SUPPLEMENTAL MATERIAL

Indirect calorimetry

A subset of WT and $DC^{hBcl\text{-}2}$ mice HFD-fed for 20 weeks was used for indirect calorimetry measurement (n = 6 per group). These mice were housed individually in metabolic cages (Phenomaster, TSE Systems GmbH, Bad Homburg, Germany). After 3 days of habituation, the measurement of food intake, drink intake, locomotor activity, O_2 consumption, CO_2 production and energy expenditure were monitored for 5 days.

Transit time

Carmine red was given by gavage to 6-h-fasted mice (10 mg/ml of water, 10 µl per g of body weight). The intestinal transit time was recorded as the time from gavage to the first appearance of the dye in the feces (minutes).

Intestinal paracellular permeability test

FITC-dextran was administered by gavage to 6-h-fasted-mice (600 mg per kg of body weight). At time 0, 1h and 3h post-gavage, blood was collected from the tip of the tail vein (40µl) into EDTA-coated tubes and centrifuged (4°C, 2 000 g for 10 min). Plasma was diluted 1:10 (v/v) in PBS (pH 7.4) and the dextran-FITC concentration was determined using a fluorescence spectrophotometer (Fluostar; SLT, Crailsheim, Germany) at 485 nm excitation and 535 nm emission wavelengths. Standard curves were obtained by diluting dextran-FITC solution in plasma from non-treated mice diluted in PBS (1:10 v/v).

Quantification of fecal SIgA by ELISA

Flat bottom 96-well plates (Immulon II, VWR) coated with 100 µL/well of goat anti-mouse IgA (5 µg/mL Bic 0.1M, pH 9.6; Southern Biotech) were incubated with serial 3-fold dilutions of either fecal supernatant (100 µL) or standard IgA (100 µL - SouthernBiotech, Birmingham, USA) for 1h30 at 37°C. After washing, fixed antibodies were detected with horseradish

peroxidase-conjugated goat anti-mouse IgA (100 µL / well - 1.5 µg/mL; Sigma-Aldrich) for 1h30 at 37°C and the reaction revealed with of 3,3'-5,5'-tetramethylbenzidine peroxidase substrate (100 µL/well - KPL, VWR, Fontenay-sous-Bois, France). Absorbencies were read at 450 nm.

16S rRNA gene sequencing and analysis

The V3-V4 region of the 16S rRNA gene was amplified with the universal primers F343 (CTTTCCCTACACGACGCTCTTCCGATCTACGGRAGGCAGCAG) and R784 (GGAGTTCAGACGTGTGCTCTTCCGATCTTACCAGGGTATCTAATCCT), using 30 amplification cycles with an annealing temperature of 65 °C. The resulting PCR products were purified and sequenced at the GeT-PlaGe Genotoul INRA platform (Toulouse, France) using 506 Illumina MiSeq technology. The sequences were demultiplexed and quality filtered using the Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0) software package [18]. We used QIIME default parameters for quality filtering (reads truncated at first low-quality base and excluded if: (1) there were more than three consecutive low quality base calls; (2) less than 75% of read length was consecutive high quality base calls; (3) at least one uncalled base was present; (4) more than 1.5 errors were present in the barcode; (5) any Phred qualities were below 20; or (6) the length was less than 75 bases). Sequences were assigned to OTUs using the UCLUST algorithm with a 97% threshold of pairwise identity and without the creation of new clusters with sequences that did not match the reference sequences. OTUs were taxonomically classified using the Greengenes 13_8 reference database. A single representative sequence for each OTU was aligned and a phylogenetic tree was built using FastTree. The phylogenetic tree was used for computing the unweighted UniFrac distances between samples. Rarefied OTU table were used to compare abundances of OTUs across samples. Principal component analysis (PCA) plots were used to assess the variation between experimental group (beta diversity), alpha diversity curves were determined for all samples using the determination

of the number of observed species, and OTU table was rarefied at various taxonomic levels using QIIME. LEfSE (LDA Effect Size) was used to investigate bacterial members that drive differences between groups.

Single-cell preparation

After surgical removal of small intestine and colon, the SILP and the CLP single-cell suspensions were obtained using the Lamina Propria Dissociation Kit (Miltenyi Biotec SAS, Paris, France). Leucocytes enrichment was then performed through a 40/80% (w/v) Percoll density gradient (GE Healthcare) centrifuged for 15min at 1900g at RT.

mLNs were surgically removed and then thoroughly smashed on a 70µm cell strainer on ice.

Single cell preparations were washed prior and resuspended in a complete media composed of DMEM-Glutamax added with 8% fetal calf serum (FCS; PAA Laboratories, Linz, Austria), HEPES (10 mM), 2-mercaptoethanol (0.05 mM), and penicillin and streptomycin (100 U/ml).

Immuno-labelling and Flow cytometry analysis

Surface staining was performed using the following antibodies (BD Biosciences): FITC-CD45 (clone 1D3), Alexa Fluor-anti-B220 (clone RA3-6B/2), v450-anti-CD19 (clone1D3), Alexa-Fluor 700-anti-MHC Class II (I-A/I-E) (clone M5/114.15.2), PE-Cy7-anti-CD11c (clone HL3), Allophycocyanin (APC)-anti-CD64 (clone X54–5/7.1), APC-Cy7-anti-CD11b (clone M1/70), PE-anti-CD103 (clone M290), PerCP-Cy5.5-anti-CD3 (clone 17A2), PerCP-eFluor710-anti-CD3 (clone 17A2), BV-711-anti-CD4 (RM4-5), PE-CF594-anti-CD8a (clone 53-6.7), PE-anti-IgA, (SouthernBiotech). For intracellular staining, cells were fixed and permeabilized with a commercially available fixation/permeabilization buffer (eBioscience). Intracellular staining was performed with PE-conjugated Foxp3 (clone FJK-16s) or with BV421-conjugated IFNg (clone XMG1.2) and APC-conjugated IL10 (clone JES516E3) and PE-cyanine7-conjugated IL17A (clone eBio17B7) or with v450-conjugated hBcl2 (clone Bcl-2/100).

Prior intracellular cytokine staining, cells were restimulated with PMA (50 ng/ml) and ionomycin (500 ng/ml) for 4 h in RPMI 1640 (Invitrogen) containing 8% FCS, HEPES (10 mM), 2-mercaptoethanol (0.05 mM), penicillin, and streptomycin (100 U/ml).

Labeled cells were analyzed with a BD LSRFortessa flow cytometer (BD Biosciences) using both Diva or Flow-Jo software. Cell sorting experiments were performed on single-cell preparations from mLNs of 8-weeks old mice before starting the HFD. After surface staining, CD103+ CD11b+ cDCs were Fluorescence-activated cell sorting (FACS)-sorted with the MoFlo Astrios EQ cell analyzer (Beckman Coulter).

SCFA analysis

Fecal or caecal samples were water extracted and proteins were precipitated with phosphotungstic acid. A volume of 0·1µl of the supernatant fraction was analyzed for SCFA on a gas-liquid chromatograph (Autosystem XL; Perkin Elmer, Saint-Quentin-en-Yvelines, France) equipped with a split-splitless injector, a flame-ionization detector, and a capillary column (15 m x 0·53 mm, 0·5µm) impregnated with SP 1000 (FSCAP Supelco, Saint-Quentin-Fallavier, France). Carrier gas (He) flow rate was 10 ml/min and inlet, column and detector temperatures were 175°C, 100°C and 280°C, respectively. 2-Ethylbutyrate was used as the internal standard [18]. Samples were analyzed in duplicate. Data were collected and peaks integrated using the Turbochrom v. 6 software (Perkin Elmer, Courtaboeuf, France).

Microarray analysis

Raw data (CEL files) were quality controlled, normalized and processed into signal intensities using the RMA algorithm with Affymetrix CDF file used for annotation. All subsequent analyses were based on the log (base 2) transformed data in Partek Genomics Suite: non-supervised analysis and ANOVA were used to detect eventual outlier samples and to identify

differentially expressed genes. Statistical and Hierarchical clustering was performed using the TIGR Multiple Experiment Viewer (MeV 4.9.0). Comparisons of the two groups were performed by a 2 two-tailed Student's t-test. Features were considered significant when the p-value was below 0.05 after Benjamini-Hochberg for false discovery rate (FDR) correction. The resulted DEGs were mapped for Gene Ontology (GO) and KEGG/BioCarta pathway analysis using ClueGO (version 2.5.5) a Cytoscape (version 3.7.0) plug-in facilitating the biological interpretation and visualization of functionally grouped GO terms in the form of networks and charts. A two-sided (enrichment/depletion) hyper-geometric distribution test with a p-value significance level of ≤ 0.05 corrected by Bonferroni was applied, together with the Kappa-statistic score threshold at 0.3 and GO levels set between 4 to 6. Datasets were derived from 8 to 12 samples per genotype. 2 to 3 samples were pooled for each genotype (corresponding to either group 1, 2, 3, or 4 in Fig.) to obtain an equivalent amount of material for further processing. Each group of pooled samples corresponds to Gp1 to Gp4 (Fig. 3D).