

Dietary Tryptophan Restriction Remodels the Skin Metabolic Environment and Drives Microbial Community Restructuring

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Abstract

Tryptophan (Trp) is an essential amino acid that links metabolism, immunity, and barrier tissue homeostasis, and plays a central role in host-microbiota interactions. However, how systemic Trp availability shapes skin biology remains incompletely understood. Here, we investigated the impact of dietary Trp depletion on skin homeostasis. Using a controlled Trp-restriction model, we show that reduced Trp availability broadly alters cutaneous Trp metabolism at the host-microbial interface, with modulation of the kynurenine, serotonin, and indole pathways. These changes were associated with selective dysregulation of aryl hydrocarbon receptor (AhR) signaling in the skin, without affecting immune cell composition at steady state.

Dietary Trp deficiency also induced early and significant alterations in the skin microbiota, characterized by reduced diversity and shifts in community structure, including the expansion of specific bacterial taxa. In contrast, microbiota changes in the gut were more limited, indicating a tissue-specific response to systemic Trp availability.

Together, our findings identify dietary Trp as a key regulator of skin metabolic and microbial homeostasis, highlighting coordinated interactions between host pathways and the skin microbiota.

Introduction

The skin is a complex barrier organ that integrates environmental, microbial, and systemic cues to maintain tissue homeostasis. Beyond its role as a physical barrier, the skin is increasingly recognized as a metabolically active interface where host- and microbiota-derived metabolites contribute to barrier integrity, cellular differentiation, and immune regulation^{1,2}. In this context, the skin microbiota forms a complex and spatially organized ecosystem in close symbiosis with the host³. In humans, this ecosystem is dominated by *Cutibacterium*, *Staphylococcus* (Firmicutes), and *Corynebacterium* (Actinobacteria), along with fungi such as *Malassezia*, reflecting adaptation to distinct cutaneous niches⁴. Murine skin microbiota shares similar high-level phyla, including Firmicutes, Actinobacteria, and Proteobacteria⁵, but differs in relative abundance. This microbial ecosystem plays a fundamental role in maintaining skin health by contributing to immune education^{6–8}, protection against pathogens⁹, and preservation of barrier integrity^{10,11}. These functions are mediated in part through the production and transformation of bioactive metabolites that regulate keratinocyte differentiation, antimicrobial peptide production, and immune activation¹. Among these metabolic pathways, tryptophan (Trp) metabolism has emerged as a key regulator of host-microbiota interactions at barrier surfaces^{12,13}. In mammals, Trp is an essential amino acid obtained exclusively from the diet and primarily metabolized through the kynurenine pathway (KP) initiated by the rate limiting indoleamine 2,3-dioxygenase (IDO) enzyme, generating immunomodulatory metabolites such as kynurenine and NAD⁺^{12,14}. In parallel, Trp can be converted into serotonin and melatonin, regulating neuroimmune and epithelial functions¹⁵. Importantly, Trp also serves as a substrate for microbial metabolism, leading to the production of indoles and

derivatives that act as ligands for the host aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor that promotes epithelial integrity and tissue homeostasis ^{12,16}.

Most current knowledge on Trp metabolism derives from studies in the gut, where dietary Trp availability, microbial metabolism, and host pathways are tightly interconnected ¹². In the gut, reduced availability of these metabolites impairs barrier function and immune regulation, and dietary Trp restriction alters microbial composition and limits AhR ligand production, resulting in dysregulated immune responses ¹⁷. Emerging evidence suggests that similar mechanisms may operate in the skin. AhR is highly expressed in keratinocytes, where it regulates differentiation, epidermal barrier formation, and inflammatory responses ^{18,19}. Alterations in Trp metabolism and AhR signaling have been reported in several inflammatory skin diseases, including atopic dermatitis, psoriasis, and hidradenitis suppurativa (HS). Notably, KP enzymes such as KYNU are strongly upregulated in psoriatic lesional skin ^{20,21}, and we previously demonstrated KP hyperactivation associated with local Trp depletion and impaired AhR signaling in HS ²². In addition, it has been demonstrated that skin commensal microbes metabolize Trp locally in the skin into indole derivatives functioning as ligands for AhR, thereby directly promoting epidermal differentiation and barrier function ^{18,23}. These findings position microbial Trp metabolism as a central determinant of cutaneous homeostasis and highlight the importance of AhR signaling in integrating metabolic and microbial cues within the skin ¹⁶.

Together, these observations suggest that Trp metabolism may regulate the balance between host- and microbiota-derived AhR ligands in the skin. However, whether dietary Trp availability directly controls this metabolic and microbial axis in the skin remains unknown. Whereas the intestine hosts a dense, metabolically active microbiota within a mucus-rich environment, the skin supports a lower biomass community adapted to a dry, oxygenated, and nutrient-limited niche ¹. These differences suggest that dietary perturbations may exert tissue-specific effects that remain insufficiently explored in the skin.

Here, we used dietary Trp restriction as a proof-of-concept to investigate whether nutrient availability directly shapes skin metabolism and microbiota. Through an integrated analysis of metabolic, microbiological, and immunological parameters across tissues, we identify the skin as a particularly sensitive target of Trp depletion. We show that dietary Trp deficiency induces a profound rewiring of Trp metabolism in the skin involving both host and microbial pathways, accompanied by selective dysregulation of AhR signaling without changes in immune cell composition at steady state. Furthermore, Trp restriction triggers early alterations in the skin microbiota, with effects that are more pronounced than those observed in the gut. Together, these findings establish that dietary Trp availability is a key determinant of skin metabolic activity and microbial ecology, providing a framework for targeting the cutaneous ecosystem through nutritional interventions.

Results

Tryptophan Depletion Rewires Skin Trp Metabolism via Host and Microbial Pathways

Since the total absence of dietary Trp causes life-threatening weight loss¹⁷, we first established a Trp-deficient mouse model alternating Trp-low and control diets (Fig. 1A). With this regime, mice showed a significant but stable weight-loss over time (Fig. 1A). We noted a more severe impact on females indicating that they are more susceptible to Trp deficiency than males (Fig. 1A). We next monitored Trp levels over 5 weeks in plasma, skin and gut (Fig. 1B & **Supplementary Fig. 1A**). Because Trp is a precursor for neuroactive metabolites and its availability is tightly regulated across tissues, including the central nervous, we extended our analysis to the brain (including brainstem and hippocampus) (Fig. 1B & **Supplementary Fig. 1A**). In females, this analysis revealed substantial temporal and tissue specific variability, with significantly reduced Trp levels in plasma, skin and hippocampus after 4 weeks of dietary intervention (Fig. 1B) while no changes were observed at earlier time points (**Supplementary Fig. 1B**). In contrast, no significant decrease was detected in the gut content and in the brainstem at the same time point (**Supplementary Fig. 1C**). In males, a similar trend toward reduced Trp level was observed in the skin, while serum and gut content levels remained unaffected (**Supplementary Fig. 1D**).

Focusing on females, to determine whether Trp depletion impacts local tissue metabolism, we performed a comprehensive and quantitative analysis of host and microbiota-derived metabolites of Trp in the skin. Specifically, the host-derived products of Trp metabolism included the KP-derived kynurenine, quinolinic acid (QA) and picolinic acid (PA); as well as serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Microbiota-derived metabolites originating from the indole pathway were also assessed, including indole-3-lactic acid (ILA), indole-3-acetic acid (IAA) and indole-3-acetamide (IAM) (Fig. 1C). A principal component analysis (PCA) of skin samples harvested after 4 weeks of diet revealed a distinctive metabolic signature in Trp-low mice (Fig. 1D). Significant decreases in levels of kynurenine, QA and 5-HIAA, along with the increased PA level indicated that both the KP and serotonin pathway were modulated after 4 weeks (Fig. 1E) while no significant changes were observed at earlier time point (**Supplementary Fig. 1E**). Looking at the Trp metabolites of microbial origin, Trp deficiency led to a sharp reduction in ILA, whereas IAM was increased (Fig. 1E). Together, these results indicate that Trp availability differentially shapes microbial Trp metabolism, with depletion favoring IAM production at the expense of ILA.

Analyses of gut and serum samples revealed distinct metabolic adaptations to Trp deficiency (Fig. 1F & **Supplementary Fig. 1F**). In the gut, most kynurenine- and indole-derived metabolites were reduced, indicating a coordinated suppression of both host- and microbiota-driven pathways, likely as a compensatory response to reduced Trp availability (**Supplementary Fig. 1G**). Serum profiles mirrored these changes in an attenuated form (Fig. 1F), suggesting that circulating Trp metabolites largely reflect intestinal processing. In contrast, Trp catabolism in the skin appeared to be rewired rather than suppressed, with local accumulation of host- and bacteria- derived metabolites including PA and IAM, respectively (Fig. 1C). Interestingly, consistent with Trp depletion in the hippocampus, serotonin levels

were markedly reduced in this region (Fig. 1G), whereas no significant changes were observed in plasma, skin or gut contents (**Supplementary Fig. 1H**). Collectively, these results highlighted a tissue-specific metabolic adaptation to Trp limitation, with a particularly distinct response in the skin.

Diet-Induced Tryptophan Deficiency Selectively Dysregulates AhR Signaling in the Skin without changing immune cell composition at homeostasis

Since PA exerts immunoregulatory effects and IAM is an AhR ligand, we next investigated the origin and effect of these metabolic changes on the immunological status of the skin. We first assessed transcriptional alterations in host enzymes involved in Trp metabolism (Fig. 2A) and in AhR downstream targets (Fig. 2B) using a microfluidic-based Biomark approach. The expression levels of selected enzymes of the KP (IDO1/2; KMO, Kynu, QPRT, 3-HAO, KYART1) and serotonin pathway (TPH1) (Fig. 2A) as well as AhR downstream targets (CYP1A1, CYP1B1 and the negative feedback regulator AHRR) (Fig. 2B) were measured in the skin after 3 and 4 weeks of dietary Trp depletion. This transcriptional analysis revealed an increased expression of the genes of the KP after 3 weeks, with significant upregulation of mRNA levels of KMO, KYAT1 and TPH1 suggesting an increased catabolism of Trp by skin cells (Fig. 2A). Concomitantly, AHRR and CYP1A1 were significantly upregulated (**Fig; 2B**), suggesting an increased AhR activity at this time point.

None of these changes were maintained after 4 weeks (Fig. 2A-B), time at which we observed significant changes in skin Trp metabolites (Fig. 1E). Together, these data suggest a biphasic response to dietary Trp restriction, with an early adaptive phase (week 3) characterized by transcriptional upregulation of Trp catabolic pathways and AhR activation, followed by a later phase (week 4) in which this compensatory response is no longer sustained and is associated with a measurable decline in Trp-derived metabolites. Overall, we conclude that the skin responds to Trp limitation by transcriptionally promoting Trp catabolism, possibly to preserve local metabolite availability and sustain AhR signaling. However, this adaptive mechanism appears to become insufficient over time, leading to reduced Trp metabolism, as evidenced by the metabolic changes observed at week 4 (Fig. 1E).

To explore potential effects of these changes on barrier homeostasis, skin sections harvested after 4 weeks of Trp-low diet were stained by hematoxylin and eosin (H&E). No structural anomaly nor variation in the epidermal thickness was noted, suggesting that epidermal architecture and progenitor cell turnover are preserved (**Supplementary Figs. 2A & 2B**). To detect potential inflammatory and /or immunological alterations, we performed targeted transcriptomic profiling and flow cytometric analysis of skin immune cell populations. Expression analysis of a panel of cytokines and chemokines, including T helper (Th)1, Th2 and Th17-type markers, as well as proinflammatory chemokines and tissue-remodeling markers, did not reveal significant differences between control and Trp-low diet after 4 weeks (**Supplementary Fig. 2C**). Consistently, flow cytometric analysis of total skin immune populations did not show significant changes in the frequency of immune (CD45⁺) cells or major immune subsets (Fig. 2C & **Supplementary Fig. 2D**). Altogether, these findings suggested that diet-induced alterations in Trp metabolism do not elicit overt tissue-level inflammation in the skin at steady-state.

Diet-Induced Tryptophan Deficiency triggers early skin microbiota alteration

Considering the profound systemic and local metabolic changes induced by dietary Trp restriction, we next assessed its impact on the organization and diversity of the cutaneous microbial community. Indeed, previous work has demonstrated that dietary Trp restriction reshapes gut microbial community composition and function after 3 weeks of diet¹⁷. More recently, it has been shown that skin-associated microbes encode enzymatic pathways capable of converting Trp into bioactive indole derivatives, thereby directly contributing to modulating cutaneous Trp metabolism¹⁸.

To have a sense of if and how early modifications of skin microbial communities occurred, we conducted an initial longitudinal taxonomic analysis of the skin microbiota along the course of the diet using 16S rRNA gene sequencing. This analysis revealed time- and diet-dependent alterations at the phylum level (Fig. 3A). Notably, the Trp-low diet induced a marked expansion of Bacillota (previously called Firmicutes), which was detectable from week 2 onward and sustained at later time points, with relative abundance reaching up to 90% of the total community in some experiment. This expansion was accompanied by a significant reduction in *alpha* diversity as early as 2 weeks and maintained at week 4 (Fig. 3B). In contrast, *alpha* diversity remained relatively stable in control animals across the same time period.

As the most pronounced alterations in Trp metabolism were observed after 4 weeks of diet, we next analyzed microbial communities in both the skin and gut at this time point. In the skin, the Trp-low diet was associated with a decrease of *alpha* diversity (**Supplementary Fig. 3A**) accompanied by a significant shift in overall microbial composition (Fig. 3C). Indeed, *beta* diversity analysis revealed a clear segregation between control and Trp-low groups based on PCA using Bray-Curtis dissimilarity, confirmed by PERMANOVA t significance test ($p = 0.001$) (Fig. 3C). In contrast, analysis of the gut microbiota revealed an increase in *alpha* diversity under the Trp-low diet (**Supplementary Fig. 3A**). Beta diversity analysis of gut communities, although less pronounced compared to the skin, also showed separation between dietary groups (Fig. 3C). Taxonomic analysis of microbial communities in the skin revealed a marked expansion of bacteria belonging to the class Bacilli under the Trp-low diet (Fig. 3D). At the genus level, this shift was exemplified by a strong increase in *Sporosarcina* abundance (Fig. 3D), suggesting the expansion of specific members within this lineage. To systematically identify taxa associated with dietary intervention, differential abundance analysis was performed using DESeq2. This analysis confirmed the significant enrichment of multiple genera within the Bacilli class, including *Lactobacillus* (family *Lactobacillaceae*), *Jeotgalicoccus* (*Staphylococcaceae*), *Sporosarcina* (*Caryophanaceae*), *Atopostipes* (*Carnobacteriaceae*), and *Eubacterium ventriosum* (*Eubacteriaceae*), in Trp-low-fed mice compared to controls (adjusted p -value < 0.05) (Fig. 3E). In contrast to the marked restructuring observed in the skin, the impact of the Trp-low diet on the gut microbiota was more limited. At the class level, a modest increase in *Desulfovibrionia* was observed, without a clear or consistent shift at the genus level (**Supplementary Fig. 3B**). Despite the absence of major compositional changes, differential abundance analysis identified the significant enrichment of specific taxa, including *Lactobacillus*,

Turcibacter, and *Mammaliicoccus*, in Trp-low-fed mice compared to controls (adjusted p-value < 0.05) (**Supplementary Fig. 3C**). Together, these findings indicate that, in the skin, dietary Trp restriction promotes the coordinated expansion of a phylogenetically related group of taxa within the Bacilli lineage, rather than isolated changes in individual genera.

This marked remodeling of the skin microbiota could have consequences on the functional capacities for Trp metabolism. Consistent with prior evidence that the microbiome regulates cutaneous Trp metabolism¹⁸, we observed that Trp deficiency impacted the abundance of some microbiota-derived metabolites in the skin (Fig. 1E). To determine whether this metabolic rewiring was microbiota-driven, we inferred Trp metabolic functions from 16S rRNA data using TrpNet (**Supplementary Fig. 3D**)²⁴. Bacterial taxa enriched under Trp deficiency were mainly positively associated with metabolites from the indole pathway and in a minor extend to KP and serotonin pathways (Fig. 3F). Given that KP and serotonin pathways are shared by host and microbes, these results suggest that the observed decrease in these pathways is primarily host-derived, with microbiota shifts potentially reflecting a compensatory response. These findings indicate that dietary Trp restriction induces selective modulation of the gut microbiota, in contrast to the more pronounced and structured remodeling observed in the skin.

Discussion

In this study, we provide evidence that dietary Trp availability shapes the skin metabolism-microbiota axis, supporting a functional link between nutrient availability and host-microbial metabolic interactions. Trp restriction was sufficient to reshape both Trp metabolic pathways and major microbial communities in the skin, highlighting a previously underappreciated role for dietary amino acids in cutaneous homeostasis.

While intestinal and serum metabolite profiles largely reflected a global suppression of host- and microbiota-driven pathways, the skin exhibited a distinct and more complex metabolic reprogramming. Host-derived metabolites, including kynurenine and 5-HIAA, were decreased, while PA accumulated, and the microbiota-derived metabolite IAM increased. These findings suggest that, under conditions of dietary restriction, the skin microbiota actively contributes to local Trp metabolite availability. Consistent with this, Trp depletion dynamically altered the expression of enzymes involved in the KP and serotonin pathways, as well as AhR-responsive genes. At week 3, the upregulation of KP (KMO, KAT1) and serotonin pathway (TPH1) enzymes suggests an early compensatory response aimed at maintaining Trp-derived signaling. However, this transcriptional adaptation was no longer sustained at later timepoints and coincided with a measurable depletion of Trp-derived metabolites, indicating that this compensatory mechanism is insufficient over time. Sustained activation of the KP may further exacerbate Trp depletion while contributing to PA accumulation in the skin.

Beyond the skin, Trp depletion also impacted central metabolism, as evidenced by reduced serotonin levels in the hippocampus but not in the brainstem. This regional specificity highlights the differential sensitivity of central serotonin synthesis to Trp availability, likely reflecting variations in Trp transport and

local metabolic regulation. Although not directly assessed here, such alterations may have implications for neurogenesis, behavior and memory ^{25,26}, and neuroimmune interactions, suggesting a broader systemic impact of dietary Trp restriction. Altogether, these data indicate that Trp availability directly influences signaling pathways linked to barrier function and immune regulation. In particular, AhR acts as a central integrator of host- and microbiota-derived Trp metabolites, including kynurenine and indole derivatives, which regulate keratinocyte differentiation, antimicrobial responses, and immune cell function ^{23,27}.

Despite these marked metabolic and microbial changes, epidermal architecture and immune cell composition remained stable, indicating that the skin maintains homeostasis under steady-state conditions, at least within the 4-week timeframe. The relative abundance of major immune populations, including T cell and myeloid subsets, was bit altered, suggesting that altered Trp metabolism alone is not sufficient to drive large-scale immune remodeling in the absence of additional stressors. This apparent resilience likely reflects the capacity of the skin immune system to buffer metabolic fluctuations under homeostatic conditions. However, it is important to consider that the immunological impact of Trp metabolism is often context-dependent. Under inflammatory or infectious conditions, activation of the KP and modulation of AhR signaling play key roles in shaping immune responses. In the gut, microbiota-derived Trp metabolites regulate IL-22 production and mucosal defense through AhR activation, and disruption of this pathway increases susceptibility to colitis ²⁸. Similarly, induction of indoleamine 2,3-dioxygenase (IDO1) during inflammation promotes Trp catabolism and contributes to immune regulation and tolerance ²⁹. In the skin, AhR signaling controls keratinocyte differentiation, antimicrobial peptide expression, and inflammatory responses, and dysregulated Trp metabolism has been implicated in diseases such as atopic dermatitis psoriasis and HS ^{22,30}. Thus, the absence of overt immune alterations in our model likely reflects a steady-state condition, and it remains possible that Trp restriction could significantly modulate cutaneous immunity in the context of inflammation or disease.

A key finding of this study is the impact of dietary Trp on the skin microbiota. While the role of Trp metabolism in shaping gut microbial communities has been already investigated ³¹, its influence on the skin microbiota has remained largely unexplored. Our data demonstrate that Trp availability significantly alters microbial diversity and community structure over time, identifying Trp as an important ecological determinant of skin-associated microbial communities. Trp restriction was associated with a reduction in several taxa, including *Coriobacteriaceae* UCG-002, *Campylobacter*, *Candidatus Saccharimonas*, *Peptococcus*, *Muribaculum*, *Bilophila*, and *Enterococcus*, suggesting a broad loss of microbial groups potentially involved in metabolic and immune interactions. In contrast, a more limited set of taxa, including *Lactobacillus*, *Jeotgalicoccus*, *Sporosarcina*, and *Aerococcus*, were enriched, possibly reflecting adaptation to nutrient-restricted conditions or alternative metabolic capabilities. Importantly, these changes likely reflect shifts in metabolic networks rather than the activity of individual taxa. Consistent with observations in the gut, Trp metabolism is regulated by complex microbial consortia and functional interactions ³¹. It is therefore plausible that reduced Trp availability reshapes the skin microbiota by altering metabolic niches, thereby influencing both community composition and metabolite production.

Notably, both skin and gut microbiota were affected, although the magnitude and nature of these changes differed, highlighting tissue-specific responses to systemic nutrient perturbation.

Together, our findings highlight diet as a previously underappreciated driver of the cutaneous ecosystem. By modulating both host Trp metabolism and microbiota-derived metabolites, particularly those linked to AhR signaling, dietary Trp modulation may influence barrier function, immune regulation, and susceptibility to inflammatory skin diseases, such as HS, where dysregulated Trp metabolism and microbial imbalance have been reported²². These results provide a conceptual framework for targeting the skin through nutritional interventions.

Study limitation

First, while we identify strong associations between dietary Trp availability, metabolic changes, and microbiota composition, causal relationships remain to be fully elucidated. Dissecting the respective contributions of host- versus microbiota-derived metabolites in shaping skin responses require further investigation. Second, our analyses were performed under steady-state conditions, thus future studies will be necessary to determine how Trp modulation influences skin responses during inflammation, infection, or disease. Finally, although our cohort included a limited number of male animals, our data suggest potential sex-specific differences in susceptibility to Trp restriction, which warrants further exploration.

Materials and Methods

Mice, dietary intervention and experimental design

Six-week-old wild-type C56BL/6J mice obtained from Janvier Labs (Le Genest-Saint-Isle, France) were maintained under specific pathogen-free conditions at the Institut Pasteur animal care facility with controlled temperature and humidity, a 12-h light/dark cycle and unrestricted access to food and water. All procedures involving animals were approved by the Institut Pasteur ethic committee for animal experimentation (dap #210003) and authorized by the French ministry of research. All experiments were performed in accordance with relevant guidelines and regulations. Upon arrival, mice were acclimated for 1 week while receiving a control diet (SAFE®, reference U8958, version 338). Subsequently, mice were switched to a tryptophan-deficient diet (SAFE®, reference U8958, version 224). To limit severe weight loss associated with Trp restriction, mice were intermittently reintroduced to the control chow every 3-4 days throughout the protocol. Body weight and clinical condition were monitored longitudinally. Clinical scoring included evaluation of locomotion, posture, breathing, and fur appearance. Movement scores were assigned as follows: 0, normal; 1, mild incoordination; or 2, marked incoordination with reduced mobility. Posture was graded as 0, normal; 1, slightly hunched with stretching behavior; or 2 markedly hunched/crouched posture. Respiratory status was scored as 0 for normal; 1 for rapid; or 2 for abdominal breathing. Fur was evaluated as 0, normal coat; 1, ruffled/hirsute; or 2 patchy alopecia. Individual scores were combined to generate a global clinical score. Humane endpoints were predefined

> 25% body weight loss, a cumulative clinical score > 3, or a maximum score of 2 in any single category. Animals reaching any of these criteria were euthanized immediately in accordance with institutional animal welfare regulations. During the experiment, fecal pellets and dorsal skin swabs were collected for microbiota analyses. At the experimental endpoint, mice received an overdose of ketamine/ xylazine (200mg.kg⁻¹ ketamine; 20mg.kg⁻¹ xylazine) followed by cervical dislocation. Blood was collected for plasma isolation. Skin, intestinal contents, hippocampus and brainstem tissues were harvested, snap-frozen in liquid nitrogen, and stored at -80 °C until analysis. These tissues were later used for targeted metabolomic profiling of Trp metabolites.

Real-time qPCR using the Biomark HD platform

Skin and gut tissues were homogenized using a Precellys® tissue homogenizer (Bertin Technologies) using 2.8 mm ceramic beads (Bertin Technologies, cat# 000911-LYSK0-A.0). Total RNA extraction was performed using the RNeasy® Fibrous Tissue Mini Kit (Qiagen, cat# 74704) according to the manufacturer's instructions. RNA concentrations were measured with the Qubit® RNA Assay Kit (Life Technologies, cat# Q32852). Reverse transcription was carried out from 500 ng RNA and the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, cat# 4368814).

cDNA pre-amplification for Fluidigm analysis

cDNA samples were pre-amplified using Fluidigm PreAmp Master Mix (Fluidigm, cat# PN 100-5580). Gene-specific primers (Delta Gene™ assays, Fluidigm) (Table 1) were pooled for target amplification by combining 1 µL of each 100 µM assay in a microcentrifuge tube and adjusting to a final volume of 200 µL with DNA Suspension Buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA) resulting in a final concentration of 500 nM. Preamplification reactions (5 µL) contained 1 µL PreAmp Master Mix, 0.5 µL pooled primers (500 nM), 2.25 µL nuclease-free water, and 1.25 µL cDNA. Thermal cycling was performed as follows: initial hold at 95 °C for 2 min, followed by 10 cycles of 95 °C for 15 s and 60 °C for 4 min, ending with a hold at 4 °C. Cleanup was then conducted with 2 µL diluted Exonuclease I (4 U/µL) (New England BioLabs®, cat# PN M0293S) and incubated at 37 °C for 30 min to remove residual primers, followed by heat inactivation at 80 °C for 15 min. Amplified products were diluted five-fold with DNA Suspension Buffer and stored at -20 °C or used immediately for real-time PCR.

Quantitative Reverse-Transcription PCR

Gene expression analysis was performed using the Fluidigm 48.48 Integrated Fluidic Circuit (IFC) and the Biomark™ HD platform. IFC priming was carried out according to the manufacturer's instructions using the IFC Controller MX system. Sample pre-mix was prepared by combining 2× SsoFast™ EvaGreen® Supermix with Low ROX (Bio-Rad, cat# PN 172-5211) and 20x DNA Binding Dye Sample Loading Reagent (Fluidigm, cat# PN 100-7609). For each sample inlet, 3.3 µL of pre-mix was combined with 2.7 µL of pre-amplified cDNA. Assay solutions were prepared by diluting pooled forward and reverse primers in assay loading reagent and DNA suspension buffer to achieve a final primer concentration of 500 nM in the reaction chamber. After loading assay and sample mixes into the IFC, the chip was

processed using the appropriate loading script and transferred to the Biomark™ HD system. Real-time PCR employed the 48.48 gene expression protocol with EvaGreen detection and ROX passive reference dye. Data collection was conducted using auto-exposure settings. Relative expression levels were calculated using the comparative threshold cycle (Ct) method ($\Delta\Delta Ct$). Ct values for each target were normalized to TFRC (transferrin receptor) expression to obtain ΔCt values ($\Delta Ct = Ct_{\text{target}} - Ct_{\text{TFRC}}$). Experimental ΔCt values were then normalized against the mean ΔCt of the control diet group to calculate $\Delta\Delta Ct$ values ($\Delta\Delta Ct = \Delta Ct_{\text{sample}} - \text{mean } \Delta Ct_{\text{control diet}}$). Relative fold change (FC) were determined using the equation $2^{-\Delta\Delta Ct}$, and expressed relative to the controls.

Preparation of skin single-cell suspension for flow cytometry

Single-cell suspensions from skin were prepared using an enzymatic digestion protocol adapted from reference ³². After euthanization, the collection area was shaved and disinfected with 70% ethanol. Skin samples were excised under sterile conditions, transferred to cold PBS and stripped off subcutaneous fat. Skin samples were cut into approximately 1 × 1 cm fragments and finely minced. Samples were incubated for 2.5 h at 37 °C under gentle agitation in a digestion buffer containing PBS supplemented with DNase I (5 U/mL), Liberase TL (25 µg/mL final), HEPES, and β-mercaptoethanol. Digestion was stopped by adding EDTA and stop solution (PBS 4% FBS and 1% HEPES). Digested tissue was mechanically dissociated, filtered through 70 µm strainers, washed extensively and centrifuged at 400 × g for 10 min at 4 °C. Cell suspensions were passed through 40 µm strainers prior to staining. For staining, 1 × 10⁶ cells were incubated with Live/dead Near IR dye (ThermoFisher, L34975) for 15 min at room temperature in the dark. Fc receptors were blocked using anti- CD16/32 antibody (Miltenyi, 130-130-189, 1:200 dilution) for 5 min on ice. Cells were then stained with fluorochrome-conjugated antibody cocktails (Table 2) for 25 min on ice protected from light. Following washing steps, cells were resuspended in PBS and analyzed on a Beckman Coulter CytoFLEX flow cytometer.

Histology

Mice fed either a control or Trp-low diet were sacrificed after 3 or 4 weeks of dietary intervention. Back skin was shaved prior to harvesting. Skin samples were fixed in 4% paraformaldehyde (PFA) for 5–6 hours at 4°C, then dehydrated in 30% sucrose at 4°C for 24 hours. Tissues were embedded in OCT compound and stored at -80°C until sectioning. Cryosections (5 µm thickness) were stained with hematoxylin and eosin (H&E) following standard procedures. Epidermal thickness was quantified on stained sections.

Quantification of Trp and Trp-derived metabolites

Tissues samples (Caecal contents, Brain, Skin). Calibration standards and quality controls were prepared in Milli-Q water containing an internal standard mixture. Tissues samples were freeze-dried for 48 h and 2.5-3 mg of dried material was weighed for analysis. Samples were homogenized in 1 mL methanol/water (1:1) containing internal standards. Homogenates were agitated for 30 min and

centrifuged at 15,000 g for 10 min at 4 °C. Supernatants (900 µL) were evaporated and dried residues reconstituted in methanol/water (1:9). The injection volume was set to 5 µL into the LC-MS/MS system as described in reference ³³.

Serum samples. Calibration standards and quality controls were prepared in PBS supplemented with 4% BSA. Internal standard solution in methanol (200µL) was added to 25µL of serum samples, standards or controls in 96 deep-well plate. After centrifugation, 175µL of supernatant was evaporated under nitrogen at 40°C for 30min. Dried extracts were reconstituted in 100µL methanol/water (1:9) and centrifuged for 10 min at 3000 rpm at 4°C. LC-MS/MS injection volume was 5 µL according to the reference ³³.

Skin swab processing and DNA extraction

Skin samples were collected using sterile dry swabs (COPAN LQ Stuart Transport Swab, COPAN Italia SpA, Brescia, Italy) rubbed repeatedly on the skin surface with consistent pressure. Swabs were immediately frozen at -80°C. For DNA extraction, swabs were thawed and vortexed for 1 minute at 2500 rpm. 1 mL of transport medium was transferred into a 96-well deep-well plate and centrifuged at 16,000 g for 10 minutes at 4°C to pellet microbial material. Genomic DNA was isolated using the NucleoSpin 96 Genomic DNA kit (Macherey-Nagel as described in references ^{34–36}. Negative controls contained extraction buffers only. Pellets were incubated with Ready-Lyse Lysozyme Solution (250 U µl⁻¹; Epicentre) for 30 min at 37 °C, followed by overnight digestion with Proteinase K buffer at 55 °C. Glycogen carrier (20 µg) was added before DNA extraction, performed according to the manufacturers' instructions. DNA was eluted in 25 µl of buffer and immediately frozen at -80 °C.

Fecal sample collection and DNA extraction

Fresh fecal pellets were collected and immediately stored at -80 °C until processing. DNA extraction was performed using the NucleoSpin® Soil kit (MACHEREY-NAGEL®) following the manufacturer's instructions as previously reported in references ^{37,38}.

16S rRNA sequencing and analysis

DNA concentration was measured using the TECAN (QuantiFluor ONE dsDNA System, Promega). DNA quality and fragment size distribution were verified with the Agilent 2100 Bioanalyzer. The V3-V4 hypervariable region of bacterial 16S rRNA was amplified as previously described in references ^{34,35,37}. This region was selected because it enables broad bacterial taxonomic resolution and facilitates comparative analyses between skin and fecal microbiota^{35,39}.

Sequence processing and statistical analyses

Sequences containing incorrect barcode or primer sequences, as well as reads containing more than one ambiguous nucleotide, were excluded. After quality filtering 13,685,059 mapped reads from 160 skin samples were retained (mean: 85,532 reads/ sample) while fecal datasets contained 6,694,650 mapped

reads from 117 samples (average of 57.219/ sample). Negative controls contained less than 500 reads, indicating minimal background contamination. Putative contaminant taxa were removed from downstream analyses. Bioinformatics analyses were performed as previously described in references^{34,35,37,40}. Briefly, amplicons were clustered into operational taxonomic units (OTUs) using VSEARCH (version 1.4) and taxonomically assigned using the SILVA database. Reads were mapped against the OTU reference tables to get abundance matrices. We used SHAMAN (SHiny application for Metagenomic Analysis (shaman.c3bi.pasteur.fr) based on R software to normalize, run statistical analyses and perform multiple visualization.

Declarations

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Author contribution

Conceptualization: CD, LGM

Methodology: All

Investigation: VAA, LGM, AL, PG

Visualization: LGM, VAA

Funding acquisition: CD

Project administration: CD

Supervision: CD, LGM

Writing – original draft: LGM, VAA

Data availability statement

RNA-seq data generated from 16S rRNA sequencing have been deposited on NCBI Sequence Read Archive under accession number (BioProject ID: PRJNA1459615; <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1459615>).

Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1: Primers used for biomark

Target	Assay ID	FP	RP
AHRR	GEP00120711	TCAGAATTGCATCCCAAACCTCA	GTTTGTCCCCTGAACAGTGAA
CXCL1	GEA00002481	CCTGAAGCTCCCTTGGTTCA	TTCTCCGTTACTTGGGGACAC
CXCL2	GEA00037775	CCCCTGGTTCAGAAAATCATCC	TCCTTTCCAGGTCAGTTAGCC
CYP1A1	GEP00119691	TCATCCTTCGTCCCCTTCAC	CAGCACCCCTTGGGGATATA
CYP1A2	GEP00120712	CCCAAGGAGCGCTGTATCTA	GGAACACAAAGGGGTCTTTCC
CYP1B1	GEP00120713	GCCAGGACACCCTTTCCA	CCACAACCTGGTCCAACTCA
GAPDH	GEP00055172	CAAGGTCATCCCAGAGCTGAA	CAGATCCACGACGGACACA
HAAO	GEP00120708	CCACTCTGAGCAATACCGAACA	GACCGTGTATTTCAGCGGGAA
IDO1	GEP00069211	ACTGCACGACATAGCTACCA	ACGTGTCTGGGTCCACAAA
IDO2	GEP00120715	GGGGCTTCATCCTAGTGACA	AATGGCCTCCATTCCCTGAA
IFNA1	GEP00065213	ACAAGAGAGCCTTGACACTCC	TGCGGGAATCCAAAGTCCTT
IFNB1	GEP00059282	TACTACTGCCTTTGCCATCCA	CCACCCAGTGCTGGAGAAA
IL10	GEA00002698	AAAGGACCAGCTGGACAACA	TAAGGCTTGGCAACCCAAGTA
IL12A	GEA00002715	AAACCAGCACATTGAAGACC	GGAAGAAGTCTCTCTAGTAGCC
IL12B	GEA00002723	ATCGTTTTTGCTGGTGTCTCC	GGAGTCCAGTCCACCTCTAC
IL17A	GEP00089147	CAGACTACCTCAACCGTTCCA	CACTGAGCTTCCCAGATCACA
IL1B	GEA00002825	TGGCAACTGTTCTGAACTCA	GGGTCCGTCAACTTCAAAGAAC
IL2	GEA00002849	CCCAGGATGCTCACCTTCAAA	CCGCAGAGGTCCAAGTTCA
IL22	GEP00116151	GCCAGCCTTGCGAGATAACAA	GTTGAGCACCTGCTTCATCA
IL23A	GEP00116152	CTGCATGCTAGCCTGGAAC	ACTGGATACGGGGCACATTA
IL3	GEP00055603	TCGTGGAAAGCCAAGGAGAA	AGATGTAGGCAGGCAACAGTTA
IL4	GEP00055426	ACGGAGATGGATGTGCCAAA	GAAGCACCTTGGAAGCCCTA
IL5	GEP00114506	GATGAGGCTTCCTGTCCCTA	TTCAGTATGTCTAGCCCCTGAA
IL6	GEP00062239	CGATGATGCACTTGCAGAAA	ACTCCAGAAGACCAGAGGAA
IL7	GEP00120714	GTGCTGCTCGCAAGTTGAA	AGTGTTTGTGTGCCTTGTGATAC
IL9	GEP00114460	CAGCTGCTTGTGTCTCTCC	TGGCATTGGTCAGCTGTAAC
KMO	GEP00120705	CCACAAGCTGTGCGAAATGCA	ATGTGACATCTCGGGGAACC
KYAT1	GEP00120706	TGCTCAACCAGTACACCTCA	CAGCAGCTTGCCAAAGAAAC

KYAT3	GEP00120707	TTTACACGTGTGCGACTCC	GGTCATCCATGCGCTTGATA
KYNU	GEP00120704	CTACTCCAAAGCGGCACAAAA	GTCCGTGAAGTTGAATCTGTGAC
MMP10	GEP00116173	ACGTACTTCTTTGTAGGGGACAA	TGTCTTGGGAAGCCTTTATCCA
MMP1A	GEA00012883	TGATGAGACGTGGACCAACA	CAAGGGAGTGACCCAGTTCA
MMP3	GEA00012885	AGGGTGGATGCTGTCTTTGAA	ACTGCGAAGATCCACTGAAGAA
PPIA	GEA00010013	AGGGTTCCTCCTTTACAGAA	TGCCGCCAGTGCCATTA
QPRT	GEP00120709	GGGATGGTGATGGTGAAAGAC	CCACCTCTACCTTCAGGGAAA
TDO2	GEP00114031	GGTGTAACAGAGCCAGCAA	TCTGAAGTTCTTGCGCATTCA
TFRC	GEA00012937	GCAAAGTCTGGCGAGATGAAC	CCATTTGACTGCACTATGGTCAC
TNF	GEP00055421	CAAATGGCCTCCCTCTCATCA	GCTACAGGCTTGTCACTCGAA
TPH1	GEP00120710	GAGAATGAAGTCGGAGGACTCA	TCCGGGACTCGATGTGTAAC
AHR	GEP00121228	ATCGGGGTACCAGTTCATCC	ACTGTCATGCCACTTTCTCCA
ARNT	GEP00121227	TGCACAGGACAGAGATCCAA	TGCTGGAGGAGATGCCTTTA
COL4A5	GEP00113674	TTGGCTGGCCACTGTTGATA	GTCCTCAAGTCTCCTGCTTTCA
IFNG	GEP00055425	CCACGGCACAGTCATTGAAA	GCCAGTTCCTCCAGATATCCAA
IL13	GEP00055427	GCTTATTGAGGAGCTGAGCAA	CAGGTCCACACTCCATACCA
IL17B	GEA00013432	ACAGCTGTGGTTGTCCAACA	AGGGATTACGCAACCCAAA
IL18	GEA00002767	CAAAGAAAGCCGCCTCAAAC	GACGCAAGAGTCTTCTGACA
KRT10	GEP00121225	CCCCGGGACTACAGCAAATA	CGTTGGCATTGTCAGTTGTCA
KRT14	GEP00112176	TGGCTGCCGATGACTTCC	CCATTGATGTGGGCCTCCA
KRT5	GEP00121230	GGAAGGAGTTGGACCAGTCA	TCCGTAGCCAGAAGAGACAC
LCE6A	GEP00121226	CAGGCTCCTCTTAGGGTGAC	GCTGTGACATTGCTCAGCTAAA
SERPINB2	GEP00121229	GGCTTTATCCTTTCCGTGTGAA	CAGCTTTGCATGGAGGAACA
TGFB1	GEA00003840	GCTGCGCTTGACAGAGATTAA	GTAACGCCAGGAATTGTTGCTA
TSLP	GEP00114137	GGAGAACAAGAGAAGCCCTCA	CTTGTGCCATTTCTGAGTACC

Table 2 : antibodies for flow cytometry

Marker	Fluorophore	clone	Final dilution	Source	Catalog
Live/dead	EF506	/	1/1000	eBioscience	65-0866-14
CD45.2	BUV395	104	1/200	BD	564279
EpCam	BV805	G8.8	1/200	BD	741996
CD3	AF700	17A2	1/100	BIOLEGEND	100215
MHCII	PE CY7 BV421 BV650	AF6-120.1	1/100	BIOLEGEND	116420
CD11c	BV421 PE Texas Red	N418	1/200	BIOLEGEND	117329
CD64	PE	X54-5/7.1	1/200	BD	558455
CD11b	BV711	M1/70	1/200	BD	553312
Siglec-F	PE-CF594	E50-2440	1/200	BD	562757
Ly6-C	FITC PE-Cy7	HK1.4	1/200	BIOLEGEND	128005
Ly6-G	APC-Cy7	1A8	1/200	Biolegend	127623
CD4	PE-Cy7	GK1.5	1/200	eBioscience	25-0041-82
CD8	APC-Cy7	53-6.7	1/200	Biolegend	100766
CD44	FITC	IM7	1/200	BD	553133
CD103	BV605	2E7	1/100	EBioscience	11-1031-82
TCRgd	PerCp	eBioGL3	1/200	eBioscience	46-5711-80

Figures

Figure 1

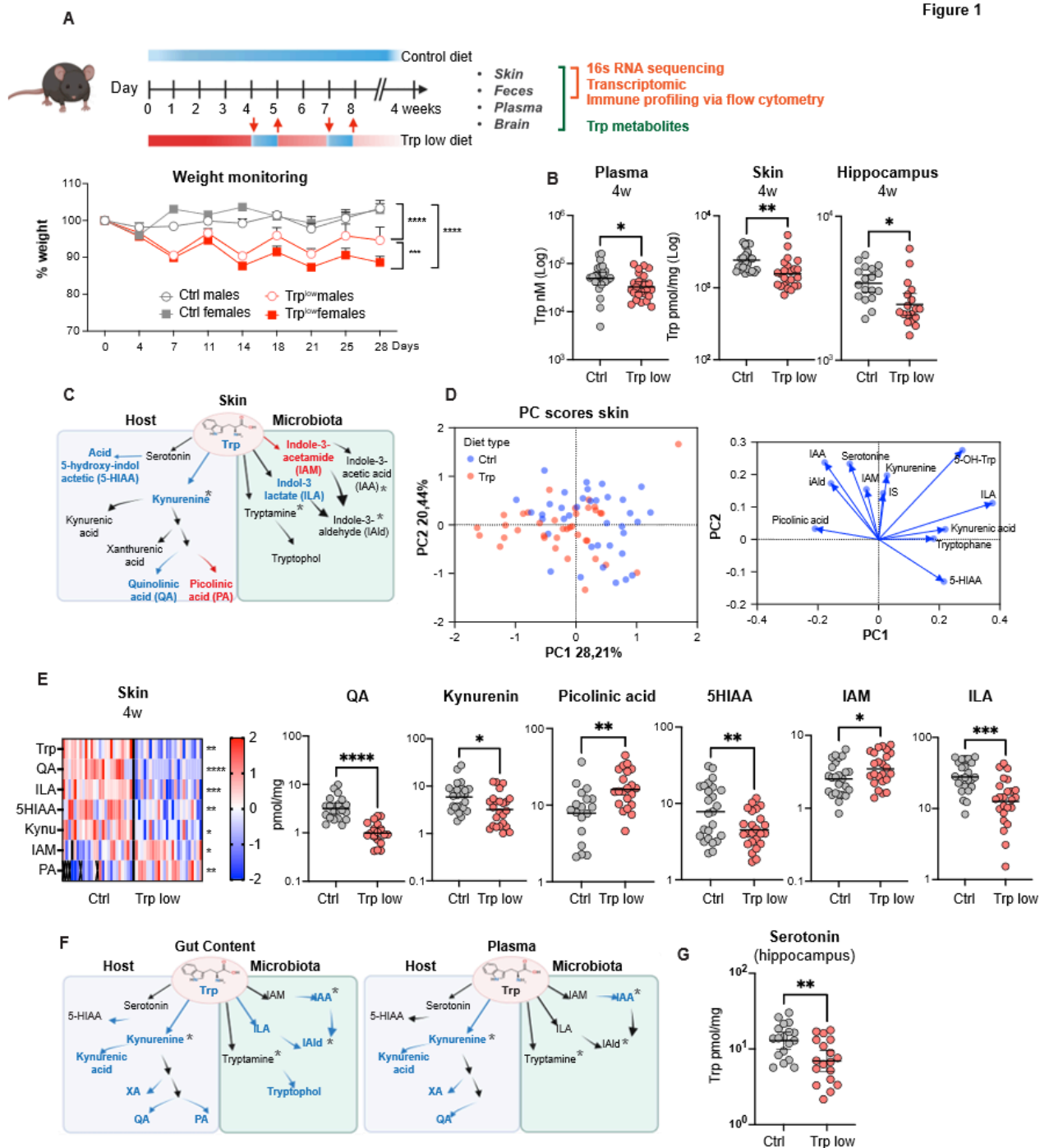


Figure 1

Diet-induced systemic and tissue-specific modulation of tryptophan levels

(A) Top: Schematic representation of the experimental design. Bottom: Body weight monitoring during the course of the tryptophan-low (Trp-low) diet compared to control diet. Males: $n = 3-13$ per group; females: $n = 13-47$ per group depending on time point. Statistical analysis: two-way ANOVA. (B) Trp abundance after 4 weeks of diet in plasma, skin, and hippocampus. Females only; $n=18$ (hippocampus) or 25 (plasma and skin) per group; pooled from 2 and 4 independent experiments respectively. Unpaired

t-test. (C) Schematic overview of Trp catabolism by host and microbiota in the skin. Metabolites significantly modulated after 4 weeks of Trp-low diet are indicated in red (upregulated) or blue (downregulated). Stars denote AhR agonists. (D) Unbiased metabolomic profiling of skin samples. Principal component analysis (PCA) of detected metabolites after 3 and 4 weeks of diet (females only). Pooled data from 4 independent experiments (N = 75). Left: PCA of centered log-transformed data. Right: corresponding loading plot. (E) Quantification of selected Trp metabolites of host origin (QA, kynurenine, PA, 5-HIAA) and microbial origin (IAM, ILA) in the skin after 4 weeks of diet. Left: Heatmap of selected skin metabolites after 4 weeks of control or Trp-low diet. Values represent z-scores calculated by centering and scaling metabolite intensities across all samples. Color scale indicates relative abundance (red: above mean; blue: below mean); Right: Absolute quantification. Females only; n = 21 per group. Unpaired t-test. (F) Schematic overview of Trp catabolism by host and microbiota in the gut content (left) and in plasma (right) and after 4 weeks of Trp-low diet. Metabolites in blue are significantly downregulated after 4 weeks of Trp-low diet. Stars denote AhR agonists. **(G)** Absolute quantification of serotonin in the hippocampus. Females only; n = 20 per group. 2 independent experiments Unpaired t-test.

Figure 2

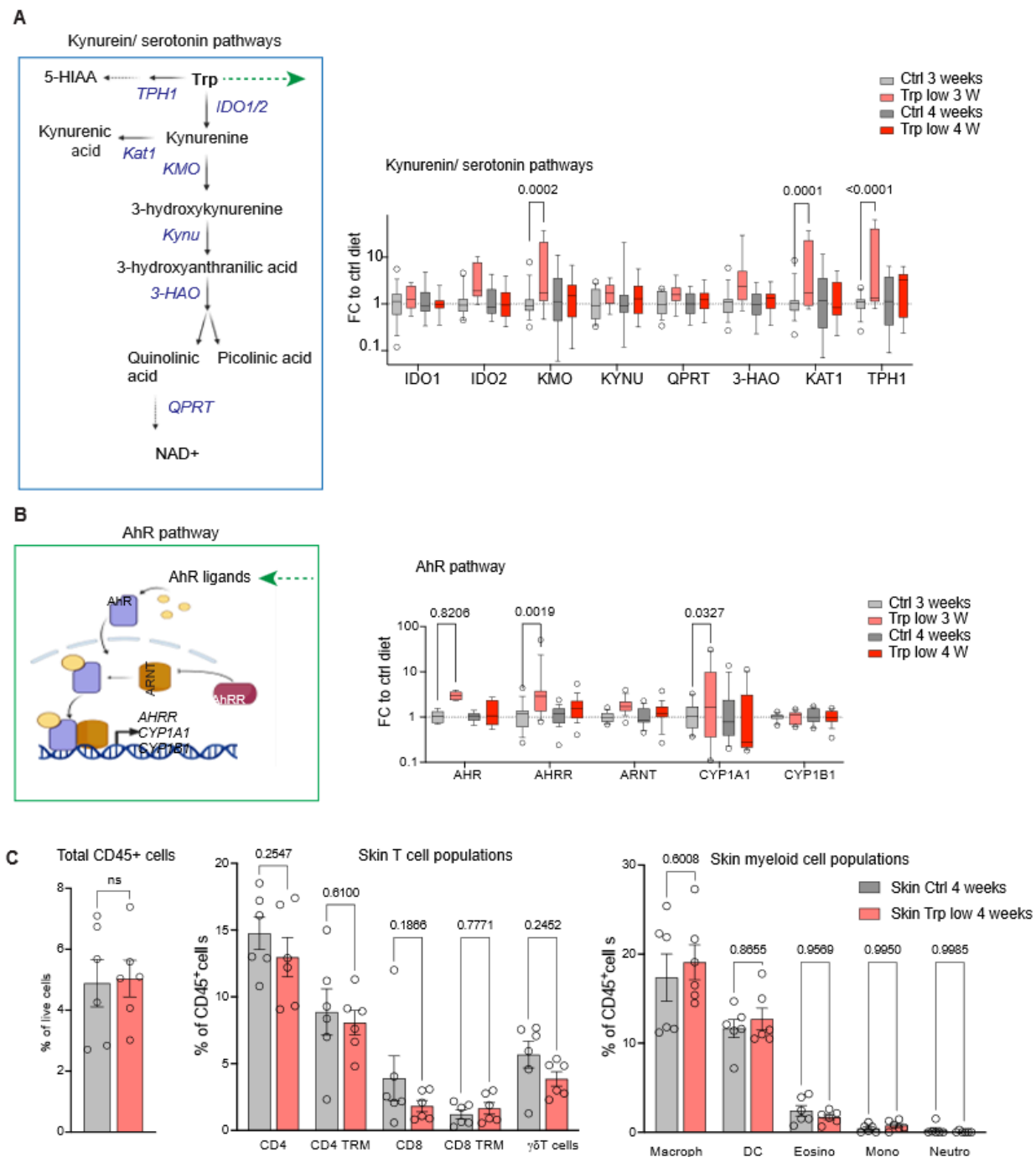


Figure 2

Tryptophan restriction modulates skin Trp catabolic pathways

(A) Left: Schematic representation of Trp catabolism through the kynurenine and serotonin pathways, (enzymes indicated in blue), green arrow indicates catabolism through the microbial indole pathway. Right: Gene expression levels of enzymes involved in kynurenine and serotonin pathways in the skin after

3 and 4 weeks of diet, measured by Biomark assay. Data are expressed as fold change relative to controls. n = 10-19 mice per group; 3 independent experiments; females only. Two-way ANOVA. (B) Left: Schematic illustration of AhR nuclear translocation following activation by AhR ligands and induction of downstream target genes. Green arrow indicates production of AhR ligands through the microbial indole pathway. Right: Differential expression of genes associated with the AhR pathway in the skin after 3 and 4 weeks of diet, measured by Biomark assay. Data are shown as fold change relative to controls. n = 5 mice per group; 3 independent experiments; females only. Two-way ANOVA. (C) Flow cytometric analysis of CD45⁺ immune cell populations in total skin after 3 weeks of diet. One experiment; n = 6 females per group. Unpaired t-test and two-way ANOVA as indicated.

Figure 3

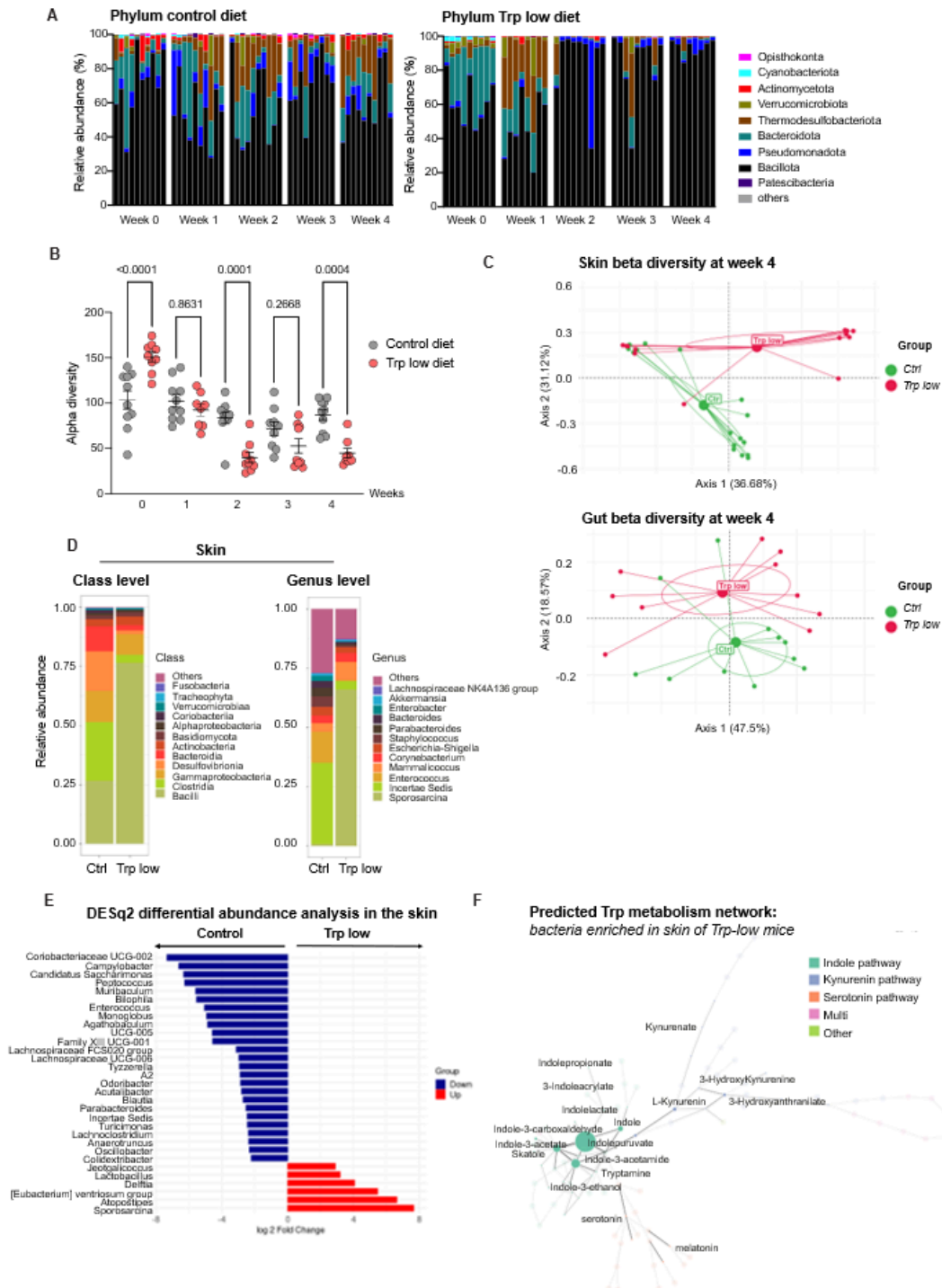


Figure 3

Dietary tryptophan restriction reshapes skin microbiota composition

(A) Bar plot showing the relative abundance of major bacterial phyla in the skin swabs from week 0 to week 4 in control ($n = 10$) and Trp-low ($n = 9$) mice. Each bar represents one mouse. **(B)** Alpha diversity of the skin microbiota from week 0 to week 4 in control and Trp-low mice. Individual values shown with

mean $\pm$ SEM. One-way ANOVA. (C) PCA of skin (top) and gut content (bottom) microbiota composition (beta diversity) at week 4 in control (n=16) and Trp-low groups (n=14). Statistical analysis: PERMANOVA (p = 0.001). Data representative of two independent experiments. **(D)** Relative abundance of major bacterial class and genera detected in the skin at week 4 in control (n = 16) and Trp-low (n = 14) mice. **(E)** Differential abundance analysis of skin microbiota at week 4 using DESeq2. Bar plot showing log₂ fold change (log₂FC) of significantly modulated taxa in Trp-low versus control groups. We used the Generalized Linear Model implemented in DESeq2 to identify the differential abundant genera. DESeq2, is a method for differential analysis of count data, using shrinkage estimation for dispersions and fold changes to improve stability and interpretability of estimates ⁴¹. **(F)** Predicted Trp metabolism network on bacteria enriched in diet-induced Trp deficiency (genus up-regulated with DESeq2 differential abundance analysis)

Supplementary Files

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