SUPPLEMENTARY METHODS

Cultivation of intestinal bacteria: According to caecal or ileal content weight measurement, ten-fold dilutions (w/v) were prepared by adding appropriate volumes of sterile PBS supplemented with peptone and cysteine (0.05 % w/v each, Fluka). Dilution series were prepared on Wilkins-Chalgren Agar supplemented with L-cysteine and dithioerythritol (0.05 % w/v each). For samples from the CONV facility, the medium was supplemented with 5 µg/ml pimaricin Sigma-Aldrich to prevent yeast growth.

Immunofluorescence Staining: Formalin-fixed paraffin-embedded tissue sections (3.5 µm) of the terminal ileum placed on Superfrost Plus slides (Thermo Scientific) were deparaffinized using a Leica ST5020 Multistainer system. Antigens were unmasked by boiling under pressure in sodium citrate buffer (pH 6, 900 W, 23 min). Tissue sections were allowed to cool down to RT and washed three times consecutively in deionized water and once in PBS (5 min each). Sections were incubated in blocking buffer (5 % normal goat or donkey serum and 0.3 % Triton X-100 in PBS) against the species of the secondary antibody (60 min; RT; in a humidified chamber). Primary antibodies (LY6G, 1:500, BD Pharmingen; lysozyme, 1:2000, Dako; Cleaved-caspase 3, 1:1000, Cell Signalling) were incubated overnight at 4°C. Fluorochrome-conjugated secondary antibodies (from Invitrogen, Dianova and Life Technologies) were diluted 1:200 and incubated for 1h at RT. Rhodamine-labeled UEA-I (Ulex europaeus agglutinin-1; Vector Laboratories) at a dilution of 1:1000 was used for detection of fucosylated cell-structures of Paneth and goblets cells. For cryptin 2 staining, paraffin-embedded tissue sections were deparaffinized and rehydrated. Antigen retrieval was performed using 0.01M citrate sodium buffer, pH 6.0. Slides were blocked with 5% BSA, 10% normal donkey serum (Dianova) and stained using a rabbit polyclonal antiserum against synthetic cryptdin 2 (LRDLVCYCRTRGCKRRERMNGTCRKGHLMYTLCCR; gift from Mats Andersson, Karolinska institute, Stockholm) and a mouse anti-E cadherin antibody (BD Bioscience), followed by Rhodamine Red-X-conjugated donkey anti-rabbit and AF647-conjugated donkey anti-mouse secondary antibodies (Dianova). Fluorescein-labelled wheat germ agglutinin (WGA; Vector) was used as a counterstaining. Nuclei counterstaining was done using DAPI (1:2000). Sections were visualized using a confocal microscope (Olympus Fluoview 1000) using the FV10-ASW software. Ly6G-positive cells per microscopic field area were counted using the Volocity® 5.51 software (Perkin Elmer) defining lamina propria as the region of interest. For each individual mouse, 3 microscopic fields at a 60-fold magnification were quantified for mean Ly6G-positive cells per mm2. Lysozyme and UEA-1 signals

were quantified by counting numbers of positive cells per crypt (description shown in Figure S1). A minimum of 12 crypts per mouse, randomly picked and distributed all over the ileal cross-section, were assessed at a 120-fold magnification.

16S ribosomal RNA (rRNA) gene sequence analysis: 16S ribosomal RNA (rRNA) gene sequence analysis: 600µl DNA stabilization solution (STRATEC biomedical), 400 µl Phenol:Chloroform:IsoAmyl alcohol (25:24:1; Sigma-Aldrich) and 500 mg autoclaved glass beads (0.1mm; Roth) were added to frozen caecal samples (approx. 200mg). Microbial cells were disrupted by mechanical lysis using a FastPrep®-24 (3 x 30 sec at maximum speed) (MP Biomedicals) fitted with a 24 x 2 ml cooling adaptor. After heat treatment (95 °C, 5 min) and centrifugation (15000xg/5 min/4°C), supernatants were treated with RNAse (0.1 µg/µl; Amresco) for 30 min at 37°C. Metagenomic DNA was purified using gDNA columns (Macherey-Nagel) following the manufacturer's instructions. Concentrations and purity were checked by NanoDrop® (Thermo Scientific) and samples were stored at 4 °C during library preparation and at -20 °C thereafter for longer storage. The V3/V4 region of 16S rRNA genes was amplified (25 cycles) from 12 ng of metagenomic DNA using the bacteria-specific primers 341F and 785R [1] following a 2-step procedure to limit amplification bias.[2] Amplicons were purified using the AMPure XP system (Beckmann), pooled in an equimolar amount, and sequenced in paired-end modus (PE275) using a MiSeq system (Illumina Inc.). A final DNA concentration of 10 pM and 15 % (v/v) PhiX standard library was used. Raw read files were demultiplexed (allowing a maximum of 2 errors in barcodes) and each sample was processed using USEARCH [3] following the UPARSE approach.[4] First, all reads were trimmed to the position of the first base with quality score <3 and then paired. The resulting sequences were size filtered, excluding those with assembled size <380 and >440 nucleotides. Paired reads with expected error >3 were further filtered out and the remaining sequences were trimmed by 10 nucleotides on each side to avoid GC bias and non-random base composition. For each sample, sequences were de-replicated and checked for chimeras with UCHIME.[5] Sequences from all samples were merged, sorted by abundance, and operational taxonomic units (OTUs) were picked at a threshold of 97 % similarity. Finally, all sequences were mapped back to the representative sequences resulting in one OTU table for all samples. Only those OTUs with a relative abundance above 0.5% total sequences per sample were kept. The final OTUs counts were normalized to the sample with the lowest number of sequences. For each OTU, the most detailed taxonomic classification among Silva,[6] RDP [7], and Greengenes [8], was manually assigned as the final OTU taxonomy. In case of different predictions, Silva was preferred over RDP, and RDP over Greengenes. For OTUs showing significant differential abundance between groups of mice, the EzTaxon classification [9] was used to identify the closest described species. For estimation of diversity within

samples (alpha-diversity), the Shannon index was calculated and transformed to the corresponding effective number of species as described by Jost.[10] For comparisons across samples (beta-diversity), phylogenetic trees across all OTU representative sequences were constructed using the maximum likelihood method in Mega6 [11] and used for calculation of samples distances with generalized Unifrac (package GUniFrac in R; [12]).

Mass spectrometry and metaproteome analysis: Colonic content was diluted in 1 ml of filter-sterilized PBS containing Protease- and Phosphatase-Inhibitor (Roche) and lysed using a FastPrep®-24 (3x40 sec at 6.5 M/s (MP Biomedicals). After centrifugation (16.000xg/4°C/5min), supernatants were frozen at -80°C until use. A total amount of 100 µg protein lysate was separated by SDS-PAGE. After electrophoresis, each lane was cut into three slices and processed by in gel proteolytic cleavage using trypsin.[13] Peptides were analyzed using an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific) coupled to a TriVersa NanoMate (Advion, Ltd.). A volume of 5 µL peptide lysate was separated with a Ultimate 3000 nano-LC system (Dionex, now Thermo Fisher Scientific) on a C18 reverse phase column (25 cm; 100 min linear gradient from 2% acetonitrile (ACN) to 55% ACN in 0.1% formic acid). Raw data files were searched with Proteome Discoverer (v1.4, Thermo Fisher Scientific) using the Mascot algorithm (v2.3 in-house server) against the taxonomy of mouse, bacteria, green plants and archaea of the NCBInr database. Only rank 1 peptides were considered to be identified with a threshold of FDR <1%. "PROteomics results Pruning & Homology group ANotation Engine" (PROPHANE) [14] was used to assign proteins to their taxonomic and functional groups. Label free quantification of protein groups were performed using the TOP-3 peptide abundance.

Monocolonization with E. Coli LF82: 100µl of an overnight culture in liquid WCA broth was gavaged (approx. 108 log cfu/ mouse) to eight week old GF WT (n=6) and TNFdeltaARE (n=9) mice. Mice were housed in isolators with mixed genotypes in each cage. No cage effects were observed. The mice were sacrificed 4 weeks after colonization.

Gene expression analysis: Primers and probes used were: *tnf* (5´-tgcctatgtctcagcctcttc-3´; 5´-gaggccatttgggaacttct-3´; Probe #49); *gapdh* (5'-tccactcatggcaaattcaa-3´; 5'-tttgatgttagtggggtctcg-3´; Probe #9); *angiogenin 4* (*ANG4*; 5´-ccccagttggaggaaagc-3´; 5'-cgtaggaatttttcgtacctttca-3´; Probe #106); *defa-5* (5'-ttttgggacctgcagaaatc-3'; 5'-cagagccgatggttgtcat-3'; Probe #84); *reg3g* (5'-accatcaccatcatgtcctg-3'; Probe #51).

References for supplementary Methods:

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