

## Supplementary Material and methods

### Cell lines

HT-29 (ATCC), a human intestinal epithelial cell line, was grown in DMEM supplemented with 10% FCS and PS. Jurkat (ATCC Clone E6-1), a human T cell line, was grown in RPMI supplemented with 10% FCS and PS. LS180 cells (ECACC), a human intestinal epithelial cell line, were grown in DMEM supplemented with 10% FCS.

### Mouse Model

All the experiments were performed in the specific pathogen-free animal facility at IERP (INRAe, Jouy-en-Josas, agreement C78-720), in a temperature-controlled environment and with a strict 12h light/dark cycle. WT mice were obtained from Janvier lab, IL-22<sup>-/-</sup> [44] from TAAM Orléans, AhR<sup>-/-</sup> [45], Rag2<sup>-/-</sup> and AhR<sup>ΔIEC</sup> from St Antoine research center animal facility (UMR S938, PHEA), and GPR35<sup>-/-</sup> [46] from IERP.

### IBD cohorts

All individuals from the Suivitheque study were recruited in the Gastroenterology Department of the Saint Antoine Hospital (Paris, France) and provided informed consent. All samples were obtained between June 2012 and November 2019. The clinical activity reported in Figure 2F was previously described [47]. **Briefly:** at each contact in the IBD unit, clinical activity is scored prospectively as follows: 0, no digestive symptoms; 1, mild symptoms that may be attributable to IBD activity, postoperative functional sequelae or associated irritable bowel syndrome; 2, symptoms that are attributable to IBD and are compatible with usual home and/or professional activities; 3, symptoms that are attributable to IBD and are not compatible with sustained home and/or professional activities; 4, hospitalization for IBD flare; 5, intestinal resection. Remission is defined by a score of 0 or 1.

### Seahorse

Following drugs concentrations were used: for Mito stress assay – Olygomycin (Olygo) 1.5µM, FCCP 1µM and Rotenone/Antimycin A (Rot/AA) 0.5µM; for Glycolytic rate assay - Rot/AA 0.5µM and 2-DG 50µM. For basal measurement and each injection, following program is repeat three times– injection compound, mixing (3min), waiting (5min), measuring (3min). Oxygen Consumption Rate (OCR, using the Mito Stress Test kit) and Extracellular Flux Analyser (ECAR, using the Glycolytic Rate Assay kit) were measured. Total protein amount was quantified with DC protein assay (BioRad, 500113-114) and used to normalize values. Analysis were performed with Wave software.

### **DSS-induced colitis model**

In all experiments, body weight, blood in stool, and stool consistency were analyzed daily. The severity of colitis was assessed using the disease activity index (DAI), and, at day twelve, colon length and histological scoring as previously described [3].

### **Nanostring**

All samples had a RIN≥8. After control, RNA was diluted at 15ng/µL. XT\_PGXMmV1 Immunology kit was used following manufacturer's instruction with 250ng per sample, prepared with nCounterPrep Station and read with nCounterPrep Analyzer thanks to Immunology Mm C2269 panel. Data were analyzed with NSolver software.

### **Scratch test**

HT-29 cells were plated at 100,000 cells per chamber in 80µL of DMEM supplemented with 10% FCS and P/S. When cells were confluent, chambers were removed, and stimulation was performed with KYNA (100µM), XANA (100µM) and FICZ (0.2pg/µL) with or without CH223191 (Sigma, 10µM), for 72h. Each dish was pictured with a ZEISS microscope and analyzed with Zen and ImageJ software. Each wound was measured on full length 60 times minimum. Oligomycin (Sigma) is used at 5µM.

### **Organoids**

For mouse organoids, colon was prepared and cleaned with washing solution (1X DPBS + 10% SVF + 2% Penicillin/streptomycin + 1% Gentamycin). Dissociation solution (1X DPBS + 8mM EDTA) was used for 15 and 30 min to harvest crypts. Crypts were resuspended and counted in DMEM/F12 completed + 2% Penicillin/Streptomycin. Fifty crypts were then seeded per well in 1:2 DMEM/F12 (supplemented with Glutamax 1X (Gibco), Penicillin/Streptomycin 1X (Gibco), HEPES 1X (Gibco), N2 1X (Gibco), B27 (Gibco), murine EGF 50ng/mL (Peprotech), recombinant mouse Noggin 100ng/mL (R&D), recombinant mouse R-Spondin1 CF 500ng/mL (R&D), mWnt-3a 100ng/mL (R&D) and CHIR99021 3 $\mu$ M (Peprotech)) and matrigel (Corning) or Ultimatrix (R&D).

Fifty crypts were then seeded per well in 1:2 DMEM/F12 (Supplemented with Glutamax 2m, HEPES 10mM, N2 1X (Thermofisher), human R-Spondin 1 $\mu$ g/mL (R&D), Nicotinamide 10mM (Sigma), B27/Vitamin A 1X (Thermofisher), human Noggin 100ng/mL (R&D), human EGF 50ng/mL (R&D), N-acetylcysteine 1mM (Sigma), Gastrin 10nM (Sigma), SB202190 10 $\mu$ M (Sigma), PGE2 0.01 $\mu$ M (Sigma), LY2157299 0.5nM (Sigma) and Wnt3a 0.5nM (R&D)) and matrigel (Corning) or Ultimatrix (R&D). Organoids were used seven days later and were treated with vehicle, KYNA (100 $\mu$ M) and XANA (100 $\mu$ M) with or without oligomycin (5 $\mu$ M) during 72h.

### Edu proliferation assay

Click-iT® EdU flow cytometry assay kit (Invitrogen, C10425) was used according to manufacturer's instructions..

### qPCR Analysis

The oligonucleotides used were as follows: Smct (F\_ ATGCATTCGTCTCTGTGGCA R\_CTGCTTTAAGACCGCCCAGT), Mct-1 (F\_CATTGGTGTTATTGGAGGTC R\_GAAAGCCTGATTAAGTGGAG), Rorc (F\_TGGCTGCAAAGAAGACCCAC R\_CCCACATTGACTTCCTCTGGT), Ifny (F\_CCATCCTTTTGCCAGTTCTC R\_ATGAACGCTACACACTGCATC), Il1 $\beta$  (F\_GCCCATCCTCTGTGACTCAT, R\_AG-

GCCACAGGTATTTTGTCTG), RegIII $\gamma$  (F\_TTCCTGTCCTCCATGATCAAAA R\_CATCCAC-CTCTGTTGGGTTCA), RegIII $\beta$  (F\_ATGCTGCTCTCCTGCCTGATG R\_CTAATGCGTGCG-GAGGGTATATTC), IL-22 (F\_CATGCAGGAGGTGGTGCCTT R\_CAGACGCAAGCATTCTCAG), Lcn2 (*Qiagen, QuantiTect GT00113407*), and Cyp1a1 (*Qiagen, QuantiTect QT00105756*). We used the  $2^{-\Delta\Delta Ct}$  quantification method with mouse *Hprt1* (*Qiagen, QuantiTect QT00166768*) as endogenous control and the WT group as a calibrator. For human cells, we used Cyp1a1 (F\_CAGCTCAGCTCAGTACCTCA R\_CTTGAGGCCCTGATTACCCA) and Gapdh (F\_AAGTGGTCGTTGAGGGCAATG R\_CTGGGCTACACTGAGCACC).

### Histology

Colon samples for histological studies were maintained at 4°C in 4% paraformaldehyde and then embedded in paraffin. 4- $\mu$ m sections (three sections per sample) were stained with hematoxylin and eosin (H&E) and then examined in a blinded manner using a BX43 Olympus microscope to determine the histological score, as described previously [41].

Immunofluorescence staining was performed according to standard staining methods on a Leica BOND RX<sup>m</sup>, including a deparaffinization and rehydration step, an alkaline antigen retrieval step (20min at 100°C in Bond<sup>TM</sup> Epitope Retrieval 2, AR9640), and blocking with 1% BSA and 10% Normal Goat Serum for 30min at room temperature.

Primary antibody anti-Ki67 (dilution 1/500, Abcam, ab15580) was applied on slides 1h at room temperature, followed by secondary antibody (dilution 1/2300, Invitrogen, A11011) with DAPI for 30 min at room temperature. Slides were mounted with Fluoromount ® (Cliniscience, 0100-01). Slides were scanned using the Panoramic SCAN II automated slide scanner (3D HISTECH) at 20X.

### FACS

All extracellular staining were realized in FACS Buffer (PBS1X + EDTA 0.5mM + FCS 2%). Staining beginning each time with a viability dye, either Fixable Viability Dye (*eBioscience*) or

Zombie Aqua Fixable Viability Kit (*Biolegend*). Cells were surface stained in FACS buffer with the following antibodies: C16/CD32 (*eBioscience*, 14-0161-85), CD45 (*Biolegend* 103147), CD4 (*Biolegend* 100552), EpCAM (*Biolegend* 118205) and CD3 $\epsilon$  (*eBioscience* 11-0031-82). For cytokines, cells were stimulated during 2 hours by PMA (50ng/mL) and ionomycin (1 $\mu$ g/mL) plus IL-1 $\beta$  (10ng/mL), IL-23 (20ng/mL) and Brefeldin-A (10 $\mu$ g/mL) before staining. For intracellular staining Cytofix/Cytoperm (*BD*) or Factor staining buffer Set (*eBioscience*, 00-5523-00) were used following manufacturer's protocols with following antibodies: IL-17A (*Biolegend* 506914), roryt (*eBioscience* 12-6988-82), tbet (*Biolegend* 644817), foxp3 (*eBioscience* 48-5773-82), IFN $\gamma$  (*Biolegend* 505850) and IL-22 (*eBioscience*, 46-7221-82). All data are acquired with LSR Fortessa X-20 (*BD Biosciences*) and analyzed with FlowJo software.

### **T cell differentiation**

24-well plates were coated overnight with 2 $\mu$ g/mL of  $\alpha$ CD3 and 10 $\mu$ g/mL of  $\alpha$ CD28. Cells from WT spleen were prepared, minced and red blood cells were lysed with a red blood lysis solution. Naïve CD4 T cells were sorted with Milteny kit (130-104-453), following manufacturer instructions. 500,000 cells were plated per well. For T<sub>H</sub>17, naïve CD4 T cells were incubated with IL-6 (0.1mg/mL) and TGF $\beta$  (0.2mg/mL) with KYNA (100 $\mu$ M), XANA (100 $\mu$ M) with or without CH223191 (Sigma, 10 $\mu$ M) during 72h. After incubation, cells were stained, with  $\alpha$ CD4,  $\alpha$ IL-17A,  $\alpha$ Roryt and viability dye, and the level of differentiation was compared with LSF Fortessa X20. 2DG (*Sigma*, D6134) was used at 10mM.

### **16S sequencing analysis**

Fecal DNA extraction was performed as previously described [3]. Gut microbiota composition and diversity were determined using 16S sequencing. Following PCR (V3-V4 region, PCR1F\_460: 5' CTTTCCCTACACGACGCTCTTCCGATCTACGGRAGGCAGCAG 3', PCR1R\_460: 5' GGAGTTCAGACGTGTGCTCTTCCGATCTTACCAGGGTATCTAATCCT 3'), amplicon quality was verified by gel electrophoresis and sent to the @BRIDGe platform

for sequencing protocol on an Illumina MiSeq (Illumina, San Diego, CA, USA). The dada2 software package (version 1.14.1)[48] in the R programming language (R version 3.6.3) was used to perform quality control, read trimming and identification of amplicon sequence variants (ASV's). The **SError! Bookmark not defined**.ilva reference database (version 138) [49] was used for taxonomic assignment. Raw sequence data are accessible in the sequence read archive (accession number pending). Bacterial ASVs that could not be assigned to a Phylum-level taxonomy were excluded. Samples with <1000 reads were excluded. Prevalence filtering excluded ASVs that were present in only one sample. The Shannon diversity index was used to estimate alpha diversity, based on the number of unique ASVs and their evenness of distribution. Statistical significance for diversity was tested using the Wilcoxon rank sum test. Beta diversity was calculated using the Bray-Curtis divergence on proportional (total sum scaled) data using the vegan package (version 2.5-6), with PERMANOVA performed using the *adonis* function (999 permutations). Data analysis was performed through the phyloseq package (version 1.30.0) [50]. Plotting was performed with ggplot2 (version 3.3.2) and ggpubr (version 0.4.0).

### Targeted quantitative metabolomics

Samples were lyophilized (3mg) and weighted. The method has been described previously [42]. Finally, 5µL were injected into the LC-MS (XEVO-TQ-XS, Waters®). A Kinetex C18 xb column (1.7µm x 150mm x 2.1mm, temperature 55°C) associated with a gradient of two mobile phases (Phase A: Water + 0.5% formic acid; Phase B: MeOH + 0.5% formic acid) at a flow rate of 0.4mL/min was used.

For each metabolite, a calibration curve was created by calculating the intensity ratio obtained between the metabolite and its internal standard. These calibration curves were then used to determine the concentrations of each metabolite in patient samples.

### Radio-Ligand Binding Assay

Cytosolic protein extracts from murine hepatoma Hepa1c1c7 cells (2mg/mL) were incubated for 2 h at room temperature with 2nM [ $^3$ H]-TCDD in the presence of KYNA (10–1000 $\mu$ M), XANA (10–1000 $\mu$ M), FICZ (100nM; positive control), or vehicle (DMSO; 0.1% V/V; corresponds to specific binding of [ $^3$ H]-TCDD = 100%). Ligand binding to the cytosolic proteins was determined by the hydroxyapatite binding protocol and scintillation counting as described elsewhere [43]. Specific binding of [ $^3$ H]-TCDD was determined as a difference between total and non-specific (TCDF; 200nM) reactions. Three independent experiments were performed, and the incubations and measurements were done in triplicates in each experiment (technical replicates). For statistical analysis One-way ANOVA followed by Dunnett's test was performed.

#### **Immunofluorescence detection of the AhR nuclear translocation [51]**

LS180 (90,000 cells/well) were grown on poly-D-lysine coated 8-well tissue culture chamber slides (Sarstedt) overnight. The cells were incubated with a vehicle (DMSO; 0.1% V/V), two positive controls (TCDD; model AhR ligand; 10nM and FICZ; endogenous AhR ligand; 10nM), KYNA (100 $\mu$ M), or XANA (100 $\mu$ M) for 90min. After the treatment, the cells were washed with PBS, fixed with 4% (V/V) formaldehyde, permeabilized using 0.1% (V/V) Triton X-100, blocked with 3% (m/V) bovine serum albumin and incubated with Alexa Fluor 488 labelled primary antibody against AhR (sc-133088, Santa Cruz Biotechnology), as described elsewhere [51]. Nuclei were stained with 4',6-diamino-2-phenylindole (DAPI) and the slides were sealed by coverslips using VectaShield® Antifade Mounting Medium (Vector Laboratories). The AhR nuclear translocation was observed using Olympus Fluoview 1000 confocal system. The experiments were performed in three consecutive cell passages. The level of AhR nuclear translocation was calculated as the proportion of fluorescence intensity of nucleus and fluorescence intensity of cytoplasm (fluorescence of nucleus/fluorescence of cytoplasm) and was expressed as a percentage of intensity of cytoplasm fluorescence. The level of nuclear translocation of AhR was estimated in 80-110 cells for every treatment in

each experiment. For statistical analysis One-way ANOVA followed by Dunnett's test was performed.

### **AADAT production**

**cloning of murine AADAT in *Escherichia coli*.** The pMA:muAADAT plasmid carrying the gene encoding murine AADAT (muAADAT) with was synthesized based on *Escherichia coli* codon usage (GeneArt). The DNA fragment coding for muAADAT was then recovered after digestion with *NheI* and *XhoI* restriction enzymes and cloned into pStaby 1 vector (DelphiGenetics). The use of pStaby 1 plasmid (**Figure S7B-C**) allows the gene transcription under the control of the phage T7 RNA polymerase promoter (T7 polymerase) and the introduction of a C-terminal six-Histidine tag (His-tag), allowing subsequent purification of muAADAT using affinity chromatography. The resulting final vector pStaby:muAADAT (**Figure S7C**) was transferred into T7 Express Competent *E. coli* (NEB) and transformants were grown at 37°C overnight (ON) in Luria-Bertani medium containing ampicillin (Amp, 100µg/mL) with shaking at 180rpm. Plasmid DNA was extracted from positive clones and sequenced to confirm their identity.

**Expression and purification of the recombinant murine AADAT protein in *E. coli*.** *E. coli* strain expressing muAADAT was cultured at 37°C ON in LB supplemented with Amp 100µg/ml and with shaking. When an optical density (OD 600nm) of 0.8-1.0 was reached, gene expression was induced by the addition of 0.25mM IPTG and the cultures incubated at 16°C ON with stirring at 180rpm (**Figure S7D**). Bacteria were harvested by centrifugation and the cell pellets washed with PBS and resuspended in Binding Buffer (PBS buffer pH 7.4-300mM NaCl supplemented with 0.1% 10X triton and 1X protease inhibitors. Roch). The cells were then sonicated in ice with an amplitude of 40%. The lysate is then centrifuged at 15,000g for 30min at 4°C. The soluble fraction containing AADAT was purified by affinity chromatography. The supernatant were incubated with Ni-NTA agarose resin (R901-15, Invitrogen) at 4°C for 1h then deposited in a BioRad column. The AADAT protein fixed to the resin was washed with PBS buffer, then PBS buffer-20mM imidazole and PBS buffer-40 mM



imidazole. The AADAT protein was eluted with PBS buffer-300mM imidazole (**Figure S7E**). The fractions 2-6 were pooled and dialyzed against PBS, 50% glycerol-300mM NaCl Spectra/Port 6. A pre-cast BioRad mini-protean TGX stain free, 4-20% gel was carried out to control the different stages of the purification.

**AADAT activity test verification.** The AADAT activity test is based on the disappearance of kynurenine which is the substrate of AADAT. A reaction mixture containing 10mM L-kynurenine, 2mM  $\alpha$ -oxoglutarate, 40 $\mu$ M PLP (pyridoxal 5'-phosphate) and 0 or 1 $\mu$ L of the purified protein sample prepared in buffer 100 mM potassium phosphate (pH 7.4). Then, the mixture was incubated at 37°C for 15min, and the reaction was stopped by the addition of an equal volume of 30% acetic acid. The supernatant of the reaction mixture, obtained by centrifugation at 3000g for 10min at 4°C, was mixed equally with Ehrlich's solution and incubated 15min at room temperature to have a colorimetric reaction. In parallel, a standard range of kynurenine from 0 $\mu$ M to 1000 $\mu$ M was made under the same conditions. The amount of kynurenine present in the sample was measured with a spectrophotometer at an OD of 492nm and calculated using the standard range (**Figure S7F**). A verification of the production of KYNA and XANA *in vitro* via the action of our enzyme during 15min in the presence of KYNU (1000  $\mu$ M) was carried out (**Figure S7F**). A dose effect can be observed from the transformation of KYNU into KYNA and XANA.

### Statistical analysis

Statistical analysis of human data was performed in the R statistical environment (R version 3.6.2). Plotting was performed with ggplot2 (version 3.3.2). In all statistical analyses, differences with p values <0.05 were considered significant. The p values were corrected using the Benjamini and Hochberg (BH) procedure to control for the false discovery rate. The other data were analyzed using Prism version 8 (Graphpad Software, San Diego, USA). Values are expressed as mean  $\pm$  Standard error of the mean (SEM). Microbiota-specific analysis is described in the 16S sequencing analysis section. For p value: \*: p<0.05, \*\*: p<0.01, \*\*\*: p<0.001, \*\*\*\*: p<0.0001.