microbiota-dependent injury and gene signatures discriminative of inflammatory disease, Urbauer E., [...], Rath E., and Haller D, *Cell Host Microbe*. 2024 Aug 14;32(8):1347-1364.e10.

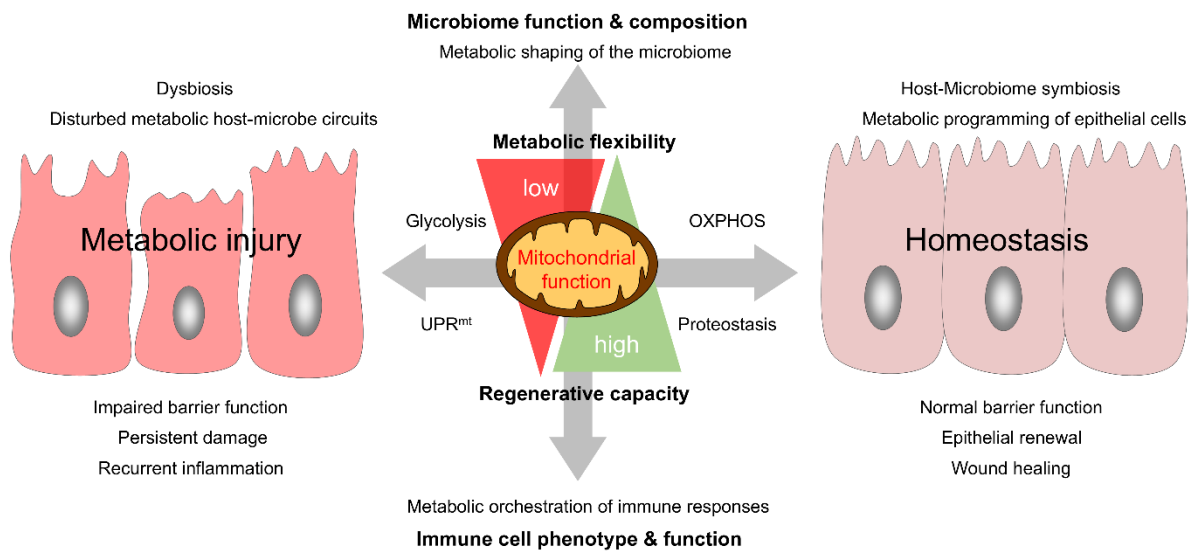


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VI. Intestinal epithelial cell metabolism at the interface of microbial dysbiosis and tissue injury, Rath E. and Haller D., *Mucosal Immunol.* 2022 Apr;15(4):595-604.



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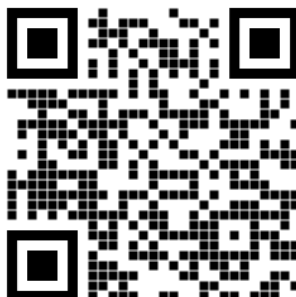


- VII. Inflammation Meets Metabolic Disease: Gut Feeling Mediated by GLP-1, Zietek T. and Rath E., *Front Immunol.* 2016 Apr 22;7:154.

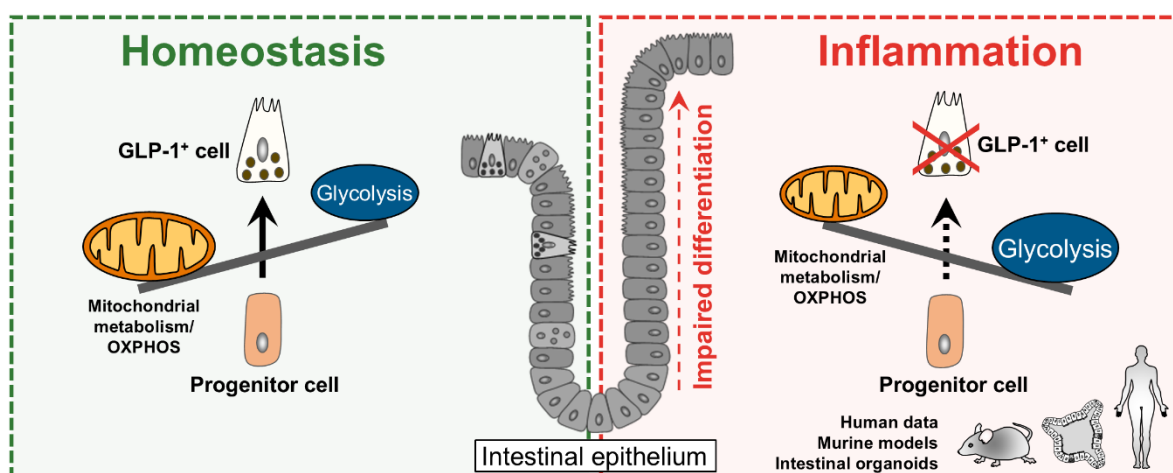


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VIII. Reduced intestinal GLP-1<sup>+</sup> cell numbers are associated with an inflammation-related epithelial metabolic signature, Urbauer E., [...], and Rath E



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- IX. Drug screening, oral bioavailability and regulatory aspects: A need for human organoids, Zietek T., Boomgaarden WAD., and Rath E., *Pharmaceutics*. 2021 Aug 17;13(8):1280.



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- X. Intestinal organoids for assessing nutrient transport, sensing and incretin secretion, Zietek T.\*, Rath E.\*, et al., *Sci Rep.* 2015 Nov 19;5:16831.



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- XI. Organoids to Study Intestinal Nutrient Transport, Drug Uptake and Metabolism – Update to the Human Model and Expansion of Applications, Zietek T., [...] and Rath E., *Front. Bioeng. Biotechnol.* 2020 8:577656.

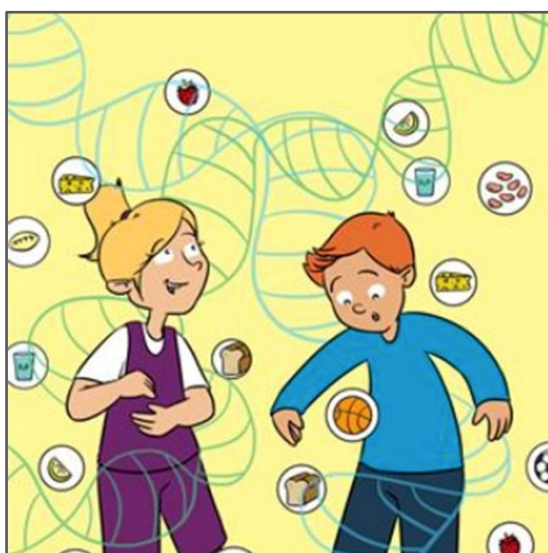


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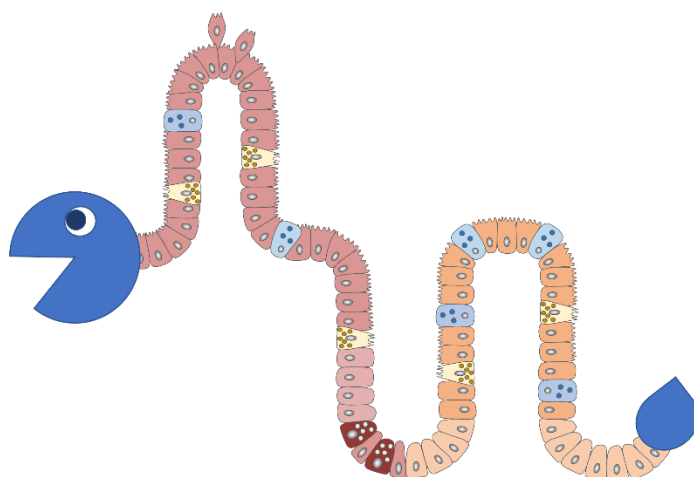
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<https://mediatum.ub.tum.de/1775380>



„Mama, heute hatten wir die komische Schlange in der Schule, die du immer malst.“  
[und es war voll langweilig]

## Collaborator publications

- (A) The gut microbiota drives the impact of bile acids and fat source in diet on mouse metabolism, Just S., [...], Rath E., et al., *Microbiome*. 2018 Aug 2;6(1):134.



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- (B) Intestinal sodium/glucose cotransporter 3 is downregulated in obese mice and humans, Soták M., Casselbrant A., Rath E., et al., *Life Sci*. 2021 Feb 15;267:118974.



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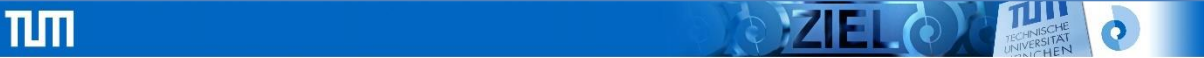
- (C) A mitochondrial unfolded protein response inhibitor suppresses prostate cancer growth in mice via HSP60, Kumar R., [...], Rath E., et al., *J Clin Invest*. 2022 Jul 1;132(13):e149906.



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Featured Poster

I. Mitochondrial stress is associated with altered mtCK expression in the intestinal epithelium (4<sup>th</sup> Seeon Conference, DGHM)



# Mitochondrial Stress Is Associated with Altered mtCK Expression in the Intestinal Epithelium

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### Background

Inflammatory bowel diseases (IBD) including Crohn's disease and ulcerative colitis are chronically relapsing disorders of the gastrointestinal tract. These diseases feature organelle stress, in particular ER- and mitochondrial stress, both crucial factors contributing to the pathogenesis of chronic intestinal inflammation. Beyond their function in cellular energy supply, mitochondria integrate cellular and extra-cellular derived signals such as the nutrient status and the pathogen load. This convergence of signals on mitochondria makes them key players when it comes to cellular life-or-death decisions. A mitochondrial protein particularly important for the switch from adaptive to apoptotic/necrotic pathways is the mitochondrial creatine kinase (mtCK). Residing at the mitochondrial transition pore (MTP), mtCK is thought to keep the MTP closed but upon exposure to reactive oxygen species (ROS), mtCK might switch from octameric to dimeric conformation leading to MTP opening and subsequently to cell death. The aim of our study was to investigate the role of mtCK during onset and perpetuation of inflammation and its regulation in the context of ER- and mitochondrial unfolded protein response.

### Conclusion

- Chronic intestinal inflammation is accompanied by a loss of mtCK in different mouse models of colitis and in patients suffering from IBD.
- During the onset of inflammation mtCK expression is induced.
- During persistent inflammation loss of mtCK is associated with massive tissue damage, whereas enhanced mtCK expression is associated with tissue protection.

- mtCK in intestinal epithelial cells is sensitive to oxidative damage caused by ROS.
- Severe ER UPR affecting cell viability is accompanied by ROS production and results in selective degradation of mtCK by C1pP.
- mtUPR leads to an induction of mtCK → confers to a protection of mitochondria + mtUPR induction does not affect the epithelial cell viability.

### Results

**Figure 1. Mitochondrial creatine kinase (mtCK) is diminished in primary IEC under inflammatory conditions.**  
 (A) Rag2<sup>-/-</sup> and Rag2<sup>-/-</sup> x IL-10<sup>-/-</sup> recipients were adoptively transferred with CD4<sup>+</sup> T cells from Wt or IL-10<sup>-/-</sup> mice (n=5). Mice were sacrificed 4 weeks later and histopathological scoring was performed by blindly assessing the inflammation between 0 (not inflamed) and 12 (highly inflamed). Bar charts show the mean histopathologic score ±SD. b different from a, f different from e, h different from g and i, ANOVA on Ranks followed by Dunn's test P<.05. Western blots show mtCK expression in isolated large IEC. β-actin served as a loading control. (B) Germ-free Wt and IL-10<sup>-/-</sup> mice were mono- or dual associated with *E. faecalis* and/or *E. coli* at 12 weeks of age. Mice were sacrificed 6 weeks later and histopathological scoring was performed by blindly assessing the inflammation between 0 (not inflamed) and 4 (highly inflamed). (C) Primary IEC were isolated from surgical specimen of patients with colorectal cancer (CC, non-inflammatory control), active Crohn's disease (CD) and ulcerative colitis (UC). UC patient 6; IEC of non-inflamed (N) and inflamed (I) tissue regions.

**Figure 2. ROS trigger a loss of mtCK in intestinal epithelial cells.**  
 The small intestinal epithelial cell line Mode K was stimulated with various concentrations of the ROS producer tert-butyl hydroperoxide (tBut) for 4 or 24 h. Western Blots show the loss of mtCK and HO1 induction. β-actin served as a loading control.

**Figure 3. Enhanced mtCK expression protects from DSS induced tissue damage.**  
 (A) 12 weeks old WT CTRL and PKR<sup>-/-</sup> mice received two times 1% sodium dextran sulfate (DSS) (n=5 per group) for 7 days each. The bar charts show the mean histopathologic score ±SD. Since DSS colitis is accompanied by a loss of epithelial cells (see IEC marker E-Cadherin), whole distal colonic tissue was lysed for Western Blotting. COX4 served as a marker to ensure equal amounts of mitochondria in all samples. (B) H&E staining of corresponding distal colonic tissue sections. (C) The box plot shows the difference in weight after DSS between CTRL and PKR<sup>-/-</sup> mice.

**Figure 4. mtCK is induced during the onset of inflammation**  
 (A) 12 weeks old CTRL and PKR<sup>-/-</sup> mice received 1% DSS for 3 days in the drinking water (n=5 per group). Mice were sacrificed, colonic IEC were isolated. The bar charts show the mean histopathologic score ±SD in the distal colon (significance levels were obtained by non-parametrical statistical tests). mtCK expression was analyzed by Western blotting in isolated large IEC. (B) H&E staining of corresponding distal colonic tissue sections. (C) Relative mRNA expression of mtCK from isolated epithelial cells were analyzed by qRT-PCR.

**Figure 5. ER stress affecting cell viability is accompanied by ROS production and leads to C1pP mediated degradation of mtCK.**  
 (A) Mode K cells were stimulated with the ER stressor tunicamycin (Tm; 500 ng/ml) for 6 h and mitochondria were isolated. Western Blots show a comparison of the whole cell lysate and isolated mitochondria. COX4 and β-actin served as mitochondrial and cytosolic marker, respectively. (B) Subsequent to a 24 h incubation of Mode K cells with Tm, a WST test was performed to analyze the effect of persistent ER stress on the cell viability. (C) After a 3 h treatment of Mode K cells with Tm, mtCK was immunoprecipitated and Western blots were performed for the protease C1pP and the chaperones Grp75 and Tid-1. The IgG heavy chain and the mtCK served as a control for equal loading.

**Figure 6. Mitochondrial stress induces the mtCK and does not affect the epithelial cell viability.**  
 Mouse embryonic fibroblasts (MEF) were transfected for 36 h with a truncated version of the mitochondrial enzyme ornithine transcarbamylase (OTCΔ) to induce the mtUPR (marked by a PKR induction). (B) Cell viability was analyzed by WST after a 48 h transfection of Mode K cells with OTCΔ.

center for diet and disease

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59

## II. Featured Poster II – Dietary fatty acids increase intestinal L cell numbers independent of the development of obesity (18<sup>th</sup> NuGOweek)



### Dietary fatty acids increase intestinal L cell numbers independent of the development of obesity

Elisabeth Urbauer<sup>1</sup>, Doriane Aguanno<sup>1</sup>, Valentina Schüppel<sup>1</sup>, Dirk Haller<sup>1,2</sup>, Eva Rath<sup>1</sup>

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#### Background & Aim

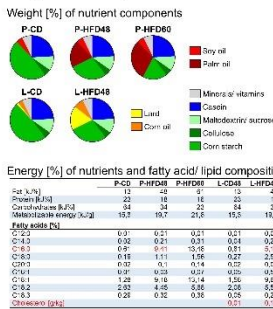
Enteroendocrine cells (EECs) comprise about 1% of the intestinal epithelial cell (IEC) population. They produce various hormones to coordinate optimal absorptive conditions following food intake, ensuring efficient postprandial assimilation of nutrients. EECs are equipped with sensors for the detection of luminal nutrients including free fatty acids, short-chain and long-chain fatty acids. Dietary habits, obesity and microbiome composition are associated with alterations in EEC function and numbers. In particular, glucagon-like peptide 1 (GLP-1)-producing L-cells seem to be affected. Ileal and colonic GLP-1 is suggested to confer location-specific functions, stimulating insulin release and reducing gastrointestinal motility, respectively.

#### Summary

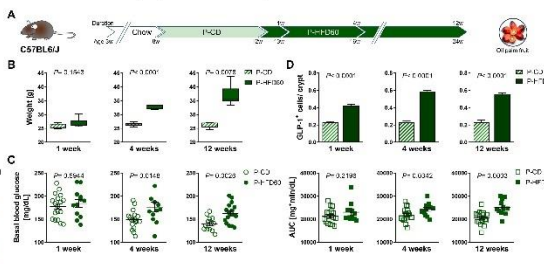
These data support the hypothesis that dietary lipids and FA composition directly impact IEC differentiation towards the enteroendocrine lineage. The effect of HFDs on metabolic parameters does not seem to affect intestinal L cell numbers. Epithelial metabolism and mitochondrial function, including FA metabolism, is increasingly recognized as important determinant of differentiation processes (see review on the left). Thus, this study highlights the potential of precise nutritional interventions in the context of metabolic diseases and underlines the necessity of careful interpretation of data from DIO models due to distinct effects of the fat source.

### Results

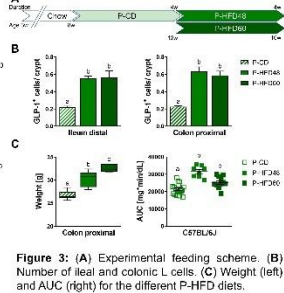
#### I. Composition of experimental diets



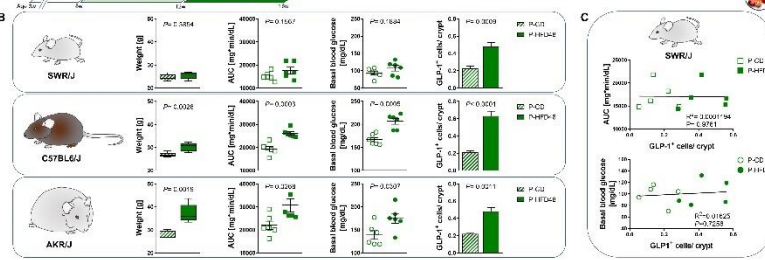
#### II. L cell numbers rapidly increase in response to P-HFD60



#### III. P-HFD48 and P-HFD60 equally increase L cell numbers



#### IV. EEC numbers increase in mouse strains with different susceptibility to diet-induced obesity in response to P-HFD48 independent of metabolic parameters



#### V. L cell and EC staining and IEC differentiation

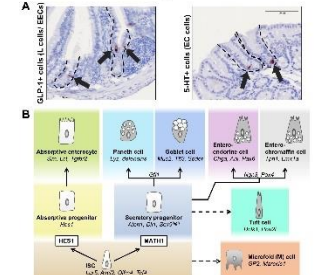
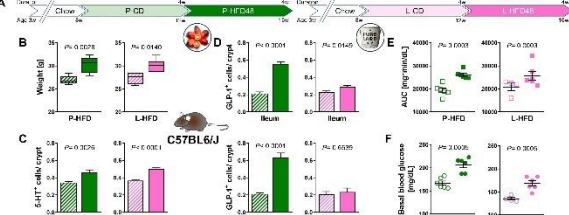


Figure 2: (A) Experimental feeding scheme. (B) Weight after the indicated time of P-HFD60-feeding (C) Basal blood glucose after 6h of fasting (left) and total area under the curve (AUC) of blood glucose levels in response to oral administration of 2.0g glucose/kg lean mass (right). (D) Number of colonic L cells.

Figure 3: (A) Experimental feeding scheme. (B) Number of ileal and colonic L cells. (C) Weight (left) and AUC (right) for the different P-HFD diets.

#### VI. P-HFD48 and L-HFD48-feeding differentially impact L cell numbers



#### VII. FA exposure does not increase EECs in intestinal organoids

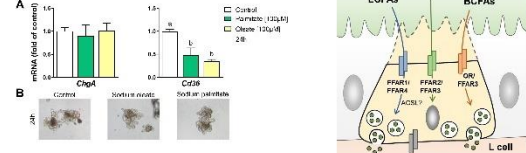


Figure 4: (A) Experimental feeding scheme. (B) Weight, AUC, basal blood glucose levels and number of colonic L cells in (upper panel) SWR/J mice with low, C57BL/6J mice with intermediate and AKR/J mice with high (lower panel) susceptibility to diet-induced obesity. (C) Colonic L cell numbers do not correlate with AUC (upper) and basal blood glucose levels (lower panel) in SWR/J mice.

Figure 7: Small intestinal organoids from WT mice were stimulated with sodium oleate and sodium palmitate (both 100µM) for 24h. (A) Gene expression analysis by qPCR indicates no changes in expression of the EECs maker *Chga* (left) but organoid responsiveness to FAs by downregulation of the FA receptor *C326*. (B) Representative pictures of intestinal organoids after FA treatment.

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25. **Rath E**.  
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20. Urbauer E\*, **Rath E**\* and Haller D.  
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19. Zietek T, Giesbertz P, Ewers M, Reichart F, Weinmüller M, Urbauer E, Haller D, Demir IE, Ceyhan GO, Kessler H and **Rath E**.  
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17. **Rath E**, Moschetta A, Haller D.  
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16. Coleman OI, Lobner EM, Bierwirth S, Sorbie A, Waldschmitt N, **Rath E**, Berger E, Lagkouvardos I, Clavel T, McCoy KD, Weber A, Heikenwalder M, Janssen KP, Haller D.  
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15. Just S, Mondot S, Ecker J, Wegner K, **Rath E**, Gau L, Streidl T, Hery-Arnaud G, Schmidt S, Lesker TR, Bieth V, Dunkel A, Strowig T, Hofmann T, Haller D, Liebisch G, Gérard P, Rohn S, Lepage P, Clavel T.  
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14. Zietek T, Waldschmitt N, **Rath E**.  
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13. Berger E, **Rath E**, Yuan D, Waldschmitt N, Khaloian S, Allgäuer M, Staszewski O, Lobner EM, Schöttl T, Giesbertz P, Coleman OI, Prinz M, Weber A, Gerhard M, Klingenspor M, Janssen KP, Heikenwalder M, Haller D.  
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12. Zietek T\*, **Rath E\***.  
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\*these authors contributed equally to the manuscript
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7. **Rath E**, Haller D.  
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6. **Rath E**, Haller D.  
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2. **Rath E**, Haller D.  
Inflammation and cellular stress – a mechanistic link between immune-mediated and metabolically-driven pathologies.  
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\*these authors contributed equally to the manuscript

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Urbauer E, Aguanno D, Kuellmer K, Metwaly A, Waldschmitt N, Ahmed M, Khaloian S, Hörmannspurger G, Planchais J, Fromme T, Sartor RB, Sokol H, Haller D, **Rath E**.  
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Developing an *In Vitro* Infant Gut Barrier Model: Sodium Glycodeoxycholate as a Promising Candidate.

### “Special” manuscript

Zietek T and **Rath E**.  
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*Front. Young Minds*. 2023 11:717455. doi: 10.3389/frym.2022.717455

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Chapter 16 - Live-Cell Calcium Imaging in 3D Intestinal Organoids.  
Editor: Caroline M. Gorvin: Calcium Signaling. Springer Protocols.  
Humana New York, doi: 10.1007/978-1-0716-4164-4, ISBN 9781071641668

Zietek T, **Rath E**.  
Chapter 3 - Intestinal organoids: Mini-guts grown in the laboratory,  
Editors: Jamie A. Davies, Melanie L. Lawrence: Organs and Organoids,  
Academic Press, 2018, Pages 43-71, ISBN 9780128126363

**Rath E**, Zietek T.  
Chapter 10 - Intestinal organoids: A model for biomedical and nutritional research  
Editors: Jamie A. Davies, Melanie L. Lawrence: Organs and Organoids,  
Academic Press, 2018, Pages 195-214, ISBN 9780128126363

## Curriculum Vitae

### Personal information

Name:	Eva Rath (Zang)	Date of birth:	4 <sup>th</sup> of May, 1979
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		Email:	eva.rath@tum.de
ORCID ID:	0000-0003-1910-1162		

### Work experience

2024 - present	Scientific Coordination Research Unit For5298 @ZIEL – Institute of Food & Health, Technische Universität München (TUM) School of Life Sciences
2021 - present	Program Management Chemical Biotechnology, Office for Academic and Study Affairs TUM Integrative Research Institute Straubing
2015 - present	Habilitation project: Chair of Nutrition and Immunology, TUM “Mitochondrial signaling as gatekeeper for intestinal health”
2011 – 2015	Postdoctoral fellow, Chair of Nutrition and Immunology, TUM
2006 – 2011	PhD Student, Chair for Biofunctionality, TUM
2007 & 2008	Research visits at the University of North Carolina, Chapel Hill, USA (four-month)
Parental leaves	05/2012 – 05/2013; 07/2014 – 08/2015; 07/2018 – 08/2019

### Advanced academic qualification

2012	Dissertation: (Dr.rer.nat.), Chair for Biofunctionality, TUM “Mitochondrial unfolded protein response in the epithelium: relevance to intestinal inflammation”, summa cum laude
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### University training and degree

2004 - 2006	Master of Science (Nutritional Sciences, focus: Biomedicine), TUM
2001 - 2004	Bachelor of Science (Nutritional Sciences), TUM
1999 - 2001	Study of Human Medicine, LMU

### Honors and awards

2013	Hans Adolf Krebs-Preis, German Nutrition Society; (DGE e. V.)
2011	Scientific Research Award, Institut Danone
2006 - 2023	11x Travel Awards to international conferences, 2x Child Care Grants 2x Presentation/Poster Awards
2006	“Preis des Oberbürgermeisters der Stadt Freising“ for the best Master’s thesis of the Department of Nutrition