Glial–cell-derived neuroregulators control type 3 innate lymphoid cells and gut defence

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Group 3 innate lymphoid cells (ILC3) are major regulators of inflammation and infection at mucosal barriers. ILC3 development is thought to be programmed, but how ILC3 perceive, integrate and respond to local environmental signals remains unclear. Here we show that ILC3 in mice sense their environment and control gut defence as part of a glial–ILC3–epithelial cell unit orchestrated by neurotrophic factors. We found that enteric ILC3 express the neuroregulatory receptor RET. ILC3–autonomous Ret ablation led to decreased innate interleukin–22 (IL–22), impaired epithelial reactivity, dysbiosis and increased susceptibility to bowel inflammation and infection. Neurotrophic factors directly controlled innate IL22 downstream of the p38 MAPK/ERK–AKT cascade and STAT3 activation. Notably, ILC3 were adjacent to neurotrophic-factor-expressing glial cells that exhibited stellate-shaped projections into ILC3 aggregates. Glial cells sensed microenvironmental cues in a MYD88-dependent manner to control neurotrophic factors and innate IL–22. Accordingly, glial–intrinsic Myd88 deletion led to impaired production of ILC3–derived IL–22 and a pronounced propensity towards gut inflammation and infection. Our work sheds light on a novel multi-tissue defence unit, revealing that glial cells are central hubs of neuron and innate immune regulation by neurotrophic factor signals.

ILC3 produce proinflammatory cytokines, and regulate mucosal homeostasis and anti-microbial defence. In addition to their well-established developmentally regulated program, ILC3 are also controlled by microbial and dietary signals, suggesting that ILC3 possess other unexpected environment-sensing strategies. Neurotrophic factors are extracellular environmental cues to neurons and include the glial-derived neurotrophic factor (GDNF) family ligands (GFL) that activate the tyrosine kinase receptor RET in the nervous system, kidney and haematopoietic progenitors.

Analysis of gut lamina propria revealed that ILC3 express high levels of Ret (Fig.1a), a finding confirmed at the protein level and by reporter Ret/GFP knock-in mice (Fig. 1b–d and Extended Data Fig. 1a–d). ILC3 subsets expressed Ret/GFP and aggregated in cryptopatches and isolated lymphoid follicles (ILF), suggesting a role of neuroregulators in ILC3 (Fig. 1b–d and Extended Data Fig. 1b–j). To explore this hypothesis, we transplanted fetal liver cells from Ret competent (RetWt/GFP) or deficient (Ret-GFP/GFP)13 animals into alymphoid Rag1−/−–γc−/− hosts. Ret-deficient chimaeras revealed unperturbed ILC3 and cryptopatch development (Fig. 1e). Notably, IL–22–expressing ILC3 were largely reduced despite normal IL–22 production by T cells (Fig.1f, g). In contrast, innate IL–17 was unaffected by Ret ablation (Fig.1f and Extended Data Fig. 2a). In agreement with this, analysis of gain–of–function RetMEN2B mice14 revealed a selective...
increase of IL-22-producing ILC3, whereas their IL-17-producing counterparts were unaffected (Fig. 1b and Extended Data Fig. 2b). To more specifically evaluate the effects of RET in ILC3, we deleted Ret in RORγt-expressing cells by breeding Rorgt-Cre with Ret<sup>fl/fl</sup> mice<sup>15,16</sup> (Extended Data Fig. 3a, b). Analysis of Rorgt-CreRet<sup>Rorgt/fl</sup> (Ret<sup>Δ</sup>) mice revealed selective and extensive reduction of ILC3-derived IL-22, but normal IL-22-producing T cells (Fig. 2a and Extended Data Fig. 3c, d). IL-22 acts on epithelial cells to induce reactivity and repair genes<sup>1</sup>. When compared to their wild-type littermate controls, the Ret<sup>Δ</sup> epithelium revealed normal morphology, proliferation and paracel-
lar permeability, but a marked reduction of epithelial reactivity and repair genes (Fig. 2b and Extended Data Fig. 3e–h). Accordingly, the Ret<sup>MEN2B</sup> epithelium displayed increased levels of these molecules in an IL-22-dependent manner (Fig. 2b and Extended Data Fig. 3i). These results indicate that RET signals selectively control innate IL-22 and shape intestinal epithelial reactivity.

To determine whether neurotrophic factors regulate intestinal defence, we tested how varying degrees of RET signals control enteric aggressions. Whereas Ret<sup>Δ</sup> mice treated with dextran sodium sulfate (DSS) had increased weight loss and inflammation, reduced IL-22-producing ILC3, decreased epithelial reactivity/repair genes and pronounced bacterial translocation from the gut, Ret<sup>MEN2B</sup> mutants were highly protected compared to their wild-type littermate controls (Fig. 2c–j and Extended Data Fig. 4). As DSS largely causes epithelial injury, we tested whether ILC3-autonomous RET signals are required to control infection. To this end, Ret<sup>Δ</sup> mice were bred with Rag1<sup>−/−</sup> mice to formally exclude adaptive T-cell effects. The resulting Rag1<sup>−/−</sup>Ret<sup>Δ</sup> mice were infected with the attaching and effacing bacteria <i>Citrobacter rodentium</i>. When compared to their littermate controls, Rag1<sup>−/−</sup>Ret<sup>Δ</sup> mice had marked gut inflammation, reduced IL-22-producing ILC3, increased <i>C. rodentium</i> infection and translocation, reduced epithelial reactivity genes, increased weight loss and reduced survival (Fig. 2k–n and Extended Data Fig. 5). Altogether, these data indicate that ILC3-intrinsic neurotrophic factor cues regulate gut defence and homeostasis.

We used a multi-tissue organoid system to show that IL-22 is the molecular link between RET-dependent ILC3 activation and epithelial reactivity. Addition of GFL to ILC3–epithelial organoids strongly induced epithelial reactivity genes in an IL-22- and RET-dependent manner (Fig. 3a, b and Extended Data Fig. 6a). To further examine how RET signals control innate IL-22, we investigated a gene signature

![Figure 2](https://example.com/figure2.png)

**Figure 2** | ILC3-intrinsic RET signals regulate gut defence. a, ILC3-derived cytokines (n = 11). b, Gene expression in the epithelium of Ret<sup>Δ</sup> and Ret<sup>MEN2B</sup> mice compared to their wild-type littermate controls (n = 7). c–f, DSS treatment in Ret<sup>Δ</sup> and Ret<sup>MEN2B</sup> mice (n = 8). g, Histopathology. d, Inflammation score and colon length. e, Innate IL-22. f, Bacterial translocation. cFU, colony-forming unit. g–j, DSS treatment in Ret<sup>Δ</sup> and Ret<sup>MEN2B</sup> mice (n = 8). g, Histopathology. h, Inflammation score and colon length. i, Innate IL-22. j, Bacterial translocation. mLN, mesenteric lymph node. k–n, <i>C. rodentium</i> infection in Rag1<sup>−/−</sup>Ret<sup>Δ</sup> (n = 15) and Rag1<sup>−/−</sup>Ret<sup>Δ</sup> (n = 17) mice. k, Histopathology. l, Inflammation score and colon length. m, Innate IL-22. n, Infection burden. Scale bars, 200 µm. Data are representative of 4 independent experiments. Error bars show s.e.m. *P < 0.05, **P < 0.01.
associated with ILC identity. Whereas the master ILC transcription factors Runx1, Id2, Gata3, Rora, Rorgt, Ahr and Stat3 were unaltered, Il22 was significantly reduced in Ret\textsuperscript{−/−} ILC3 (Fig. 3c and Extended Data Fig. 6b). Accordingly, activation of ILC3 with all or distinct GFL–GF\textsubscript{R}α pairs in trans efficiently increased Il22 despite normal expression of other ILC3-related genes (Fig. 3d and Extended Data Fig. 6c). Activation of RET by GFL leads to p38 MAPK/ERK-AKT cascade activation in neurons, whereas phosphorylation of STAT3 shapes Il22 expression. Analysis of Ret\textsuperscript{−/−} ILC3 revealed hypo-phosphorylated ERK1/2, AKT, p38/MAP kinase and STAT3 (Fig. 3e and Extended Data Fig. 6d). Accordingly, GFL-induced RET activation in ILC3 led to rapid ERK1/2, AKT, p38/MAP kinase and STAT3 phosphorylation and increased Il22 transcription (Fig. 3d, f and Extended Data Fig. 6e, f). Accordingly, inhibition of ERK, AKT or p38/MAP kinase upon GFL activation led to impaired STAT3 activation and Il22 expression (Fig. 3g, h). Finally, inhibition of STAT3 upon GFL-induced RET activation led to decreased Il22 (Fig. 3h). To examine whether GFL directly regulate Il22, we performed chromatin immunoprecipitation (ChIP)\textsuperscript{18} (Fig. 3i, j). Stimulation of ILC3 with GFL resulted in increased binding of pSTAT3 in the Il22 promoter and increased trimethyl-H3K36 at the 3’ end of Il22, indicating active Il22-transcribed regions (Fig. 3d, j). Thus, cell-autonomous RET signals control ILC3 function and gut defence by direct regulation of Il22 downstream of STAT3 activation.

Propensity towards inflammation and dysregulation of intestinal homeostasis have been associated with dysbiosis\textsuperscript{20,21}. When compared to their wild-type littermates, Ret\textsuperscript{−/−} mice have altered microbial communities as evidenced by quantitative analysis, weighted UniFrac analysis and significantly altered levels of Sutterella, unclassified Clostridiales and Bacteroides (Fig. 4a and Extended Data Fig. 7). Discrete microbial communities may have transmissible colitogenic properties. Recent studies have shown that glial cells express pattern recognition receptors on innate IL-22 production, we deleted TLR2, TLR4, and the alarmins IL-1β, IL-18, and IL-33\textsuperscript{19}. Glial-derived neurotrophic factors operate in an ILC3-intrinsic manner orchestrated by neurotrophic factors (Extended Data Fig. 10).

**Figure 4 | Glial cells set GFL expression and innate IL-22 via MYD88-dependent sensing of the microenvironment.**

a, Weighted UniFrac PCA analysis and genus-level comparisons from co-housed Ret\textsuperscript{−/−} (white circles) and Ret\textsuperscript{+/+} (black circles) littermates (n = 5). Purple, unclassified S247; red, Bacteroidetes; green, Sutterella; blue, unclassified Clostridiales; grey, other. b–d, DSS treatment of germ-free (GF) mice (n = 5) colonized with microbiota from Ret\textsuperscript{−/−} or Ret\textsuperscript{+/+} mice. b, Histopathology. c, Inflammation score. d, Innate IL-22. e, Innate IL-22 after antibiotic treatment (n = 8). f, Ret\textsuperscript{−/−}Gfp–Cre-Rosa26\textsuperscript{ERT2} mice. Green, RET/GFP; red, GFAP/RFP. g, h, Glial cell activation with TLR2, TLR4, IL-13 receptor and IL-33 receptor ligands (n = 6). i, TLR ligands, IL-1β and IL-33 activation of co-cultured ILC3 with wild-type (white bars) or Myd88−/− glial cells (black bars) (n = 6). j–m, DSS treatment of Gfp–Cre-Myd88\textsuperscript{−/−} mice (n = 12). j, Histopathology. k, Inflammation score and colon length. l, Innate IL-22. m, Body weight. Scale bars, 200 μm (b, j); 10 μm (f). Data are representative of 3–4 independent experiments. Error bars show s.e.m. *P < 0.05, **P < 0.01.

Defining the mechanisms by which ILC3 integrate environmental cues is critical to understanding mucosal homeostasis. Our work sheds light on the relationships between ILC3 and their microenvironment, notably through decoding a novel glial–ILC3–epithelial cell unit orchestrated by neurotrophic factors (Extended Data Fig. 10). Glial-derived neurotrophic factors operate in an ILC3-intrinsic manner by activating the tyrosine kinase RET, which directly regulates innate IL-22 downstream of p38 MAPK/ERK-AKT and STAT3 phosphorylation (Extended Data Fig. 10). Future studies will further elucidate the mechanisms inducing RET expression in ILC3. Our data demonstrate that, in addition to their well-established capacity to integrate dendritic-cell-derived cytokines, ILC3 perceive distinct multi-tissue regulatory signals leading to STAT3 activity and IL-22 expression, notably by integration of glial-cell-derived neuroregulators. Thus, rather than providing hard-wired signals for downstream of MYD88-dependent sensing of commensal products and alarmins.
ILC3-immunity, we propose that RET signalling induces fine-tuned innate IL-22 production that leads to efficient gut homeostasis and defence.

Previous studies demonstrated that neurons may indirectly shape fetal lymphoid tissue inducer cell aggregation via regulation of mesenchymal cells and that ablation of glial cells leads to gut inflammation\(^{25,26}\); here we reveal that glial cells are central hubs of neuronal and innate immune regulation. Notably, neurotrophic factors are the molecular link between glial cell sensing, innate IL-22 and intestinal epithelial defence. Thus, it is tempting to speculate that glial–immune cell units might be also critical to the homeostasis of other barriers, notably in the skin, lung and brain\(^\text{10}\). From an evolutionary perspective, coordination of innate immunity and neuronal function may ensure efficient mucosal homeostasis and a co-regulated neuro-immune response to various environmental challenges, including xenobiotics, intestinal infection, dietary aggressions and cancer.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**METHODS**

**Data reporting.** The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment, unless stated otherwise. Power analysis was used to estimate sample size.

**Mice.** C57BL/6j mice were purchased from Charles River. RefGRP (ref. 13), Rag1−/− (refs 31, 32), RefMIBM (ref. 14), Rosa26RFP (ref. 33), Rosa26RFP (ref. 16), RoGer-Cre; Il1b-fluc (ref. 35) and MyD88−/− (ref. 36) were in a full C57BL/6J background. Gfp-Cre-E1a; Myd88−/− (ref. 27) were in F8–F9 to a C57B/6J background. All lines were bred and maintained at IMI Lisboa animal facility. Mice were systematically compared with co-housed littermate controls. Both males and females were used in this study. Randomization and blinding were not used unless stated otherwise. All animal experiments were approved by national and institutional ethical committees, respectively. Direção Geral de Veterinária and iMM Lisboa ethical committee. Germ-free mice were housed at Instituto Gulbenkian de Ciência, Portugal, and Institut Pasteur, France, in accordance to institutional guidelines for animal care. Power analysis was performed to estimate the number of experimental mice.

**Generation of fetal liver chimeras.** For reconstitution experiments, 5 × 106 fetal liver cells were isolated from E15.5 RefWTGRFP or RefGRFP mice and injected intravenously into non-lethally irradiated (200 rad) alaphympod Rag1−/−/− mice. Mice were analysed 8 weeks after transplantation.

**DSS-induced colitis.** Dextran sodium sulfate (DSS) (molecular mass 36,000–50,000 Da; MP Biomedicals) was added into drinking water 3% (w/v) for 5 days followed by 2 days of regular water. Mice were analysed at day 7. Body weight, presence of blood and stool consistency was assessed daily.

**Citrobacter rodentium infection.** Infection with Citrobacter rodentium ICC180 (derived from DBS101 strain) was performed by gavage inoculation of 109 colony-forming units.\(^4,8\) Acquisition and quantification of luciferase signal was performed in a IVIS system (Caliper Life Sciences). Infection with Citrobacter rodentium was monitored by live imaging for user verification of cell-counting results and co-expression analysis (https://imm.medicina.ulisboa.pt/en/servicos-e-recursos/technical-facilities/bioimaging).

**Histopathology analysis.** Colon samples were fixed in 10% neutral buffered formalin. The colon was prepared in multiple cross-sections or ‘swiss roll’ technique\(^8\), routine-processed for paraffin embedding and 3–5 μm sections were stained with haematoxylin and eosin. Enteric lesions were scored by a pathologist blinded to experimental groups, according to previously published criteria\(^21–22\). Briefly, lesions were individually scored (0–4 increasing severity) for the following criteria: 1, mucosal loss; 2, mucosal epithelial hyperplasia; 3, degree of inflammation; 4, extent of the section affected in any manner; and 5, extent of the section affected in the most severe manner. Final scores were derived by summing the individual lesion and the extent scores. The internal diameter of the crypts was measured in at least five fields (10× magnification), corresponding to the hotspots in which the most severe changes in crypt architecture were seen. Measurements were performed in an average of 35 crypts per mouse, from proximal to distal colon. Villous villus height was measured in the jejunum. Measurements were performed in slides scanned using a Hamamatsu Nanozoomer SQ digital slide scanner running NDP Scan software.

**Enteric gial cell isolation.** Enteric gial cell isolation was adapted from previously described protocols\(^23–24\). Briefly, the muscularis layer was separated from the submucosa with surgical forceps under a dissection microscope (SteREO Lumar. V12, Zeiss). The lamina propria was scraped mechanically from the underlying submucosa using 1.5-mm cover-slips (Thermo Scientific). Isolated tissues were collected and digested with Liberase TM (7.5 μg ml\(^{−1}\); Roche) and Dnase I (0.1 mg ml\(^{−1}\); Roche) in RPMI supplemented with 1% HEPS, sodium pyruvate, glutamine, streptomycin and penicillin and 0.1% β-mercaptoethanol ( Gibco) for approximately 40 min at 37°C. Single-cell suspensions were passed through a 100-μm cell strainer (BD Biosciences) to eliminate clumps and debris.

**Flow cytometry and cell sorting.** Lamina propria cells were isolated as previously described\(^25\). Briefly, intestines were digested with collagenase D (0.5 mg ml\(^{−1}\); Roche) and Dnase I (0.1 mg ml\(^{−1}\); Roche) in RPMI supplemented with 10% FBS, 1% HEPS, sodium pyruvate, glutamine, streptomycin and penicillin and 0.1% β-mercaptoethanol ( Gibco) for approximately 30 min at 37°C under gentle agitation. For cytokine analysis, cell suspensions were incubated 4 h in PMA/ionomycin (Sigma-Aldrich) and brefeldin A (E Bioscience) at 37°C. Intracellular staining was performed using IC fixation/permeabilization kit (E Bioscience). Cells were stained using PBS, 1% FBS, 1% HEPES and 0.6% EDTA (Gibco). Flow cytometry analysis and cell sorting was performed using FORTESSA and FACSaria flow cytometers (BD Biosciences). Data analysis was performed using FlowJo software (Tristar). Sorted populations were 95–99% pure. Cell suspensions were stained with anti-CD45 (30-F11), anti-TER119 (TERT-19), TCRβ (H57-597), anti-CD8α (eBio500A2), anti-CD19 (eBioD3), anti-NK1.1 (PK136), anti-CD11c (N418), anti-Gr1 (RB6-8C5), anti-CD11b (Mi70), anti-CCR6 (29-2L17), anti-CD127 (IL-7Rα), A7R34, anti-Thy1.2 (52.1-3), anti-CD49D (DX5), anti-Tcrγδ (GL3), anti-NKp46 (29A1.4), anti-IL-17 (eBio17B7), anti-IL-22 (H9P8WSR), rat IgG1 isotype control (eBGRG1) antibodies, 7AAD viability dye, anti-mouse CD16/CD32 (Fc block), anti-RO5-α (AKF3K-9); rat IgG2a, isotype control (eBR2a) and streptavidin fluorochrome conjugates all from E bioscience; anti-CD4 (GK1.5), anti-CD31 (390), anti-CD68 (53-6.7), anti-CD24 (M1/69), anti-Epcam (G8.8) antibodies were purchased from Biolegend. Anti-RC (IC78A) antibody was purchased from R&D Systems. LIVE/DEAD Fixable Aqua Dead Cell Stain Kit was purchased from Invitrogen. Cell populations were defined as: ILC3 - CD45+ Lin- Thy1.2+IL7Rα+ROR-γt. For ILC3 subsets, additional markers were employed: LTI - CCR6+NKp46+; ILC3 NCR - CCR6+NKp46+; ILC3 NCR+ CCR6+NKp46+. Lineage was composed from CD3ε, CD8α, TCRγδ, CD19, Gr1, CD11c and TER119. Glial cells - CD45+ CD31+ TERT19+ CD49b+ (ref. 47); T cells - CD45+ CD3ε+γδ T cells - CD45+ CD3ε+γδ TCRγδ; B cells - CD45+ CD19+ B220+ macrophages - CD45+ CD11b+F4/80+ dendritic cells - CD45+ CD19+ CD3ε+MHCIIC+CD11c+ enteric neurons - CD45+ RET/GFP (ref. 13); epithelial cells - CD45+ CD24+ EpCAM. **Quantitative RT-PCR.** Total RNA was extracted using RNeasy micro kit (Qiagen) or Trizol (Invitrogen) according to the manufacturer’s protocol. RNA concentration was determined using Nanodrop Spectrophotometer (Nanodrop Technological). Quantitative real-time reverse transcription (RT)–PCR was performed as previously described\(^26,27\). Hprt and Gapdh were used as housekeeping genes. For Taqman assays (Applied Biosystems), RNA was retro-transcribed using a High Capacity RNA-to-cDNA Kit (Applied Biosystems), followed by a pre-amplification PCR using Taqman PreAmp Master Mix (Applied Biosystems). TaqMan Gene Expression Master Mix (Applied Biosystems) was used in real-time PCR Taqman Gene Expression Assays (Applied Biosystems) were the following: Gapdh Mm99999915_g1; Hprt Mm00449698_m1; Artm Mm00578545_m1; Nrtin Mm00324002_m1; Gdfn Mm00599849_m1; Gfrα1 Mm00439086_m1; Gfrα2 Mm00433584_m1; Gfrα3 Mm00495849_m1; Ret Mm00436304_m1; Il22 Mm01226722_g1; Il7a Mm00439618_m1; Il23r Mm00519943_m1; Rorgt Mm01261022_m1; Il7ra Mm00434295_m1; Ahr Mm00478392_m1; Stat3 Mm00129775_m1; Cxcr6 Mm02620517_s1; Hkbz Mm00605622_m1; Reg3q Mm001181787_m1; Reg3b Mm00406616_g1; Reg3g Mm00441127_m1;
primer sequences were previously described,48–50, IL-22 control IgG (Abcam) or H3K36me3 (07-030; Millipore). Immunoprecipitates were generated using a polyclonal antibody against anti-pSTAT3 (Cell Signalling Technology), rabbit anti-mouse Ki-67 antibody (BioLegend) were used.

Cells were administrated by gavage after overnight starvation. Plasma was collected 4 hours of Dextran-FITC administration using a Microplate Reader (Sigma-Aldrich). Colonies were counted after 2 days of culture at 37 °C. To address the microbial composition of the intestine, DNA samples were processed using mothur version 1.25.0 (ref. 52) and QIIME version 1.8 (ref. 53). Chimaeric sequences were removed with ChimeraSlayer.44, Operational taxonomic units (OTUs) were defined with CD-HIT using 97% sequence similarity as a cut-off. Only OTUs containing ≥2 sequences were retained; OTUs assigned to cyano bacteria or those which were not assigned to any phylum were removed from the analysis. Taxonomy was assigned using the Ribosomal Database Project (RDP) classifier v 2.2 (ref. 56), multiple sequence assignment was performed with PhyNAST (v 1.2.3),57 and FastTree was used to build the phylogenetic tree. Samples were rarified to 22,000 sequences per sample for alpha- and beta-diversity analyses. Taxonomic relative abundances are reported as the median with standard deviation. P values were calculated using the Wilcoxon rank-sum test. Statistical tests were conducted in R version 3.2.0. To determine which factors were associated with microbial community composition, statistical tests were performed using the non-parametric analysis of similarities (ANOSIM) with weighted UniFrac distance metrics.59

Data accession. The sequencing data generated in this study have been submitted to the NCBI Sequence Read Archive under BioProject PRJNA314493 (SRA: http://www.ncbi.nlm.nih.gov/sra/?term=PRJNA314493).

Intestinal organoids. Intestinal organoid cultures were obtained by dissociating the epithelium of the small bowel, and propagated as organoids according to previously described procedures. Briefly, 100 μg ml−1 of a 96-well round-bottom plate. After Matrigel solidification 100 μg ml−1 of growth medium (100 μM −1 penicillin/streptomycin) was added and replaced every 3 days. Organoids were grown at 37 °C with 5% CO2 and passed according to the manufacturer’s instructions. Freshly sorted intestinal ILC3 were added to 5–8 days old epithelial organoids after plating for 24 hours with or without anti-mouse IL-22 antibody (R&D Systems) (IL-22 blocker).

IL-22 agonist administration in vivo. 150 μg of anti-IL-22 antibody (8E11; gift from Genentech) or mouse IgG1 isotype control (MOPC-21; Bio X Cell) was administered by intraperitoneal injection to ReDerm1/2 mice every 2 days. Animals were analysed 2 weeks after the first administration.

Neosporon-derived glial cells. Neosporon-derived glial cells were obtained by previously described60,61, briefly, total intestines from E14.5 C57BL/6J and Mydb8 mice were digested with collagenase D (0.5 mg ml−1; Roche) and DNase I (0.1 mg ml−1; Roche) in DMEM/F-12, GlutaMAX, supplemented with 1% HEPES, streptomycin/penicillin and 0.1% ß-mercaptoethanol ( Gibco) at 37 °C. Cells were then plated at a density of 5,000–7,000 crypts per ml. 15 μg ml−1 of this mix was plated per well of a 96-well round-bottom plate. After Matrigel solidification 100 μg ml−1 of growth medium (100 μM penicillin/streptomycin) was added and replaced every 3 days. Organoids were grown at 37 °C with 5% CO2 and passed according to the manufacturer’s instructions. Freshly sorted intestinal ILC3 were added to 5–8 days old epithelial organoids after plating for 24 hours with or without anti-mouse IL-22 antibody (R&D Systems). IL-22 agonist administration in vivo. 150 μg of anti-IL-22 antibody (8E11; gift from Genentech) or mouse IgG1 isotype control (MOPC-21; Bio X Cell) was administered by intraperitoneal injection to ReDerm1/2 mice every 2 days. Animals were analysed 2 weeks after the first administration.

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**Statistics.** Results are shown as mean ± s.e.m. Statistical analysis used Microsoft Excel. Variance was analysed using F-test. Student’s t-test was performed on homoscedastic populations, and Student’s t-test with Welch correction was applied on samples with different variances. Analysis of survival curves was performed using a MANtel–Cox test. Results were considered significant at *P* ≤ 0.05 and **P** ≤ 0.01. Statistical treatment of metagenomics analysis is described in the Methods section ‘16S rRNA gene sequencing and analysis.’

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Extended Data Figure 1 | ILC3 selectively express the neurotrophic factor receptor RET. a, Expression of RET protein in gut CD45<sup>+</sup>Lin<sup>−</sup>Thy1.2<sup>+</sup>IL7R<sup>+</sup>ROR<sup>+</sup>γ<sup>t</sup> ILC3. b, Analysis of gut ILC3 from Ret<sup>GFP</sup> mice. Embryonic day 14.5 (E14.5). c, d, Analysis of enteric ILC3 subsets from Ret<sup>GFP</sup> mice. e, Analysis of cytokine-producing ILC3 from Ret<sup>GFP</sup> mice. f, Pregnant Ret<sup>GFP</sup> mice were provided with antibiotic cocktails that were maintained after birth until analysis at 6 weeks of age. Left, RET/GFP (white); right, flow cytometry analysis of RET/GFP expression in ILC3. Thin line, Ab-treated; bold line, specific pathogen free (SPF). g, Ret expression in enteric ILC3 from germ-free (GF) mice and SPF controls (n = 4). h, Analysis of lamina propria populations from Ret<sup>GFP</sup> mice. i, Enteric ILC3 clusters. Green, RET/GFP, blue, ROR<sup>+</sup>γ<sup>t</sup>; red, B220. Bottom, quantification analysis for RET/GFP and ROR<sup>+</sup>γ<sup>t</sup> co-expression (79.97 ± 4.72%). j, Rare RET-expressing ILC3 in intestinal villi. Green, RET/GFP; blue, ROR<sup>+</sup>γ<sup>t</sup>; red, CD3ε. Scale bars, 10μm. Data are representative of 4 independent experiments. Error bars show s.e.m.
Extended Data Figure 2 | T cell-derived IL-22 and IL-17 in Ret<sup>GFP</sup> chimaeras and Ret<sup>MEN2B</sup> mice. a, T-cell-derived IL-17 in Ret<sup>GFP</sup> chimaeras. Ret<sup>WT/GFP</sup>, n = 25; Ret<sup>GFP/GFP</sup>, n = 22. b, T-cell-derived IL-22 and IL-17 in the intestine of Ret<sup>MEN2B</sup> mice and their wild-type littermate controls (n = 7). Data are representative of 4 independent experiments. Error bars show s.e.m.
Extended Data Figure 3 | Enteric homeostasis in steady-state RetΔ mice.  

a. *Rorgt*-Cre mice were bred to Rosa26<sup>YFP</sup> mice. Analysis of Rosa26<sup>YFP</sup> expression in gut ILC3 from *Rorgt*-Cre*Rosa26<sup>YFP</sup> mice. b. Number of Peyer’s patches (PP) (n = 10). c. T-cell-derived IL-22 in Ret<sup>Δ</sup> mice and their wild-type littermate controls. (n = 11). d. γδ T-cell-derived IL-22 in Ret<sup>Δ</sup> mice and their wild-type littermate controls (n = 4). e. Intestinal villus and crypt morphology (n = 6). f. Epithelial cell proliferation (n = 5). g. Intestinal paracellular permeability measured by Dextran-Fitc in the plasma (n = 5). h. Tissue repair genes in Ret<sup>Δ</sup> intestinal epithelium in comparison to their wild-type littermate controls (n = 8). i. Reactivity genes in Ret<sup>MEN2B</sup> mice treated with anti-IL-22 blocking antibodies compared to Ret<sup>MEN2B</sup> intestinal epithelium. Ret<sup>MEN2B</sup>, n = 4; Ret<sup>MEN2B</sup> + anti-IL-22, n = 4. Data are representative of 3 independent experiments. Error bars show s.e.m.
Extended Data Figure 4 | Enteric inflammation in mice with altered RET signals. Mice were treated with DSS in the drinking water. a, Weight loss of DSS-treated RetΔ mice and their littermate controls (n = 8). b, T-cell-derived IL-22 in RetΔ mice and their wild-type littermate controls after DSS treatment (n = 8). c, Weight loss of DSS treated RetMEN2B mice and their wild-type littermate controls (n = 8). d, T-cell-derived IL-22 in RetMEN2B mice and their wild-type littermate controls (n = 8). e, Intestinal villi and crypt morphology (n = 6). f, Epithelial reactivity gene expression in DSS treated RetΔ mice in comparison to their wild-type littermate controls (n = 8). g, Tissue repair gene expression in DSS treated RetΔ mice in comparison to their wild-type littermate controls (n = 4). Data are representative of 3–4 independent experiments. Error bars show s.e.m. *P < 0.05; **P < 0.01.
Extended Data Figure 5  |  Citrobacter rodentium infection in RetΔ mice. a, C. rodentium translocation to the liver of Rag1−/− RetΔ and their Rag1−/− Retfl littermate controls at day 6 after infection (n = 15). b, MacConkey plates of liver cell suspensions from Rag1−/− RetΔ and their Rag1−/− Retfl littermate controls at day 6 after C. rodentium infection. c, Whole-body imaging of Rag1−/− RetΔ and their Rag1−/− Retfl littermate controls at day 6 after luciferase-expressing C. rodentium infection. d, Epithelial reactivity gene expression in C. rodentium infected Rag1−/− RetΔ mice (n = 17) and their Rag1−/− Retfl littermate controls (n = 15). e, Weight loss in C. rodentium-infected Rag1−/− RetΔ mice and their Rag1−/− Retfl littermate controls (n = 8). f, Survival curves in C. rodentium infected Rag1−/− RetΔ mice and their Rag1−/− Retfl littermate controls (n = 8). g, C. rodentium translocation to the liver of RetΔ and their Retfl littermate controls at day 6 after infection (n = 6). h, MacConkey plates of liver cell suspensions from RetΔ and their Retfl littermate controls at day 6 after C. rodentium infection. i, Whole-body imaging of RetΔ and their Retfl littermate controls at day 6 after luciferase-expressing C. rodentium infection. j, C. rodentium infection burden (n = 8). k, Innate IL-22 in in C. rodentium infected RetΔ mice and their Retfl littermate controls (n = 8). Data are representative of 3–4 independent experiments. Error bars show s.e.m. ns, not significant. *P < 0.05; **P < 0.01.
Extended Data Figure 6 | Glial-derived neurotrophic factor family ligand (GFL) signals in ILC3. a, Multi-tissue intestinal organoid system. Scale bar, 20 μm. Black arrows, ILC3. b, Expression of ILC-related genes in ILC3 from RetΔ mice in comparison to their littermate controls (n = 4). c, ILC3 activation with all GFL/GFRα pairs (GFL); single GDNF family ligand (GDNF, ARTN or NRTN); or single GFL/GFRα pairs (GDNF/GFRα1, ARTN/GFRα3 or NRTN/GFRα2) compared to vehicle BSA (n = 5). d, ILC3 from RetΔ mice (open black) and their littermate controls (open dash). Isotype (closed grey). e, 30-min activation of ILC3 by GFL (open black) compared to vehicle BSA (open dash). Isotype (closed grey). f, 10-min activation of ILC3 by GFL. pERK, n = 8; pAKT, n = 8; phosphorylated p38/MAP kinase, n = 8; pSTAT3, n = 8. Similar results were obtained in at least 3–4 independent experiments. Error bars show s.e.m. *P < 0.05; **P < 0.01.
Extended Data Figure 7 | Alterations in the diversity of intestinal commensal bacteria of RetΔmice. a, Quantitative PCR analysis at the phylum level in stool bacteria from co-housed Retfl and RetΔ littermates in steady state (n = 5). b, Metagenomic phylum level comparisons in stool bacterial from co-housed Retfl and RetΔ littermates in steady state (left) and after DSS treatment (right) (n = 5). c, Genus-level comparisons in stool bacteria from co-housed Retfl and RetΔ littermates in steady state (left) and after DSS treatment (right) (n = 5). Error bars show s.e.m. *P < 0.05; **P < 0.01.
Extended Data Figure 8 | GFL-expressing glial cells anatomically co-localize with ILC3. a, Intestine of RetGFP mice. Green, RET/GFP; red, GFAP; blue, RORγt. Similar results were obtained in 3 independent experiments. b, Purified lamina propria LTi, NCR− and NCR+ ILC3 subsets, T cells (T), B cells (B), dendritic cells (Dc), macrophages (Mø), enteric neurons (N) and mucosal glial cells (G). c, Neurosphere-derived glial cells. d, Activation of neurosphere-derived glial cells with TLR2 (Pam3CSK4), TLR3 (Poli I:C), TLR4 (LPS) and TLR9 (DsDNA-EC) ligands, as well as IL-1β, IL-18 and IL-33 (n = 6). M, medium. e, Il22 in co-cultures of glial and ILC3 using single or combined GFL antagonists (n = 6). f, Il22 in co-cultures of ILC3 and glial cells from Il1b−/− or their wild-type controls (n = 3). g, Gdnf, Artn and Nrtn expression in glial cells and ILC3 upon TLR2 stimulation (n = 3). Scale bar, 30 μm. Similar results were obtained in at least 4 independent experiments.
Extended Data Figure 9 | Glial cell sensing via MYD88 signals.

a–c, Intestinal glial cells were purified by flow cytometry. a, Germ-free and their respective SPF controls (n = 3). b, Myd88−/− and their respective wild-type littermate controls (n = 3). c, Gfap-CreMyd88Δ and their littermate controls (Myd88fl) (n = 3). d, Total lamina propria cells of Gfap-CreMyd88Δ and their littermate controls (Myd88fl) (n = 6).

e–h, Citrobacter rodentium infection of Gfap-CreMyd88Δ mice and their littermate controls (Myd88fl) (n = 6). e, Innate IL-22. f, Citrobacter rodentium translocation. g, Infection burden. h, Weight loss. Data are representative of 3 independent experiments. Error bars show s.e.m. *P < 0.05; **P < 0.01.
Extended Data Figure 10 | A novel glial-ILC3-epithelial cell unit orchestrated by neurotrophic factors. Lamina propria glial cells sense microenvironmental products that control neurotrophic factor expression. Glial-derived neurotrophic factors operate in an ILC3-intrinsic manner by activating the tyrosine kinase RET, which directly regulates innate IL-22 downstream of a p38 MAPK/ERK-AKT cascade and STAT3 phosphorylation. GFL induced innate IL-22 acts on epithelial cells to induce reactivity gene expression (CBP, commensal bacterial products; AMP, antimicrobial peptides; Muc, mucins). Thus, neurotrophic factors are the molecular link between glial cell sensing, innate IL-22 production and intestinal epithelial barrier defence.