Neuronal regulation of type 2 innate lymphoid cells via neuromedin U

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Group 2 innate lymphoid cells (ILC2s) regulate inflammation, tissue repair and metabolic homeostasis1, and are activated by host-derived cytokines and alarmins1. Discrete subsets of immune cells integrate nervous system cues2–4, but it remains unclear whether neuron-derived signals control ILC2s. Here we show that neuromedin U (NMU) in mice is a fast and potent regulator of type 2 innate immunity in the context of a functional neuron–ILC2 unit. We found that ILC2s selectively express neuromedin U receptor 1 (Nmur1), and mucosal neurons express NMU. Cell-autonomous activation of ILC2s with NMU resulted in immediate and strong NMUR1-dependent production of innate inflammatory and tissue repair cytokines. NMU controls ILC2s downstream of extracellular signal-regulated kinase and calcium-influx-dependent activation of both calcineurin and nuclear factor of activated T cells (NFAT). NMU treatment in vivo resulted in immediate protective type 2 responses. Accordingly, ILC2-autonomous ablation of Nmur1 led to impaired type 2 responses and poor control of worm infection. Notably, mucosal neurons were found adjacent to ILC2s, and these neurons directly sensed worm products and alarmins to induce NMU and to control innate type 2 cytokines. Our work reveals that neuron–ILC2 cell units confer immediate tissue protection through coordinated neuroimmune sensory responses.

ILC2s are abundant at mucosal barriers and act as key initiators of type 2 inflammation and tissue repair1. ILC2s are activated by cell-extrinsic cytokines, including interleukin 25 (IL-25), IL-33 and thymic stromal lymphopoietin (TSLP)1. Previous reports indicated that discrete lymphocyte subsets and haematopoietic progenitors are controlled by dietary signals and neuroregulators2–4,11,14,15, suggesting that ILC2s may exert their function in the context of neuroimmune cell units5.

To investigate whether ILC2s directly and selectively perceive neuronal-derived molecules, we used genome-wide transcriptional profiling of ILC2s to compare them to their adaptive (T helper cells) and innate (ILC1 and ILC3) counterparts12 (Fig. 1a, b). This analysis identified the gene Nmur1 as being selectively enriched in ILC2s when compared to ILC1s, ILC3s and T helper cells (Fig. 1a, b, Extended Data Fig. 1a, b). This finding was confirmed by independent quantitative expression assays in multiple subsets of immune cells, including ILC1s, ILC3s, natural killer cells, eosinophils, mast cells, macrophages, neutrophils, dendritic cells, T cell subsets and B cells (Fig. 1c, Extended Data Fig. 1c, d). Notably, human ILC2 also expressed Nmur1 (Fig. 1d). This gene encodes a transmembrane receptor for NMU. The latter is a secreted neuropeptide found in the brain and highly expressed in the gastrointestinal tract13–15,16. As such, NMU acts as a neuronal-derived regulator in diverse physiologic processes16. NMU was shown to be produced by cholinergic enteric neurons, which also express the neurotrophic factor receptor RET13–15,17. In agreement, the NMU gene (Nmu) was mainly expressed by neurons in the lamina propria, whereas these transcripts were not detectable in enteric neuroglia and epithelial cells (Fig. 1e). Similarly, all analysed immune cell subsets, including dendritic cells, macrophages and B cells, had no significant Nmu expression (Fig. 1e, Extended Data Fig. 1e). Notably, the use of reporter mice for total neurons (Rosagfp) or for cholinergic neurons (Chat-cre-Rosa26RFP)18–20 revealed that CD3+ KLRG1+ candidate ILC2s are adjacent to the intestinal neuronal network (4.716±0.656 (mean±s.e.m.)), and lung ILC2s could also be found in the vicinity of local neurons (Fig. 1f, g, Extended Data Fig. 1f–h). Taken together, these data suggest a paracrine neuron–ILC2 crosstalk that is orchestrated by NMU–NMUR1 interactions.

To explore this hypothesis, intestine- and lung-derived ILC2s were purified and activated with recombinant mouse peptide NMU (NmU23) (Fig. 2a–e). Interestingly, cell-autonomous activation of ILC2s with NmuU23 resulted in prompt and potent expression of the pro-inflammatory and tissue-protective type 2 cytokines genes Il5, Il13, amphiregulin (Areg) and colony stimulating factor 2 (Gsfs2) (Fig. 2a, b, Extended Data Fig. 2a). NmuU23-dependent activation of ILC2s also increased ILC2 proliferation, as measured by Ki67 expression (Extended Data Fig. 2b–d). NMU was shown to bind with similar affinity to two orphan class A G-protein-coupled receptors19, NMUR1 and NMUR2. Genetic ablation of Nmur1 showed that NMUR1 activation provides the molecular link between NMU-dependent ILC2 activation and cytokine production. Activation of purified ILC2s with NmuU23 led to potent expression of the type 2 cytokine proteins IL-5 and IL-13 in an NMUR1-dependent manner (Fig. 2c, d, Extended Data Fig. 2e–h). Notably, and in contrast to the canonical ILC2-activating cytokines (IL-33 and IL-25), NmuU23 led to an immediate strong expression of innate IL-5, IL-13 and AREG, indicating that NMU is a uniquely fast and potent regulator of ILC2-derived type 2 cytokines (Fig. 2e, Extended Data Fig. 3a, b). In agreement, in vivo administration of the neuropeptide NmuU23 resulted in immediate and selective type 2 cytokine production from ILC2s, whereas their adaptive T-helper-cell-derived counterparts were upregulated (Fig. 2f, g, Extended Data Fig. 3c). Although NMU restored ILC2 numbers in vivo in the absence of IL-33 and IL-25 receptor signals, this neuropeptide failed to efficiently induce innate cytokines in the similar contexts (Extended Data Fig. 4a–d). This suggests that NMU signals immediately trigger innate type 2 cytokines, whereas the canonical IL-25–IL-33 signalling axis regulates ILC2 homeostasis and function with comparatively delayed kinetics (Extended Data Fig. 4a–d). To establish the link between ILC2-autonomous NMUR1 activation and innate type 2 cytokine production, we generated mixed bone marrow chimerae with Nmur1–sufficient and -deficient bone marrow progenitors. Whereas NMUR1 signals were dispensable for ILC2 homeostasis (Extended Data Fig. 5a–f), an intact NMU–NMUR1 axis was critical for production of ILC2-derived cytokines. Notably, NmuU23 administration to bone marrow chimerae

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revealed that Nmur1-deficient ILC2s had reduced innate IL-5 and IL-13 expression as compared to Nmur1-sufficient counterparts (Fig. 2h, i). In contrast, T-helper-cell-derived cytokines were unperturbed in Nmur1-deficient cells (Extended Data Fig. 5g, h). These data indicate that NMU is a rapid and potent cell-autonomous regulator of innate type 2 inflammatory and tissue repair cytokines, through NMUR1 activation.

To further examine how NMU controls innate type 2 responses, we investigated the signalling cues provided by activated NMUR1 in ILC2s. In neurons, activation of NMU receptors leads to increased Ca²⁺ influx and extracellular signal-related kinase (ERK)1/2 activation, whereas NFAT activity is required for type 2 cytokine production²¹–²³. Stimulation of ILC2s with NMU led to immediate and efficient ERK1/2 activation, whereas inhibition of ERK activity upon NmuU23-induced ILC2 activation resulted in impaired type 2 cytokine gene expression (Fig. 3a, b). Analysis of NMU-induced activation of ILC2s also led to immediate and robust Ca²⁺ influx, suggesting a role for the calcium-dependent serine/threonine protein phosphatase calcineurin in NmuU23-induced type 2 responses (Fig. 3c). Accordingly, inhibition of calcineurin upon NmuU23 activation led to impaired innate Il5, Il13 and Csf2 expression, whereas NMU23 activation led to rapid NFAT nuclear translocation in ILC2s (Fig. 3d–f, Extended Data Fig. 6). Finally, inhibition of NFAT activity on NmuU23-induced NMUR1 activation led to a similar decrease in Il5, Il13 and Csf2 expression (Fig. 3g). Thus, we conclude that the
neuropeptide NMU can operate in an ILC2-intrinsic manner by activating NMUR1, which regulates innate type 2 cytokines downstream of a Ca²⁺–calcineurin–NFAT cascade and ERK1/2 phosphorylation.

To test whether neuronal peptides can regulate mucosal defense we tested the effect of varying degrees of NMUR1 signals during infection with the helminth parasite *Nippostrongylus brasiliensis*. To test whether neuronal peptides can regulate mucosal defense we tested the effect of varying degrees of NMUR1 signals during infection with the helminth parasite *N. brasiliensis*. To test whether neuronal peptides can regulate mucosal defense we tested the effect of varying degrees of NMUR1 signals during infection with the helminth parasite *N. brasiliensis*. To test whether neuronal peptides can regulate mucosal defense we tested the effect of varying degrees of NMUR1 signals during infection with the helminth parasite *N. brasiliensis*.

**Figure 2** | NMU is a potent ILC2-intrinsic regulator of type 2 cytokines, through NMUR1 activation. a, b, Type 2 cytokine gene expression in gut (a) and lung (b) ILC2s after NmU23 activation (n = 6). c, d, Type 2 cytokine protein expression in ILC2s after NmU23 activation. e, Intestinal ILC2s. IL-5 (n = 6), IL-13 (n = 6), AREG (n = 3). f, G. In vivo administration of NmU23. f, Gut ILC2-derived type 2 cytokines (n = 6). g, Intestinal T(H)2-cell-derived type 2 cytokines (n = 6). h, i, Bone marrow mixed chimaeras with Nmur1+/– and their Nmur1+/+ wild-type littermate controls upon NmU23 administration. Percentage (h) and number (i) of lung ILC2s. Nmur1+/+ (n = 5); Nmur1+/– (n = 4). Data are representative of 2–6 independent experiments. Error bars show s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant.

**Figure 3** | NMU regulates ILC2-derived cytokines through ERK1/2 and a Ca²⁺–calcineurin–NFAT cascade. Intestinal ILC2 activation by NMU. a, Top, p-ERK. Bottom, percentage of p-ERK cells (n = 4). Mean fluorescence intensity (MFI) of p-ERK expression (n = 4). b, IL5, IL13 and Csf2 expression in ILC2s cultured with medium (control), NmU23 or NmU23 and ERK inhibitor PD98059 (n = 3). c, Left and centre, Ca²⁺ influx, represented by Fluo-4–AM intensity. NmU23 was added 60 s after ILC2 baseline acquisition (arrow). Right, mean intensity of Ca²⁺ influx (n = 3). d, IL5, IL13 and Csf2 expression in ILC2s cultured with medium (control), NmU23 or NmU23 and calcineurin inhibitor FK506 (n = 12). e, Nuclear translocation of NFAT (red) upon NmU23 activation in ILC2s. f, Left, percentage of ILC2s with nuclear NFAT (n = 3). Right, NFAT nuclear fluorescence intensity. Control (n = 16), NmU23 (n = 7), P + I (n = 8). g, IL5, IL13 and Csf2 expression in ILC2s cultured with medium (control), NmU23 or NmU23 and NFAT inhibitor 11R-VIVIT (n = 6). Scale bars, 5 μm. Data are representative of 2–4 independent experiments. Error bars show s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant.

Data Fig. 7c, d), decreased tissue haemorrhage (Fig. 4c, Extended Data Fig. 7e, f) with subsequent increased eosinophil and mast cell infiltrates in the lung, and reduced infection burden when compared to their vehicle (PBS)-treated counterparts (Fig. 4d, e). To further explore the role of NMUR1 in innate type 2 responses, we infected Nmur1-deficient mice and their wild-type littermate controls with *N. brasiliensis* (Fig. 4f–i). When compared to their wild-type littermate counterparts, Nmur1-deficient mice had decreased type 2 responses, notably reduced ILC2-derived cytokines (Fig. 4f, Extended Data Fig. 8a), increased tissue haemorrhage (Fig. 4g, Extended Data Fig. 8b, c) and impaired eosinophil and mastocyte infiltrates (Fig. 4h). In line with these findings, Nmur1-deficient mice had increased *N. brasiliensis* infection burden at the peak of lung and gut infection phases, whereas late worm expulsion was intact (Fig. 4i, Extended Data Fig. 8d). To more specifically define the link between ILC2, the NMU–NMUR1 signalling axis and protection against worm infection, we generated chimaeras of Nmur1-sufficient and Nmur1-deficient ILC2s in allogeneic host mice. Infection of such chimaeras with *N. brasiliensis* revealed that Nmur1-knockout ILC2s had decreased type 2 cytokine responses compared to their wild-type counterparts (Fig. 4j). Accordingly,
of innate type 2 cytokines, and intranasal challenge with NES led to similar increases of ILC2-derived cytokines in the lung (Extended Data Fig. 9a, b). Enteric neurons have been previously shown to express Toll-like receptors. Activation of neuronal organoids with NES or IL-33 induced Nmu expression in an MYD88-dependent manner (Fig. 4m). To demonstrate the physiological importance of MYD88-dependent neuronal sensing in ILC2 function, we deleted Myd88 in cholinergic neurons by breeding Chat-Cre to Myd88−/− mice. Notably, cholinergic-neuron–intrinsic deletion of Myd88 resulted in impaired cytokine-producing ILC2s during N. brasiliensis infection (Fig. 4n). Taken together, these data indicate that neurons can orchestrate innate type 2 cytokines through direct sensing of worm products and alarmins.

Determining the mechanisms by which ILC2s perceive, integrate and respond to environmental signals is critical for understanding tissue and organ homeostasis. In our work, we establish unexpected relationships between ILC2s and their environment and identify a neuron–ILC2 cell unit orchestrated by NMU (Extended Data Fig. 10). Mucosal neurons can directly sense alarm and worm products to produce NMU. NMU activates ILC2s, via NMR1, resulting in potent and immediate production of innate type 2 cytokine downstream of ERK phosphorylation and activation of a Ca2+-calcineurin–NFAT signalling cascade (Extended Data Fig. 10).

Although it is well established that ILC2s integrate cytokine signals, including IL-25, IL-33 and TSLP, here we demonstrate that ILC2s can integrate signals from different germ-layer–derived tissues to immediately trigger inflammatory and tissue repair type 2 responses. Thus, neuron–ILC2 cell units might be poised to confer immediate tissue protection through coordinated neuroimmune sensory responses (Extended Data Fig. 10). Afferent extrinsic nervous cues might also affect local neuroimmune cell units, but future studies using emerging technology platforms are required to interrogate these putative interactions.

Previous studies demonstrated that ILC2s contribute to multiple homeostatic processes, including nutrient sensing, metabolism, tissue repair and infection control. Here we show that NMU is the molecular link between neuronal sensing, innate type 2 responses and mucosal protection. Coupling neuronal activity and ILC2-dependent immune regulation may have ensured potent, efficient and integrated multi–tissue responses to environmental challenges throughout evolution. Notably, coordinated NMU-induced smooth muscle contraction and type 2 innate immunity may have coevolved to control worms that have been intimate evolution partners of mammals. In line with this hypothesis, NMU is highly conserved across mammalian, amphibian, avian and fish species. Finally, our current data and other independent studies indicate that the mucosal nervous system partners with immune cells to ensure local tissue regulation; thus, it is tempting to speculate that neuroimmune sensoryunits regulate physiology and homeostasis at an organismal level.

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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**Author Contributions** VC. and J.C designed, performed and analysed the experiments in Figs 1–4 and Extended Data Figs 1–10. H.R. contributed to the design of the experiments in Figs 1a, b and Extended Data Figs 1a, b. T.B., K.S. and N.H. contributed to the experiments in Fig. 4c, g and Extended Data Figs 7e, f, 8b, c. N.L.B.-M. analysed the experiments in Figs 1f, g, 3e, f and Extended Data Fig. 1f–g. T.C. analysed the experiments in Fig. 4a and managed the animal colony. B. G.-C. contributed to the experiments in Figs 1–4 and Extended Data Figs 1–10. H.R. provided technical assistance. V.C was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal; J.C. by Fondation pour la Recherche Médicale (FRM), France, and by Marie Skłodowska-Curie fellowship (750030), EU; B.G.-C. by FP7 (289720), EU. N.L.B.-M. is supported by FCT, Portugal, and European Molecular Biology Organisation (EMBO), N.H. by Swiss National Science Foundation (310030_156517), H.V.-F. by ERC (647274), EU; Kenneth Rainin Foundation, USA; Crohn’s and Colitis Foundation of America, USA; and FCT, Portugal.

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METHODS

Data reporting. No statistical methods were used to predetermine sample size, unless stated otherwise. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment, unless stated otherwise.

Mice. C57BL/6J (B6) mice were purchased from Charles River. Nod/Scid/Gamma (NSG) mice were bought from The Jackson Laboratory. Sperm from the strain C57BL/6-Nmur1tm1.1(KOMP)Vlcg, which contains a Nmur1 deletion, was obtained from the KOMP Repository, University of California Davis and Children’s Hospital Oakland Research Institute, USA. Nmur1−/− mice were generated by in vitro fertilization at the Champalimaud Centre for the Unknown, Portugal. Chat-Cre28, Rosa26S-PP (ref. 19), Rag2−/−Il2rg−/− (refs 31,32), Myd88−/− (ref. 33) and RetGfp (ref. 18) and Myd88−/− (ref. 33) mice were on a C57BL/6J background. Mice were maintained at the Champalimaud Centre for the Unknown and IMM Lisbon animal facilities under specific pathogen free conditions. Il11r1−/− Il17r1−/− mice43,45 and their wild-type controls were on a BALB/c background and were bred and maintained at Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland.

Mice were systematically compared with co-housed littermate controls unless stated otherwise, 7–12-week-old males and females were used in this study. All animal experiments were approved by national and institutional ethical committees, respectively Direção Geral de Veterinária and Service de la Consommation et des Affaires Vétérinaires Federal (Canton Vaud, Switzerland), Champalimaud Centre for the Unknown, IMM Lisbon and EPFL ethical committees. Randomisation and blinding were not used unless stated otherwise. Power analysis was performed to estimate the number of experimental mice.

Analysis of gene expression microarray data. The expression profile of 239 genes related to neural pathways was performed in mouse lymphoid cells based on the Affymetrix Mouse Gene 1.0 ST Array data set (GEO accession number GSE37448). Preprocessing of microarray data (including background correction and normalization) was performed applying the robust multiarray analysis (RMA) method46, included in the Bioconductor package affy47 for the statistical software environment R48. Linear models and the B (empirical Bayes) statistic were employed in differential gene expression analysis, using Bioconductor package limma49. Plots associated with the microarray data analyses were generated in R.

In vitro and in vivo ILC2 activation. For in vitro experiments, purified lung and small intestine lamina propria ILC2s were cultured in complete RPMI (supplemented with 10% fetal bovine serum (FBS), 1% hepes, sodium pyruvate, glutamine, streptomycin and penicillin (300 U ml−1) streptomycin and penicillin (300 U ml−1) 10 ng ml−1; R&D Systems) for 1 week. 24 h after the last challenge lung cells were enzymatically recovered for analysis. Digestive organs were disrupted by passage through a 100-μm cell strainer (BD Biosciences). A 40–80% percoll gradient centrifugation was used for additional leukocyte purification from lung and small intestine cell suspensions. Erythrocytes from lung, small intestine and bone marrow preparations were lysed with RBC lysis buffer (eBioscience).

Flow cytometry and cell sorting. For cytokine protein analysis ex vivo, cells were incubated with PMA (50 ng ml−1), ionomycin (500 ng ml−1) (Sigma) and brefeldin A (eBioscience) for 4 h before intracellular staining. Intracellular staining was performed using IC fixation/permeabilization kit (eBioscience). Cell suspensions were stained with anti-CD45 (30-F11), anti-TER119 (TEN-19), TCRγ (H57-597), anti-CD3 (eBio500A2), anti-CD19 (eBio1D3), anti-NK1.1 (PK1.3), anti-CD11c (N418), anti-Gr1 (RB6-8C5), anti-CD11b (M1/70), anti-CD127 (IL-7Rα; A2R34), anti-IL-12 (1D4; R4-6A2) and anti-IL-12 (1 μg ml−1; C17.8 (Biolegend). Polyclonal IgG2a cells were analysed after 4 days of differentiation.

Cell isolation. Lungs were perfused with a solution of cold PBS with 2% heparin through the right ventricle of the heart and were subsequently finely minced and digested in complete RPMI supplemented with collagenase D (0.1 mg ml−1; Roche) and DNase I (20 μg ml−1; Affymetrix) for 1 h at 37 °C under gentle agitation. For isolation of small intestine lamina propria cells, intestines were thoroughly rinsed with PBS, cut in 1-cm pieces, and shaken for 30 min in PBS containing 2% FBS and 5 mM EDTA to remove intraepithelial and epithelial cells. Intestines were then digested with collagenase D (0.5 mg ml−1; Roche) and DNase I (20 μg ml−1; Affymetrix) in complete RPMI for 30 min at 37 °C, under gentle agitation. Enteric neurons and glial cells were isolated as previously described31,41. Briefly, isolated tissues were digested with Liberase TM (7.5 μg ml−1; Roche) and DNase I (20 μg ml−1; Affymetrix) in complete RPMI for 30 min at 37 °C, under gentle agitation. Digested organs were disrupted by passage through a 100-μm cell strainer (BD Biosciences). A 40–80% percoll gradient centrifugation was used for additional leukocyte purification from lung and small intestine cell suspensions. Erythrocytes from lung, small intestine and bone marrow preparations were lysed with RBC lysis buffer (eBioscience).

Bone marrow transplantation. Bone marrow cells were flushed out from femurs and tibiae of Nmur1−/−, Il11r1−/− Il17r1−/− mice and their respective wild-type controls. Bone marrow cells were CD3-depleted using Dynabeads Biotin Binder (Thermo Fisher Scientific) according to the manufacturer's instructions. 105 cells of each genotype (CD45.2) were injected intravenously alone or in direct competition with 105 cells of the respective wild-type competitor (CD45.1/CD45.2), in a 1:1 ratio into non-lethally irradiated (150 Rad) NSG mice (CD45.1). Mice were analysed after 8 weeks at transplantation.

ILC2 adoptive transfer. For adoptive cell transfer, small intestine ILC2s from Nmur1−/− and Nmur1−/− littermate control mice were purified and expanded in vitro in supplemented RPMI in the presence of recombinant mouse IL-2, IL-7 (10 ng ml−1; Peprotech) and IL-33 (10 ng ml−1; R&D Systems) for 1 week. 2 × 105 expanded ILC2s were injected intravenously into Rag2−/− Il2rg−/− recipients. Mice were infected with 400 Nippostrongylus brasiliensis larvae 1 week after ILC2 transplantation.

Parasite infection. Nippostrongylus brasiliensis was maintained by monthly passages in Lewis rats as previously described60. Infective (IL3) worms were provided by N. Harris (EPFL, Switzerland). IL3 larvae were treated for 15 min with streptomycin and penicillin (300 U ml−1; Thermo Fisher Scientific), gentamicin (1.5 mg ml−1; Sigma) and tetracyclin (30 μg ml−1; Sigma), washed with PBS and counted under a stereomicroscope. Mice were injected subcutaneously with 400 IL3 in 200 μl of sterile PBS using a 21G needle. Mice were killed at days 1, 2, 6 and 9 after infection and lungs, bronchoalveolar lavage (BAL) and small intestine were collected and analysed.

Infection burden. Lung and small intestine parasite burden was quantified in minced lungs and small intestine as previously described60. Briefly, lungs and small intestine were placed on sterile cheesecloth and suspended in a 50 ml tube containing PBS at 37 °C for at least 4 h. Viable worms that migrate out into the bottom of the tube were counted under a stereomicroscope (stereO Lumar V12; Zeiss).

Airway challenge. C57BL/6J mice were anaesthetized by injection of isoflurane. Mice were challenged with a single intranasal dose of Nippostrongylus brasiliensis secretory/secretory products (NES) (5 μg) (provided by N. Harris (EPFL)) or with PBS in a volume of 30 μl for 4 consecutive days. 24 h after the last challenge lung and BAL were recovered for analysis.

Differential of naive T helper cells. Enteric naive T helper cells were purified and cultured in an anti-CD3 (2 μg ml−1; 17A2; Biolegend) pre-coated plate. For T12 differentiation, cells were stimulated in the presence of soluble anti-CD28 (1 μg ml−1; Roche) and DNase I (20 μg ml−1; Affymetrix) in complete RPMI for 30 min at 37 °C, under gentle agitation. Enteric neurons and glial cells were isolated as previously described31,41. Briefly, isolated tissues were digested with Liberase TM (7.5 μg ml−1; Roche) and DNase I (20 μg ml−1; Affymetrix) in complete RPMI for 30 min at 37 °C, under gentle agitation. Digested organs were disrupted by passage through a 100-μm cell strainer (BD Biosciences). A 40–80% percoll gradient centrifugation was used for additional leukocyte purification from lung and small intestine cell suspensions. Erythrocytes from lung, small intestine and bone marrow preparations were lysed with RBC lysis buffer (eBioscience).
gated in live CD45^−Lin^−Thy1.2^− cells, unless stated otherwise. Sorted populations were >95% pure. Data analysis was done using FlowJo software (Tristar).

**Enteric neurosphere-derived neurons.** Enteric neurosphere-derived neurons were obtained as previously described. Briefly, total intestines from embryonic day 14.5 (E14.5) to E18.5 Miy88L/− mice and control embryos were digested with collagenase D (0.5 mg ml^−1; Roche) and DNase I (10 μg ml^−1; Affymetrix) in supplemented DMEM/F-12, GlutaMAX (1% hepes, penicillin and streptomycin and 0.1% 1-mercaptoethanol) (Gibco) for 1 h at 37 °C under gentle agitation. Cells were mechanically disrupted, washed and cultured in a non-nutrient media for 1 week in a CO_2_ incubator at 37 °C in supplemented DMEM/F-12, GlutaMAX with B27 (Gibco), EGF and FGF2 (20 ng ml^−1; R&D Systems). After neurosphere formation, cells were dissociated using NeuroCult (STEMCELL Technologies) chemical dissociation kit, according to the manufacturer's instructions. Dissociated neurospheres were plated on a 24-well plate previously coated with PDL (100 μg ml^−1; Sigma) and cultured in supplemented NEUROBASAL media (1% hepes, glucose, penicillin, and streptomycin) (Gibco) with B27 for neuronal differentiation. After 7 days, neuronal organoids were used either for activation or immunostaining. Enteric neurosphere-derived neurons were activated with LPS (5 μg ml^−1; Invivogen), recombint mouse IL-33 (100 ng ml^−1; R&D Systems) and NES (10 μg ml^−1), for 24 h followed by RNA extraction. For ILC2 activation experiments, conditioned media from NES activated enteric neurons was collected and applied overnight to purified ILC2s.

**Purification of human ILC2s and T helper cells.** For purification of human peripheral ILC2s and T helper cells, peripheral blood mononuclear cells (PBMCs) were isolated from human buffy coats by diluting the blood 1:2 in PBS FBS 2%. The blood was layered on top of Lymphoprep (STEMCELL Technologies) and centrifuged at 800×g for 30 min at room temperature without brake. Human PBMCs were then washed and stained with antibodies against human lineage markers namely, CD3 (GTX3), α/β-TCR (αβ-TCR) (B1.1), CD19 (HB19), CD14 (61D3), CD11c (3.9), CD16 (eBioCB16) (eBioscience); and CD45 (HI30), CD127 (A019D5), CD161 (HP-3G10) (Bioscience), CRT2H (BM16) (BD Biosciences) and CD4 (OKT4; Biolegend). Human ILC2s were defined as CD45^+ (A019D5), CD161 (HP-3G10) (Biolegend), CRTH2 (BM16) (BD Biosciences) and then counterstained for 5 min with DAPI. Samples were mounted in Mowiol (Covance). Neuronal organoids were sequentially incubated at room temperature in a PBS solution containing 0.3% collagenase D (0.5 mg ml^−1; Sigma) and 4% low-melting temperature agarose (Invitrogen). Samples were blocked and permeabilized at room temperature for 30 min with a PBS solution containing 0.3% Triton X-100 and 2% BSA and then incubated for 1–2 days at room temperature with the following antibodies: mouse monoclonal anti-KLRG1 (2F1; KLRG1; Biolegend); anti-CD3 (17A2; Biolegend) and anti-tubulin-3 (3; TUBB3) (TU1; Covance) for small intestine imaging and anti-Thy-1.2 (53-2.1; eBioscience), anti-CD45.1 (A20; Biolegend) and anti-TUBB3 (TU1; Covance) for lung imaging. Alexa Fluor 647 goat anti-hamster, Alexa Fluor 568 goat anti-rat and Alexa Fluor 633 goat anti-mouse (Invitrogen) were used as secondary antibodies overnight at room temperature. Distances between neurons and ILC2 were measured using Imaris software. For NEUFA2 imaging, ILC2s were sorted from small intestine of C57Bl/6 mice. ILC2s were FBS starved for 2 h and treated for 90 min with NMU23 (100 ng ml^−1). After stimulation, cells were plated in a pre-coated 0.01% of poly-l-lysine (Sigma) coverslip and let to adhere. Cells were fixed with 2% FPA for 30 min at room temperature. Coverslips were blocked and permeabilized at room temperature for 30 min with a PBS solution containing 0.3% Triton X-100 and 1% BSA. Cells were stained at room temperature for 150 min with mouse monoclonal anti-NFAT2 antibody (7A6; Abcam). ILC2s were incubated subsequently at room temperature in the dark for 30 min with Alexa Fluor 488 goat anti-mouse antibody (Invitrogen) and then counterstained for 5 min with DAPI. For quantification of NEUFA2 fluorescence intensity single-cell ILC2 nuclei were identified by DAPI staining. Regions of interest (ROIs) were defined from each cell nucleus and NFAT signal was measured using ImageJ software. Enteric neurosphere-derived neurons were fixed in 2% FPA for 30 min at room temperature. Cells were blocked and permeabilized at room temperature or 30 min with a PBS solution containing 0.3% Triton X-100 and 1% BSA. Cells were stained at room temperature for 150 min with mouse monoclonal anti-NFAT2 antibody (7A6; Abcam). ILC2s were incubated subsequently at room temperature in the dark for 30 min with Alexa Fluor 488 goat anti-mouse antibody (Invitrogen) and then counterstained for 5 min with DAPI. For quantification of NEUFA2 fluorescence intensity single-cell ILC2 nuclei were identified by DAPI staining. Regions of interest (ROIs) were defined from each cell nucleus and NFAT signal was measured using ImageJ software. Enteric neurosphere-derived neurons were fixed in 2% FPA for 30 min at room temperature. Cells were blocked and permeabilized at room temperature for 30 min. Differentiated neurons were stained at room temperature for 150 min with anti-TUBB3 (TU1; Covance). Neuronal organoids were sequentially incubated at room temperature in the dark for 30 min with Alexa Fluor 488 goat anti-mouse antibody (Invitrogen) and then counterstained for 5 min with DAPI. Samples were mounted in Mowiol and were acquired on a Zeiss LSM710 confocal microscope using Plan Apochromat 20 × 0.8 M27 objective and EC Plan-Neofluar 40 × /1.30 and Plan-Apochromat 63 × /1.4 Plan-Apochromat oil immersion (1.4) objectives.

**Statistics.** Results are shown as mean ± s.e.m. Statistical analysis was performed with GraphPad Prism software (GraphPad Software). Student's t-test was performed on homoscedastic populations. Unpaired t-test was applied on samples with different variances. Results were considered significant at *P* < 0.05, **P** < 0.01, ***P*** < 0.001.

**Data availability.** The data that support the findings of this study are available on request from the corresponding author (H.V.-F.). The data are not publicly available due to patent application request.
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Extended Data Figure 1 | Genome-wide ILC2 transcriptional profiling and neuron-ILC2 interactions. a, Weighted UniFrac PCoA analysis of ILC2s, Th cells, ILC1s and ILC3s. b, Levels of Nmur1 expression in ILC2s, Th cells, ILC1 and ILC3 populations. c, Nmur1 expression in lung ILC2s, eosinophils (Eo), mast cells (Mast), macrophages (Mø), neutrophils (Neu), dendritic cells (DC), helper T (Th), B cells (B) and glial cells (G) (n = 3). d, Nmur2 expression in intestinal ILC2s, eosinophils, mast cells, macrophages, neutrophils, dendritic cells, helper T, B cells, glial cells, neurons and brain (n = 3). e, Nmu expression in lung immune cell subsets (n = 3). f, Distance of T cells and ILC2 to adjacent enteric neurons. T cells (n = 22), ILC2 (n = 28). g, Confocal analysis of lung. Red, neurons (TUBB3); green, Thy1.2; blue, DAPI. Green arrows, candidate ILC2s; red arrow, neuron. Scale bar, 5 μm. h, Confocal analysis of lung. Red, neurons (Chat-Cre.Rosa26RFP); green, CD45.1; blue, DAPI. Green arrows, candidate ILC2s; red arrow, neuron. Scale bar, 5 μm. Error bars show s.e.m. *P < 0.05.
Extended Data Figure 2 | Neuromedin U is a potent regulator of innate type 2 cytokines, via NMUR1 activation. a, ILC2 and Th2 cells were activated with NmU23; ILC2s (n = 6), Th2 cells (n = 3). b, Proliferation (as measured by Ki67 expression) of gut ILC2s upon NmU23 activation in the presence or absence of IL-3 and IL-7 in vitro (n = 3). c, Percentage of Ki67 expression in enteric ILC2 upon NmU23 administration in vivo (n = 5). d, Dot plots representing Ki67 expression in gut ILC2 upon NmU23 administration in vivo. e, Lung innate type 2 cytokines after NmU23 in vitro stimulation (n = 3). f, Dot plots representing lung ILC2-derived type 2 cytokines after NmU23 in vitro activation. g, Enteric ILC2-derived type 2 cytokines upon NmU23 stimulation over different incubation times or PMA + ionomycin (P + I) activation for 4 h. h, Dot plots representing gut ILC2-derived type 2 cytokines upon NmU23 stimulation over different incubation time periods. Error bars show s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant.
NMU is a fast and potent inducer of ILC2 cytokines. 

**Extended Data Figure 3**

**a,** Dot plots representing enteric ILC2-derived type 2 cytokines upon activation with increasing concentrations (10, 50 and 100 ng ml$^{-1}$) of IL-33, IL-25 and NmU23 for 20 h.

**b,** Gut ILC2-derived cytokines after stimulation with increasing concentrations (10, 50 and 100 ng ml$^{-1}$) of IL-33, IL-25, NmU23 and PMA+ionomycin (P+I) for 4 h ($n=3$).

**c,** Lung ILC2- and TH-cell-derived type 2 cytokines after in vivo administration of NmU23 ($n=3$). Error bars show s.e.m. *$P<0.05$; **$P<0.01$; NS, not significant.

IL-33, IL-25, NmU23 and PMA+ionomycin (P+I) for 4 h ($n=3$). c, Lung ILC2- and TH-cell-derived type 2 cytokines after in vivo administration of NmU23 ($n=3$). Error bars show s.e.m. *$P<0.05$; **$P<0.01$; NS, not significant.
Extended Data Figure 4 | Activation of ILC2s by NMU and IL-25/IL-33 signals. a, Lung ILC2s from Il1rl1<sup>−/−</sup>Il17rb<sup>−/−</sup> (DKO) and their wild-type controls after NmU23 stimulation (n = 6). b, Type 2 cytokines in Nmur1 sufficient and deficient ILC2s after IL-33 and IL-25 (10 ng ml<sup>−1</sup>) activation for 24 h (n = 3). c, Intestinal ILC2-derived cytokines after NmU23 administration in Il1rl1<sup>−/−</sup>Il17rb<sup>−/−</sup> (DKO) and their wild-type controls. Left panel represents ILC2 percentage gated in total live cells (n = 5). d, Lung ILC2-derived cytokines in wild-type BALB/c and Il1rl1<sup>−/−</sup>Il17rb<sup>−/−</sup> bone marrow chimaeras upon NmU23 administration (n = 5). Error bars show s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant.
Extended Data Figure 5 | ILC2-autonomous Nmur1 signals.

a–d. ILC2-derived type 2 cytokines in Nmur1−/− and in their Nmur1+/+ wild-type littermate controls. a, Percentage of intestinal ILC2s and their signature cytokines. Nmur1−/− (n = 6), Nmur1+/+ (n = 9). b, Number of intestinal ILC2s and their signature cytokines. Nmur1−/− (n = 6); Nmur1+/+ (n = 9). c, Percentage of lung ILC2s and their signature cytokines. Nmur1−/− (n = 6), Nmur1+/+ (n = 9). d, Number of lung ILC2s and their signature cytokines. Nmur1−/− (n = 6), Nmur1+/+ (n = 9).

e–h. Competitive bone marrow chimaeras. 10⁶ cells of each genotype (CD45.2) were injected intravenously in direct competition with a third-party wild-type competitor (CD45.1/CD45.2), in a 1:1 ratio, into non-lethally irradiated (150 rad) NSG mice (CD45.1). e, Percentage and number of donor ILC2s in the intestine. Nmur1−/− (n = 8), Nmur1+/+ (n = 6). f, Percentage and number of donor ILC2s in the lung. Nmur1+/+ (n = 12), Nmur1+/− (n = 13). g, h, Bone marrow mixed chimaeras upon NmuU23 administration. g, Percentage of lung Th-cell-expressing type 2 cytokines. Nmur1−/− (n = 5), Nmur1+/− (n = 4). h, Number of lung Th-cell-expressing type 2 cytokines. Nmur1+/+ (n = 5), Nmur1+/− (n = 4).

Error bars show s.e.m. NS, not significant.
Extended Data Figure 6 | Calcineurin inhibition during NMU-dependent ILC2 activation. Intestinal ILC2 activation with NMU23. Il5, Il13 and Csf2 expression in ILC2s cultured with medium (control), NMU23 or NMU23 and calcineurin inhibitor cyclosporine (CsA) (n = 3). Error bars show s.e.m. ***P < 0.001.
Extended Data Figure 7 | Neuromedin U administration during worm infection. a–f, Mice were infected with N. brasiiliensis larvae and treated with NmU23. 

a, Nmur1 expression in lung ILC2 at day 6 after infection (n = 6). 
b, Nmur1 expression in lung immune populations (n = 3). 
c, Number of lung ILC2s at day 1 after infection in NmU23 treated and control animals (n = 5). 
d, Lung T helper cells at day 1 after infection in NmU23 treated and control animals (n = 5). 
e, Myeloperoxidase (MPO)- (granulocytes) and Luna-stained (eosinophils) lung sections at day 2 after infection. 
f, Lung granulocyte and eosinophilic cell counts (cells mm$^{-2}$) at day 2 after infection (n = 8). Scale bar, 50 μm. Error bars show s.e.m. 

*P < 0.05; **P < 0.01; NS, not significant.
Extended Data Figure 8 | Worm infection in Nmur1 deficient mice. Nmur1−/− and in their Nmur1+/+ wild-type littermate control mice were infected with *N. brasiliensis*. a, Number of lung ILC2s and their cytokines at day 6 after infection. Nmur1+/+(n = 6), Nmur1−/−(n = 8). b, Myeloperoxidase (MPO)- (granulocytes) and Luna-stained (eosinophils) lung sections at day 2 after infection. Scale bar, 50 μm. c, Lung granulocyte and eosinophil cell counts (cells mm−2) at day 2 after infection (n = 8). d, Worm infection burden at day 6 and 9 after infection in the small intestine of Nmur1 sufficient and deficient mice. Day 6 (n = 6), day 9 (n = 5). Error bars show s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant.
Extended Data Figure 9 | Secretory worm products induce ILC2-derived type 2 cytokines. **a**, ILC2-derived cytokines after stimulation with *Nippostrongylus brasiliensis* excretory/secretory products (NES) alone or with NES-activated neurosphere-derived enteric neurons conditioned media (SN NES). Control (*n* = 3), NES (*n* = 3), SN NES (*n* = 3).

**b**, Percentage and number of lung ILC2s and their signature cytokines after intranasal NES administration to wild-type mice. PBS (*n* = 5), NES (*n* = 5). Error bars show s.e.m. *P* < 0.05; **P** < 0.01; NS, not significant.
A novel neuron-ILC2 unit orchestrated by Neuromedin U. Mucosal neurons can directly sense worm products (NES) and the host alarmin (IL-33) to control neuromedin U expression. Neuromedin U activates ILC2s in a cell-autonomous and NMUR1 dependent manner, resulting in a fast and potent production of inflammatory and tissue repair cytokines that confer immediate protection to worm infection. Neuromedin U activates NMUR1 inducing type 2 cytokine expression downstream of ERK phosphorylation and activation of a Ca^{2+}–calcineurin–NFAT cascade. This model indicates that neuron-ILC2 cell units are poised to uniquely ensure potent and immediate type 2 responses in a neuromedin U-dependent manner.
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### Experimental design

1. **Sample size**
   - Describe how sample size was determined.
   - Power analysis was performed to estimate sample size (Methods section, page 15).

2. **Data exclusions**
   - Describe any data exclusions.
   - No data exclusions

3. **Replication**
   - Describe whether the experimental findings were reliably reproduced.
   - The experimental findings were reliably reproduced

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - No randomization was performed. Animals were compared with co-housed control litter-mate controls

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - Blinding was performed for experiments in Fig.4c,g; Extended Data Fig.7e,f; and Extended Data Fig.8b,c

   Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).
   - n/a Confirmed
      - The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
      - A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
      - A statement indicating how many times each experiment was replicated
      - The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
      - A description of any assumptions or corrections, such as an adjustment for multiple comparisons
      - The test results (e.g. p values) given as exact values whenever possible and with confidence intervals noted
      - A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
      - Clearly defined error bars

   See the web collection on statistics for biologists for further resources and guidance.

### Software

Policy information about availability of computer code

7. **Software**
   - Describe the software used to analyze the data in this study.
   - Results are shown as mean ± s.e.m. Statistical analysis was performed with GraphPad Prism software (GraphPad Software, version 6, La Jolla, Calif).
Student's t-test was performed on homoscedastic populations. Unpaired t-test was applied on samples with different variances. Results were considered significant at *P<0.05, **P<0.01, ***P<0.001. Flow cytometry data was analysed using FlowJo (Tristar). Bioimaging data was analysed with ImageJ or Imaris software.

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The Nature Methods guidance for providing algorithms and software for publication may be useful for any submission.

### Materials and reagents

#### Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

| Not applicable |

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

| Cell suspensions were stained with anti-CD45 (30-F11), anti-TER119 (TER-119), TCRβ (H57-597), anti-CD3e (eBio500A2), anti-CD19 (eBio1D3), anti-NK1.1 (PK136), anti-CD11c (N418), anti-Gr1 (eBio500A2), anti-CD11b (Mi/70), anti-CD127 (IL-7Ra; A7R34), anti-α4β7 (DATK32), anti-Flt3 (A2F10), anti-CD25 (PC61.5), anti-c-Kit (2B8), anti-Thy1.2 (53-2.1), anti-CD49b (DX5), anti-TCRγδ (GL3), anti-Nkp46 (29A1.4), anti-CD4 (GK1.5), anti-CD31 (390), anti-IL-13 (eBio13A), anti-F4/80 (BM8), anti-FcεR1 (MAR-1), anti-CD44 (IM7), anti-CD62L (MEL-14), 7AAD viability dye, anti-CD16/CD32 (93) from eBioscience; anti-CD8α (53-6.7), anti-KLRG1 (2F1/KLRG1), anti-Scal (D7), anti-CCR3 (J073E), anti-MHC-II (M5/114.15.2), anti-Ki67 (16A8), anti-streptavidin and anti-CD326 (G8.8) from Biolegend, anti-IL-5 (MH9A3) from BD Biosciences, anti-amphiregulin (R&D Systems). LIVE/DEAD Fixable Aqua Dead Cell Stain Kit was purchased from Invitrogen. All the reagents were optimized and validated (i.e assay and species) by the companies. |

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

| Not applicable |

b. Describe the method of cell line authentication used.

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c. Report whether the cell lines were tested for mycoplasma contamination.

| Not applicable |

d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

| Not applicable |

### Animals and human research participants

#### Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

| C57BL/6j (B6) mice were purchased from Charles River. Nod/Scid/Gamma (NSG) mice were bought from The Jackson Laboratory. Sperm from the strain C57BL/6-Nmur1tm1(1KOMP)Vlcg, which contains a Nmur1 deletion, was obtained from the KOMP Repository, University of California Davis and Children’s Hospital Oakland Research Institute, US. Nmur1-/- mice were generated by in vitro fertilisation at the Champalimaud Centre for the Unknown, Portugal. Chat-Cre, Rosa26RFP, Rag2-/-; Il2rg-/-, Myd88-/- and RetGFP and Myd88fl/fl mice were on a C57BL/6J background. Mice were bred and maintained at the Champalimaud Centre for the Unknown and iMM Lisboa animal facilities under specific pathogen free conditions (SPF). Il1rl1-/-; Il17rb-/- mice and their WT controls were on a BALB/c background and were bred and maintained at École Polytechnique Fédérale de Lausanne (EPFL), Switzerland. Mice were systematically compared with co-housed littermate controls unless stated otherwise. Both males and females between (7-12 weeks) were used in this study. |

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The Nature Methods guidance for providing algorithms and software for publication may be useful for any submission.
Policy information about studies involving human research participants

12. Description of human research participants
Describe the covariate-relevant population characteristics of the human research participants.

Not applicable
Flow Cytometry Reporting Summary

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Data presentation

For all flow cytometry data, confirm that:

1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
3. All plots are contour plots with outliers or pseudocolor plots.
4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.

Lungs were perfused with a solution of cold PBS with 2% heparin through the right ventricle of the heart and were subsequently finely minced and digested in complete RPMI supplemented with collagenase D (0.1mg/mL; Roche) and DNase I (20U/mL; Affymetrix) for 1h at 37°C under gentle agitation. For isolation of small intestine lamina propria cells, intestines were thoroughly rinsed with PBS, cut in 1cm pieces, and shaken for 30 minutes in PBS containing 2% FBS, 1% hepes and 5mM EDTA to remove intraepithelial and epithelial cells. Intestines were then digested with collagenase D (0.5mg/mL; Roche) and DNase I (20U/mL; Affymetrix) in complete RPMI for 30 minutes at 37°C, under gentle agitation. Enteric neurons and glial cells were isolated as previously described3,41. Briefly, isolated tissues were digested with Liberase TM (7.5μg/mL; Roche) and DNase I (20U/mL; Affymetrix) in complete RPMI for 30 minutes at 37°C, under gentle agitation. Digested organs were disrupted by passage through a 100μm cell strainer (BD Biosciences). A 40-80% percoll gradient centrifugation was used for additional leukocyte purification from lung and small intestine cell suspensions. Erythrocytes from lung, small intestine and bone marrow preparations were lysed with RBC lysis buffer (eBioscience).

6. Identify the instrument used for data collection.

LSRFortessa, FACSAria and FACSFusion (BD Biosciences) flow cytometers.

7. Describe the software used to collect and analyze the flow cytometry data.

Data analysis was done using FlowJo software (Tristar).

8. Describe the abundance of the relevant cell populations within post-sort fractions.

Sorted populations were >95% pure.

9. Describe the gating strategy used.

Cell populations were defined as: Common Lymphoid Progenitor (CLP) - Lin−CD127+Flt3+Sca1int–Kitint; Common Helper Innate Lymphoid Progenitor (CHILP) - Lin−CD127+α4β7+Flt3−CD25−; ILC2 precursor (ILC2P) - Lin−CD127+α4β7+Flt3−CD25+; ILC2 - CD45+Lin−
Thy1.2+KLRG1+Sca1+; ILC1 - CD45+Lin-NKp46+NK1.1+CD49b-CD127+; ILC3 - CD45+Lin-Thy1.2hiKLRG1-; for ILC3 subsets additional markers were employed: ILC3 CD4+ - NKp46-CD4+; ILC3 NCR - NKp46-CD4-; ILC3 NCR+ - NKp46+CD4+; NK cells - CD45+Lin-NKp46+NK1.1+CD49b+CD127-; Lineage was composed by CD3ε, CD8α, TCRβ, TCRγδ, CD19, Gr1, CD11c and TER119; Eosinophils (Eo) - MHC-II-CCR3hiGR1int; Mast cells (Mast) - CD3-FcεR1+; Macrophages (MØ) - CD3-MHC-II+F4/80+; Neutrophils (Neu) - MHC-II-CCR3-GR1hi; Dendritic cells (DC) – CD45+MHC-II+F4/80-CD11c+; T cells - CD45+CD3+TCRβ+; T helper (Th) cells - CD45+CD3+TCRβ+CD4-; Th naïve (Thn) cells - CD45+CD3+CD4+CD44loCD62Lhi; memory Th (Thm) cells - CD45+CD3+CD4+CD44hiCD62Llo; B cells - CD45+CD19+; enteric glial cells (G) - CD45-CD31-TER119-CD49b+; enteric neurons (N) - CD45-CD31-TER119-RETGFP+/; Epithelial cells (Ep) – CD45-CD31-CD326+. Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.