The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation

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The type 2 cytokines interleukin (IL)-4, IL-5, IL-9 and IL-13 have important roles in stimulating innate and adaptive immune responses that are required for resistance to helminth infection, promotion of allergic inflammation, metabolic homeostasis and tissue repair1–3. Group 2 innate lymphoid cells (ILC2s) produce type 2 cytokines, and although advances have been made in understanding the cytokine milieu that promotes ILC2 responses4–9, how ILC2 responses are regulated by other stimuli remains poorly understood. Here we demonstrate that ILC2s in the mouse gastrointestinal tract co-localize with cholinergic neurons that express the neuropeptide neuromedin U (NMU)10,11. In contrast to other haematopoietic cells, ILC2s selectively express neurons that express the neuropeptide neuromedin U (NMU)10,11. In vitro stimulation of ILC2s with NMU induced rapid cell activation, proliferation, and secretion of the type 2 cytokines IL-5, IL-9 and IL-13 that was dependent on cell-intrinsic expression of NMUR1 and Gαq protein. In vivo administration of NMU triggered potent type 2 cytokine responses characterized by ILC2 activation, proliferation and eosinophil recruitment that was associated with accelerated expulsion of the gastrointestinal nematode *Nippostrongylus brasiliensis* or induction of lung inflammation. Conversely, worm burden was higher in *Nmur1*–/– mice than in control mice. Furthermore, use of gene-deficient mice and adoptive cell transfer experiments revealed that ILC2s were necessary and sufficient to mount NMU-elicited type 2 cytokine responses. Together, these data indicate that the NMU–NMUR1 neuronal signalling circuit provides a selective mechanism through which the enteric nervous system and innate immune system integrate to promote rapid type 2 cytokine responses that can induce anti-microbial, inflammatory and tissue-protective type 2 responses at mucosal sites.

In addition to being populated by cells of the innate and adaptive immune systems, mucosal sites are heavily innervated12,13. Immunofluorescence staining of the neuronal marker SNAP-25, the ILC2 marker KLRG114, and CD3ε revealed that ILC2s and T cells were closely associated with SNAP-25+ neurons in the intestine (Fig. 1a). Surface reconstruction suggested that neurons and ILC2s form a network of neuromedin-U-expressing neurons co-localizes with NMUR1+ ILC2s. a, Immunofluorescence staining of the intestinal submucosa. Scale bar, 20 μm. b, RNA-seq volcano plot of differential expression between ILC2s (positive log(fold change (FC))) and ILC3s (negative log(FC)). Genes belonging to the KEGG pathway ‘neuroactive-receptor-ligand interaction’ (nmur04080) are shown in red and are enriched among the genes differentially expressed in ILC2s (corrected P < 0.01). c, Heatmap showing expression Z-scores of the indicated genes in small intestine ILC2s and ILC3s, as measured by RNA-seq. For Nmur2, Z-scores of 0 indicate read counts of zero in all samples. d, Expression of Nmur1 and Nmur2 in the indicated sorted lymphocyte populations as determined by qPCR analysis (n = 3). ILC2p, ILC2 progenitor; n.d., not detectable; SI, small intestine. e, Expression of NMUR1 in the indicated sorted lymphocyte populations from human intestine as determined by qPCR analysis (n = 7). f, Dot plots show expression of Nmur1 as measured by conversion of the fluorescent LacZ substrate fluorescein di-β-D-galactopyranoside (FDG). Lineage 1: CD11b, CD11c and B220 (all APC-eF780); lineage 2: CD3, CD5 (both PerCP-Cy5.5) and FcεRI PerCP-eF710. g, CLARITY staining of the small intestine. h, Immunofluorescence staining of the intestinal muscularis mucosae. i, Immunofluorescence staining of the intestinal submucosa. Scale bar, 100 μm (g, h), 50 μm (i). Error bars, mean ± s.d. Data in a and f–i are representative of three independent experiments with similar results. Data are based on three (b–d) or seven (e) biological replicates per group.

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close inter-cellular contacts (Extended Data Fig. 1). To test whether neuron-derived signals might be sensed by ILC2s, we performed RNA sequencing (RNA-seq) and KEGG pathway analysis of ILC subsets. Multiple genes associated with neuroactive receptor–ligand interactions were differentially expressed between ILC2s and ILC3s (Fig. 1b). The most differentially expressed gene in ILC2s in this category was \textit{Nmur1}, a receptor for the neuropeptide NMU, which has previously been reported to provoke anorectic effects in the central nervous system and promote cutaneous inflammation\textsuperscript{10,11,15,16}. We compared \textit{Nmur1} expression to several highly differentially expressed genes found in ILC3s (Rorc, Il17f, Il1r1 and Il22) or in ILC2s (Gata3 and Il1r1). \textit{Nmur1} expression segregated in ILC2s to a comparable degree to that noted for other ILC2-associated genes (Fig. 1c). The selective expression of \textit{Nmur1} in ILC2s, but not in other innate and adaptive lymphocyte lineages or myeloid cells, was confirmed by quantitative PCR (qPCR) analysis (Fig. 1d, Extended Data Fig. 2a). In contrast, a second receptor for NMU (\textit{Nmur2}), which is predominantly expressed in the central nervous system\textsuperscript{10,11}, was not detected in any of the immune cell populations examined (Fig. 1c, d). Expression of \textit{NMUR1} was also detectable in human intestinal ILCs, but not in B cells, suggesting that human ILCs sense neuronal signals via \textit{NMUR1} (Fig. 1e).

Analysis of NMUR1 protein expression using a LacZ reporter revealed that 2% of all CD45\(^{+}\) cells in the small intestine were NMUR1\(^{\text{+}}\) \textit{Nmur1}mice but not in control \textit{Nmur1}mice that lacked the reporter (Fig. 1f). Analysis of \textit{NMUR1} cells revealed that 97% of NMUR1\(^{+}\) cells were negative for cell lineage markers such as CD3, CD5, FcRI, CD11b, CD11c and B220, but expressed the ILC2 marker CD127 and KLRG1 (Fig. 1f). Consistent with mRNA expression, ILC2s expressed LacZ under the control of the \textit{Nmur1} promoter, whereas no expression was detected in B cells, T cells, ILC1s, ILC3s, eosinophils, mast cells, macrophages and basophils (Extended Data Fig. 2b–g). Thus, \textit{Nmur1} is selectively expressed in ILC2s.

The ligand of NMUR1 is the 23-amino-acid-long neuropeptide NMU\textsuperscript{10,11}. \textit{Nm} was not detectable by qPCR in the epithelial or lamina propria fractions of the small intestine isolated from mouse or human specimens but was expressed in the parenchymal tissue of the small intestine, suggesting that haematopoietic cells do not express NMU (Extended Data Fig. 2h, i). Immunofluorescence staining of intestinal tissue or use of \textit{NmuGFP} reporter mice revealed a network of NMUR1 neurons including the plexus myentericus, plexus submucosus and nerve fibres that are located below the tips of the villi (Fig. 1g, Extended Data Fig. 2j–l). Notably, NMU was expressed in cholinergic (marked by choline acetyltransferase (ChAT) expression) but not in catecholaminergic neurons (marked by tyrosine hydroxylase (TH) expression) (Fig. 1h, Extended Data Fig. 2m). NMU\(^{+}\) neurons co-localized with ILC2s, and 68% of ILC2s had overlapping pixels with neurons (Fig. 1i, Extended Data Fig. 3a, b). Further, co-culture of ILC2s with enteric neurons that expressed NMU led to increased proliferation and blastic of ILC2s, suggesting that enteric nerve-derived bioactive factors directly activate ILC2s (Extended Data Fig. 3c–e). Together, these data indicate that NMUR1 is specifically expressed by ILC2s, which co-localize with NMU\(^{+}\) cholinergic neurons in the intestine and that ILC2s might sense nerve-derived signals.

To test whether NMU stimulates ILC2s, we isolated lamina propria lymphocytes (LPLs) from IL-13 reporter mice\textsuperscript{17} and incubated them \textit{in vitro} with or without NMU. NMU potently activated ILC2s as measured by IL-13 expression (Fig. 2a, b; for gating see Extended Data Fig. 4a). We confirmed the capacity of NMU to induce IL-5 and IL-13 production by intracellular cytokine staining (Fig. 2c, d) and ELISA assays (Extended Data Fig. 4b, c). Further, a comparison of stimulation of ILC2s with NMU versus combinations of IL-2, IL-7, IL-25 and IL-33 or PMA and ionomycin (all stimuli known to stimulate ILC2s\textsuperscript{14,18–20}), revealed that NMU-induced expression of IL-5 and IL-13 in ILC2s was stronger than the effects of cytokines previously known to activate ILC2s. Indeed, only a combination of IL-2, IL-7, IL-25 and IL-33 or PMA and ionomycin stimulation resulted in comparable ILC2 activation as NMU stimulation (Fig. 2e, f). As NMU could trigger potent cytokine production from ILC2s lacking IL-33 responsiveness, we conclude that NMU can activate ILC2s independently of IL-33 (Extended Data Fig. 4d).

In order to investigate whether NMU activation of ILC2s requires NMUR1, we examined \textit{Nmur1}−/− mice. ILC2s developed in comparable proportions in \textit{Nmur1}+/− mice and did not exhibit obvious defects at steady-state (Extended Data Fig. 4e, f). However, only ILC2s from \textit{Nmur1}−/− but not from \textit{Nmur1}+/− mice were activated by NMU to produce type 2 cytokines (Fig. 2g). In contrast, ILC2s from both genotypes could be activated by PMA and ionomycin, demonstrating that ILC2s from \textit{Nmur1}−/− were responsive to stimulation but had a specific defect in the NMUR1 signalling pathway (Fig. 2g). In addition, chemical inhibition of G\textsubscript{q} family of proteins, which mediate NMUR1 signal transduction\textsuperscript{21}, completely abolished activation of ILC2s but had only modest effects on ILC2 stimulation with IL-2, IL-7, IL-25 and IL-33 (Fig. 2h, Extended Data Fig. 4g). Thus, NMU appears to trigger a NMUR1- and G\textsubscript{q}-dependent signalling pathway in ILC2s.

To test whether the stimulation of NMUR1 signalling by NMU is ILC2-intrinsic, we isolated LPLs from CD45.1:\textit{Nmur1}+/+ CD45.2:\textit{Nmur1}−/− mixed bone marrow chimaeras and stimulated...
NMU stimulates ILC2s in vivo and promotes worm expulsion. a–c, RNA was extracted from sort-purified ILC2s one day after PBS or NMU administration and sequenced (n = 3). Principal component (PC) analysis. Ellipses show, for each group, the curve at which the fitted bivariate normal distribution equals 0.68 (a). Heatmap showing level of significance of GO enrichment tests, as measured by −log10(Pcorrection). Blue, not significant (Pcorrected > 0.01) (b). Heatmap showing expression Z-scores of selected differentially expressed genes between PBS- or NMU-treated mice (c). d–f, PBS or NMU was injected daily for two days in C57BL/6 mice (d, e) or in CD45.1:Nmur1+/−/CD45.2:Nmur1−/− chimaeiras (f). One day later, ILC2s from the small intestine were analysed by flow cytometry for KLRG1 and Ki67 expression. Plots are gated on Lin−CD45+CD25−CD127−ILC2+ cells (%). Histogram overlay for IL-13 (g) is gated on Lin−CD45+CD25+KLRG1+ lymphocytes (h). FMI of KLRG1 expression (g). Graph representation for ILC-13 (h) is gated on Lin−CD45+CD127+CD25+KLRG1+ lymphocytes and percentage (n = 3) of IL-13 YFP+ KLRG1+ cells among all KLRG1+ cells (i). Worm burden (n = 8) of NMU-constituted Rag2−/−Il2rg−/− mice (j) and percentage of KLRG1+ST2+ cells was measured by flow cytometry in the lung (s). t–v, Worm burden (Nmur1+/−, n = 7 and Nmur1−/−, n = 8) of ILC2-reconstituted Rag2−/−Il2rg−/− mice (t) and percentage of KLRG1+ST2+ cells (u) or eosinophils (v) was measured by flow cytometry in the lung. Error bars, mean ± s.d. Data are representative of two (f, q) or three independent experiments (d, e, g–o) with similar results. Data are pooled from two (r–v) or three (p) independent experiments. RNA-seq data in a–c are based on three biological replicates per group.

Processes related to cell cycle regulation and cell division (Fig. 3b), including expression of the cell-cycle-associated genes Kntc1, Chafa1a, Cdc6, Spag5, Brcal, Mki67 and Ccda3 (Fig. 3c). Flow cytometric analysis for Ki67, the protein encoded by Mki67, demonstrated that NMU administration resulted in significantly increased proliferation of ILC2s, but not of ILC3s (Fig. 3d, e), Extended Data Fig. 5a). Injection of NMU into CD45.1:Nmur1+/−/CD45.2:Nmur1−/− mixed bone marrow chimaeras revealed an ILC2-intrinsic requirement for Nmur1 to respond to NMU in vivo (Fig. 3f, Extended Data Fig. 5b–d). In addition, in vivo delivery of NMU induced blasting (Extended Data Fig. 5e) and maturation (Fig. 3g) of ILC2s that was associated with prototypic type 2 inflammation marked by production of IL-13 (Fig. 3h, i) and goblet cell hyperplasia (Fig. 3j, k).

The intestine is highly innervated by a network of NMU+ neurons (Fig. 1i), we investigated the role of NMU in immunity to the helminth...
parasite Nippostrongylus brasiliensis, an intestinal parasite infection model, in which immunity is critically dependent on activation of ILC2s\textsuperscript{17,22,23}. Consistent with a role for NMU in regulating ILC2 responses during helminth infection, NMU expression was upregulated following infection with *N. brasiliensis* and was a conserved response following exposure to the related nematode parasites *Trichuris muris* and *Heligmosomooides polygyrus* (Fig. 3l, Extended Data Fig. 5f, g). As NMUR1 is specifically expressed on ILC2s at steady state (Fig. 1d, f, Extended Data Fig. 2b–g), we tested whether NMUR1 is induced on other cell types following exposure to *N. brasiliensis* infection. Although increased Nmur1 expression was detected on ILC2s (Extended Data Fig. 5h), Nmur1 expression was still specific to ILC2s and not detected on sizable proportions of other immune cell populations including mast cells, basophils, macrophages, T cells or IL-4-producing T\textsuperscript{H}2 cells, indicating that NMU is not acting directly on other hematopoietic cells (Fig. 3m, Extended Data Fig. 5i–l). However, in order to rule out that some of the effects of NMU in vivo require mast cells or eosinophils, in which Nmur1 was detected in previous publications\textsuperscript{15,24}, we injected NMU in mast-cell-deficient or eosinophil-deficient mice. Consistent with our Nmur1 expression results, NMU elicited ILC2 activation in mast-cell-deficient Cpa\textsuperscript{−/−} and eosinophil-deficient ΔdblGATA1 mice, demonstrating that ILC2 activation by NMU is independent of eosinophils and mast cells in vivo (Extended Data Fig. 5m–o).

Delivery of NMU during *N. brasiliensis* infection was associated with elevated eosinophilia and accelerated worm expulsion (Fig. 3n, o). *N. brasiliensis*-infected Nmur1-deficient mice and Nmur1\textsuperscript{−/−} bone marrow chimaeras exhibited elevated worm burden and reduced ILC2 responses (Fig. 3p, q, Extended Data Fig. 5p). To test whether there is a cell-intrinsic role for NMUR1 on ILC2s, we reconstituted Rag2\textsuperscript{−/−}Il2rg\textsuperscript{−/−} mice with ILC2 progenitors derived from either Nmur1\textsuperscript{+/+} or Nmur1\textsuperscript{−/−} mice. Accelerated worm expulsion and increased ILC2 numbers were observed in recipients reconstituted with Nmur1\textsuperscript{+/+} ILC2 progenitors compared to those reconstituted with Nmur1\textsuperscript{−/−} ILC2 progenitors (Fig. 3r, s). Administration of NMU to Rag2\textsuperscript{−/−}Il2rg\textsuperscript{−/−} mice reconstituted with ILC2 progenitors derived from either Nmur1\textsuperscript{+/+} or Nmur1\textsuperscript{−/−} mice resulted in enhanced worm expulsion and type 2 response in mice reconstituted with Nmur1\textsuperscript{+/+} precursors, indicating that NMU stimulates ILC2s in vivo and promotes ILC2-dependent immunity to infection (Fig. 3t–v, Extended Data Fig. 5q).

To investigate whether NMU can influence ILC2 responses at other mucosal barriers, we delivered NMU intranasally. NMU administration stimulated maturation, cytokine expression and proliferation of lung ILC2s (Fig. 4a–c, Extended Data Fig. 6a). Associated with this, NMU administration resulted in increased eosinophilia in bronchoalveolar lavage (BAL) and lung tissue and enhanced mucus production compared to PBS-treated control animals (Fig. 4d, e, Extended Data Fig. 6b). To directly test whether NMU-induced lung inflammation is dependent on ILC2s, NMU was administered to Rag2\textsuperscript{−/−} versus ILC2-deficient Rag2\textsuperscript{−/−}Il2rg\textsuperscript{−/−} mice. NMU administration to Rag2\textsuperscript{−/−} mice resulted in robust ILC2 activation, eosinophil recruitment and airway inflammation compared to PBS-treated Rag2\textsuperscript{−/−} mice, whereas no recruitment of eosinophils was observed in Rag2\textsuperscript{−/−}Il2rg\textsuperscript{−/−} mice (Fig. 4f, g, Extended Data Fig. 6c). Reconstitution of Rag2\textsuperscript{−/−}Il2rg\textsuperscript{−/−} mice with ILC2 progenitors restored ILC2 populations in the lung (Extended Data Fig. 6d) and delivery of NMU resulted in robust ILC2 proliferation and eosinophilia that was absent in ILC2-deficient recipients (Fig. 4h, i). Lastly, delivery of NMU to Nmur1\textsuperscript{+/+} or Nmur1\textsuperscript{−/−} mice demonstrated the requirement for NMUR1 in eliciting NMU-dependent ILC2 responses in vivo (Fig. 4j, k, Extended Data Fig. 6e).

Taken together, these findings elaborate a previously unrecognized pathway that enables neuronal–immune system cross-talk and identify a role for cholinergic neuron-derived NMU in promoting activation, proliferation and effector functions of ILC2s at mucosal sites. The discovery that NMU can promote ILC2 responses and accelerate type 2 inflammation suggests that beyond their capacity to respond to cytokines and alarmins, ILC2s may have selectively co-evolved with cholinergic neurons. NMU is a conserved response to infectious and foreign stimuli at barrier surfaces. We propose that cholinergic enteric neuron–derived neuromedin U functions as a neuronal amplifier that primes ILC2s for rapid and optimal activation by promoting their entry into the cell cycle or their responsiveness to other host-derived or environmental stimuli, which are essential activation and/or survival factors for ILC2s. Moreover, the appreciation that the immediate response capability of the nervous system can elicit a short, rapid and selective activation of ILC2s provides new insights into the pathophysiology of innate type 2 cytokine responses associated with exposure to multiple infectious agents, environmental allergens and pollutants at mucosal sites.

\[e^{\text{NMU}}\text{Induces ILC2-dependent lung inflammation. a–c, PBS or NMU was intranasally administered to Il13\textsuperscript{−/−}\text{-a, b) or C57BL/6 (c) mice daily for four days. One day later, ILC2s from the lung were analysed by flow cytometry. Mean fluorescence intensity (n = 3) of KLRG1 expression (a). Percentage (n = 3) of IL-13 YFP* ILC2s (b). Percentage (n = 5) of Ki67\textsuperscript{+} KLRG1\textsuperscript{+} cells (c, d, e, PBS or NMU was delivered intranasally to C57BL/6 mice daily for five days and lung infiltration was examined three days later. Percentage (n = 5) of CD11b\textsuperscript{+} SiglecF\textsuperscript{+} CD11c\textsuperscript{−} eosinophils in the lung (d). PAS staining of lung sections (scale bars, 100 μm (left), 50 μm (right)). Electronically magnified images from the left are shown on the right (e, f–k). PBS or NMU was intranasally administered to Rag2\textsuperscript{−/−} or Rag2\textsuperscript{−/−}Il2rg\textsuperscript{−/−} mice (f, g). ILC2-reconstituted Rag2\textsuperscript{−/−}Il2rg\textsuperscript{−/−} mice (h, i), or Nmur1\textsuperscript{+/+} or Nmur1\textsuperscript{−/−} mice (j, k) for four days. One day later, ILC2s and eosinophils from the lung were analysed by flow cytometry. Percentage (n = 4) (Rag2\textsuperscript{−/−}) or (Rag2\textsuperscript{−/−}Il2rg\textsuperscript{−/−}) (f, g), n = 4 (Rag2\textsuperscript{−/−}Il2rg\textsuperscript{−/−}), n = 5 (Rag2\textsuperscript{−/−}Il2rg\textsuperscript{−/−} + ILC2p) (h, i), n = 3 (PBS), n = 5 (NMU) n = 4 (NMU Nmur1\textsuperscript{−/−}) (j, k) of Ki67\textsuperscript{+} KLRG1\textsuperscript{+} cells (f, h, j) or percentage of CD11b\textsuperscript{+} SiglecF\textsuperscript{+} CD11c\textsuperscript{−} eosinophils (g, i, k) (n.a., not applicable). Error bars, mean + s.d. Data are representative of two (h–k) or three independent (a–g), experiments with similar results.\]
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METHODS

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

Mouse strains. C57BL/6 mice and CD45.1 mice on C57BL/6 background (B6.SJ-Pprrca Pepcb/Boy) were purchased from The Jackson Laboratory. Rag2−/− and Rag2−/−Il2g−/− on a C57BL/6 background were from Taconic Farms. Chat−/− (ref. 25), ChatCre−/− (ref. 26), AID−/− (ref. 27), Il2rg−/− (ref. 28) on a C57BL/6 background and II10−/− (ref. 17), Il1rl1−/− (ref. 29) and ΔβiGATA1A1 (ref. 30) on a BALB/c background were originally from The Jackson Laboratory. These strains, together with Cpa3loxP−/− (ref. 31), Il1r1−/− (ref. 32) and the strains mentioned below, were bred at Weill Cornell Medicine. Nmur1LacZ+/− (Nmur1tm1.KOMP)Vlcg were generated by Velocigene using C57BL/6 embryonic stem cells in which the Nmur1 gene was replaced by LacZ and a floxed neomycin cassette. The neomycin cassette was removed by crossing the mice to Cre deleter mice. Breeding of Nmur1LacZ+/− to homozygosity resulted in Nmur1−/− deficient (Nmur1lox/loxlox) mice referred to as Nmur1−/−. Nmur2GFP were generated by Gensat and provided on a Swiss Webster background. Sex- and age-matched animals between 6 and 16 weeks of age were used for experiments if not otherwise indicated. We did not use randomization to assign animals to experimental groups. Investigators were not blinded to group allocation during experiments. No animals were excluded from the analysis unless clearly indicated. All animal experiments were approved and are in accordance with the Institutional Animal Care and Use Committee guidelines at Weill Cornell Medicine.

Isolation of cells from the lamina propria and the lung. Small intestine was removed, cleaned from remaining fat tissue and washed in ice-cold PBS (Corning). Peyers' patches were identified and eliminated. Small intestine was opened longitudinally and washed in ice-cold PBS. Dissection of epithelial cells was performed by incubation on a shaking at 37 °C in HBSS (Sigma-Aldrich) containing 10 mM Hepes and 5 mM EDTA (both Thermo Fisher Scientific) or 1 mM DTT (Sigma-Aldrich) two times for 15 min. After each step, samples were vortexed and the epithelial fraction discarded. Afterwards, remaining tissue was chopped into small pieces and enzymatic digestion was performed using dispase (0.4 U/ml; Thermo Fisher Scientific), collagenase III (1 mg/ml; Worthington) and DNase (20 μg/ml; Sigma-Aldrich). Leukocytes were further enriched by a 40%/80% Percoll gradient centrifugation (Sigma-Aldrich).

One PBS-perfused lung lobe was minced and incubated using same conditions and enzymatic cocktail as for the intestine for 45 min at 37 °C. Afterwards lung was vortexed, mashed through a 70-μm cell strainer and purified over a 40%/80% Percoll gradient. Mesenteric lymph nodes were chopped and incubated in RPMI medium supplemented with 1% BSA (Sigma-Aldrich), DNase (20 μg/ml) and collagenase II (1 mg/ml; Sigma-Aldrich) for 20 min at 37 °C. Specimens were mechanically dissociated and filtered through a 70-μm cell strainer.

Flow cytometry and cell sorting. After saturating the Fc-receptors with CD16/ CD32-blocking antibody (Biolegend), single-cell suspensions were incubated on ice with conjugated antibodies in PBS (Ca2+ and Mg2+-free). Dead cells were routinely excluded with Fixable Aqua Dead Cell Stain or SYTOX Blue Dead Cell Stain (Thermo Fisher Scientific). Lineage-positive cells were excluded by staining CD32-blocking antibody (Biolegend), single-cell suspensions were incubated with Flow cytometry and cell sorting.

μPercoll gradient. One PBS-perfused lung lobe was minced and incubated using same conditions and enzymatic cocktail as for the intestine for 45 min at 37 °C. Afterwards lung was removed and cleaned in PBS, after which RNA was extracted for qPCR. For H. polygyrus, 250 infective L3 larvae were administered by oral gavage. At day 18, a piece from the proximal colon was removed and cleaned in PBS, after which RNA was extracted for qPCR.

RNA-seq analysis. For the RNA-seq data presented in Fig. 1, ILC2 (Lin−CD45+CD90+CD127+KLRG1−) and ILC3 (Lin−CD45+CD90+CD127+CCR6+) lymphocytes were sort-purified from the small intestine of C57BL/6 mice. For the RNA-seq data presented in Fig. 3, PBS or NMU (20 μg, Phoenix Pharmaceuticals) was injected i.p. on day 2, 4 and 6 of infection. At day 7 of infection, mice were killed and analyzed if not otherwise indicated. For T. muris, infection with 200 embryonated eggs was administered by oral gavage. At day 18, a piece from the duodenum was removed and cleaned in PBS, after which RNA was extracted for qPCR.

μnmuGFP mice were generated by Gensat and provided on a Swiss Webster background. Sex- and age-matched animals between 6 and 16 weeks of age were used for experiments if not otherwise indicated. We did not use randomization to assign animals to experimental groups. Investigators were not blinded to group allocation during experiments. No animals were excluded from the analysis unless clearly indicated. All animal experiments were approved and are in accordance with the Institutional Animal Care and Use Committee guidelines at Weill Cornell Medicine.

In vivo stimulation and ELISA. Bulk LPLs or sort-purified ILC2s were incubated in DMEM with high glucose supplemented with 10% FCS, 10 mM Hepes, 1 mM sodium pyruvate, non-essential amino acids, 80 μM 2-mercaptoethanol, 2 mM glutamine, 100 μM penicillin and 100 μg/ml streptomycin (both from Gibco) in 96-well microtiter plates (Corning) for 4 h at 37 °C and 5% CO2. Neuromedin U-23 (Phoenix Pharmaceuticals or Alpha Diagnostics) or a control peptide (Alpha Diagnostics) were added at 0.1 (Fig. 2e, f, h), 1 (Fig. 2a–d, g) or 10 μg/ml (Fig. 2i–m) if not otherwise indicated. If indicated, the culture was supplemented with phorbol 12-myristate 13-acetate (PMA, 1 μg/ml) and ionomycin (Sigma-Aldrich, 1 μg/ml). IL-2, IL-7, IL-33 (R&D, 100 ng/ml) and/or IL-25 (ebiScience, 100 ng/ml). The inhibitor of GSK3 proteins FR900359 was purified at the University of Bonn and used at 1 μM concentration. In experiments in which intracellular cytokine staining was performed, brefeldin A was added (Sigma-Aldrich, 10 μg/ml).

Cytokines in the supernatants were detected with a sandwich ELISA using IL-5 (TRFK5) or IL-13 (eBio13a) as capture antibodies and IL-5 (TRFK4) or IL-13 (eBio1316H) as detection antibodies (all from eBioscience) or the Legendplex bead-based assay (Biolegend) for IL-5, IL-9 and IL-13 according to the manufacturer's protocol.

NMU-induced inflammation. Third-stage larvae (L3) of N. brasiliensis were purified from a banana apparatus. After washing three times in PBS, living worms were counted. 500 purified larvae were injected subcutaneously in PBS. In addition, in some experiments PBS or NMU (20 μg, Phoenix Pharmaceuticals) was injected i.p. on day 2, 4 and 6 of infection. At day 7 of infection, mice were killed and analyzed if not otherwise indicated. For T. muris, infection with 200 embryonated eggs was administered by oral gavage. At day 18, a piece from the proximal colon was removed and cleaned in PBS, after which RNA was extracted for qPCR.
either Nmur1−/− or Nmur1+/− bone marrow. Antibiotics (sulfamethoxazol and trimethoprim) were delivered for 2 weeks after bone marrow transplantation in the drinking water and mice were reconstituted for at least 6 weeks before experimentation.

**Reconstitution of Rag2−/−Il2rg−/− mice.** Bones were cleaned, washed in 70% EtOH and subsequently in PBS and crushed using a pestle. Bones were rinsed with PBS and bone marrow was resuspended in PBS and filtered through a 70-μm cell strainer. Red blood cells were lysed using ACK buffer (Lonza) and lineage-positive cells were depleted using the Dynabeads untouched mouse CD4 cells kit according to the manufacturer’s protocol (Thermo Fisher Scientific). Remaining cells were stained and ILC2 progenitors were sort-purified as Lin− Sca-1+ CD127+ CD25+ cells. ×10⁴ sort-purified ILC2 progenitors were injected i.v. and mice were used at least 4 weeks after adoptive transfer.

**Clarity imaging and immunofluorescence.** Clarity imaging was performed following a modified protocol of Chung et al. Briefly, mice were killed and transcardially perfused with 20 ml of ice-cold PBS followed by hydrogel solution combining 1% acrylamide (40%, BioRad), 0.025% bis-acrylamide (2%, BioRad), 0.25% VA-044 initiator (Wako) and 4% PFA in PBS. Intestines were immediately excised, opened longitudinally and cleaned. Tissue was placed in a 50 ml conical tube including 20 ml of cold hydrogel solution and incubated slowly shaking at 4°C for 2 days. The tube was degassed in a desiccation chamber to replace air with nitrogen gas and submersed in a 37°C water bath for 4 h until polymerization of the hydrogel solution. Tissue was extracted and cleaned of excess gel followed by washing in clearing solution (200 mM Boric Acid, 4% Sodium Dodecyl Sulphate (pH 8.5; both Sigma-Aldrich) at 37 °C on a shaker. Clearing solution was changed every 2 days until tissue became transparent. Tissue was washed twice for 24 h in 0.1% Triton X-100 in PBS and stained with indicated antibodies.

For whole mount staining small intestine was cleaned, opened longitudinally and washed ice-cold HBSS with 5% FCS and HBSS with 1 mM DTT. The muscular mucosa was then mechanically separated from the mucosa using forceps and washed ice-cold HBSS with 5% FCS. The tissue was fixed in 4% PFA for 2 h at room temperature. Afterwards the tissue was washed in ice-cold PBS and blocked with PBS 0.1% Triton X-100 and 10% serum and stained. The following antibodies were used: rabbit anti-NMU (Santa Cruz), chicken anti-tyrosine-hydroxylase (Abcam), mouse anti-SNAP25 (S1M 81, Biologend), rat anti-CD3ε (17A2, Biologend), hamster anti-KLRG1 (2F1, eBioscience) followed by goat anti-rabbit Alexa 488, donkey anti-rabbit Alexa 647, goat anti-chicken Alexa 555, goat anti-hamster Alexa 546, all Thermo Fisher Scientific, donkey anti-rat Alexa 647 (Jackson Immuno Research), and images of representative tissue were captured under a LSM 880 confocal microscope and analysed with Zen software (Zeiss). Surface reconstruction was performed by using Imaris software (Bitplane). For quantification of ILC2-neuron co-localization we performed background subtraction, Gaussian smoothing. Then a surface was created and small objects were filtered out for the CD3ε channel. Afterwards, the KLRG1 channel was masked with CD3ε objects in order to remove these from the KLRG1 channel. Background subtraction, Gaussian smoothing, creation of surface and filtering of small objects were performed for KLRG1 channel. This mask was used to select pixels in the NMU channel that overlapped with KLRG1. For the masked NMU channel, a surface was created and small objects were filtered out. The objects with and without overlap of the KLRG1 and NMU channel were manually counted.

**Enteric neuron culture.** Neosphere culture and differentiation was essentially carried out as described before. In brief, the intestine were dissected from embryos approximately 18 days p.c., minced and washed four times in HBSS/2% FBS (4 min, at 400g). Tissue was digested for 15 min at 37 °C in HBSS supplemented with 0.05% Trypsin–EDTA solution (Gibco) and 50µg/ml DNaseI. After digestion the solution was vortexed and filtered through a 70-μm cell strainer. Cells were plated on an ultralow adherent plate (Corning) in DMEM/F12 supplemented with N2 and Antibiotic–Antimycotic solution (all Gibco) and expanded for 4–5 days. EGF and FGF (20 ng/ml, both R&D) were added to the culture. For differentiation, cells were cultured on a 96-well flat-bottom adherent plate (Corning) coated with fibronectin (20 µg/ml in PBS, Sigma) in Neurobasal medium supplemented with B27 and antibiotic–antimycotic solution (all Gibco) for 15 days. For coculture, sort-purified ILC2s were added to the culture with IL-2 and IL-7 (20 ng/ml each).

**Human tissues.** Human tissues were obtained through an approved research protocol and material transfer agreement with LiveOnNY as described before. Donors were tested to be HIV−, hepatitis B−, hepatitis C− and did not have chronic disease or cancer. This work does not qualify as ‘human subject’ research as confirmed by the institutional review board of Columbia University. Tissues were collected after the donor organs were flushed with preservation solution. The intestinal tissue was washed in PBS, cleaned from fat tissues and cut in smaller pieces, which were incubated in HBSS supplemented with 10 mM Hepes, 5 mM EDTA and 1 mM DTT for 1 h at 37°C in a shaking incubator. The specimens were vortexed and filtered through a cell strainer. Epithelium and intraepithelial lymphocytes (IELs) were purified with a 20%/40% Percoll gradient. Digestion solution (collagenase D, Roche, 2 mg/ml, trypsin inhibitor (Thermo Fisher, 1 mg/ml), and DNasel (0.1 mg/ml) in RPMI medium (Corning), was injected submucosally into the remaining tissue pieces. After incubation at 37°C for 30 min, the specimens were washed and moved to shaking incubator for another 30 min at 37°C. Afterwards, specimens were vortexed, filtered through a tissue sieve and purified over a 40%/80% Percoll gradient. Recovered LPLs were stained with fluorescent-label coupled antibodies and sort-purified. In addition, RNA was extracted from fractioned epithelium, intraepithelial lymphocytes, lamina propria lymphocytes, remaining parenchyma or whole unfractioned tissue using Trizol.

**Statistical analysis.** P value of mouse data sets was determined by paired or unpaired two-tailed Student’s t-test with 95% confidence interval. Normal distribution was assumed. If equal variances between two groups could not be assumed, Welch’s correction was performed. Data from human samples were analysed using a two-tailed Mann–Whitney test with 95% confidence interval. All statistical tests were performed with Graph Pad Prism V7 software. (P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant).

**Data availability.** RNA-seq data are deposited under accession number GSE101625 in the Gene Expression Omnibus database.
Extended Data Figure 1 | ILC2s and neurons co-localize. Surface reconstruction of immunofluorescence staining from the intestinal submucosa shown in Fig. 1a. Scale bar, 30 μm. Data are representative of three independent experiments with similar results.
Extended Data Figure 2 | ILC2s selectively express NMUR1.

a, Expression of Nmru1 in the indicated sorted cell populations as determined by qPCR analysis (n = 5). MC, mast cells; Mϕ, macrophages; PMϕ, peritoneal macrophages. MC and PMϕ were obtained by peritoneal lavage; Mϕ and ILC2s were purified from the small intestine. 

b–d, f, Histograms and dot plots show expression of Nmru1 as measured by conversion of the fluorescent LacZ substrate FDG. Histograms are gated on Lin−CD45+ cells and CD127+ KLRG1+ (ILC2s), CD127+CCR6− NKp46+ NK1.1+ (ILC1s), CD3+ (T cells), CD19+ (B cells), CD3−CD19−CD11b+ SiglecF+ (Eosinophils) from the small intestine (b, c). Gating for mast cells and basophils from the lung is shown (d). Percentage (n = 3) of FDG+ cells from the indicated population of the small intestine. Eos, eosinophils (f). e, g, Flow cytometry analysis of the indicated immune cell populations (top row) for Nmu1 (bottom row). MC and PMϕ were obtained by peritoneal lavage; Mϕ and ILC2 were purified from the small intestine (e). Percentage (n = 3) of FDG+ cells (g). 

h, Expression of Nmu (n = 3 (LPL), n = 5 (all others)), as determined by qPCR from the indicated fractions of the murine small intestine. i, Expression of NMU (n = 9 (epithelium), n = 8 (whole jejunum and LPL), n = 7 (IEL), n = 6 (parenchyma)) as determined by qPCR from the indicated fractions of the human jejunum. 

j, k, CLARITY staining of the small intestine (j) or colon (k) for NMU. l, Image of the intestinal muscularis mucosae from NmuGFP mice. m, Immunofluorescence staining of the intestinal mucosa from ChatCre x Ai14 mice for NMU. Scale bar, 100μm (j–m). Error bars, mean ± s.d. Data are representative of two (e, g) or three independent experiments (b–f, j–m) with similar results. Data in a, h, i are based on the indicated number of biological replicates per group.
Extended Data Figure 3 | ILC2s and NMU+ neurons co-localize.

a, Surface reconstruction of immunofluorescent staining from the intestinal submucosa shown Fig. 1i. Scale bar, 50 μm. b, Percentage (n = 4) of ILC2s, that have overlapping pixels with neurons. A total of 348 cells were counted and 236 cells exhibited pixels overlapping with NMU staining. c, Expression of Nmu as determined by qPCR in enteric neuron (n = 5, pooled from two independent experiments) cultures and compared to the epithelial fraction or parenchyma of the small intestine (n = 3). d, e, Sort-purified ILC2s (3 × 10⁴) were cultured with or without enteric neurons for 5 days. Absolute number (d) and FSC (e) (n = 3) of ILC2s are shown. Error bars, mean ± s.d. Data are representative of three independent experiments (a, d, e) or on the indicated number of biological replicates per group (b, c).
Extended Data Figure 4 | NMU activates ILC2s. a, Gating strategy for flow cytometric analysis of bulk LPLs cytokine assays. Lineage 1: CD11b, CD11c and B220 (all APC-eF780); lineage 2: CD3, CD5 (both PerCP-Cy5.5) and FcεRI PerCP-eF710. b, c, Concentration (n = 3) of IL-13 (b) or IL-5 (c) in the culture supernatant after 4 h stimulation of bulk LPLs with a control peptide or NMU as determined by ELISA (n.d., not detectable). d, e, Bulk LPLs from Il1rl1+/+ and Il1rl1−/− mice were incubated in medium with or without NMU for 4 h in vitro. Percentage (n = 3) of IL-5+KLRG1+ cells. f, g, LPLs from Nmur1+/+ or Nmur1−/− mice were analysed by flow cytometry. Plots are gated on Lin−CD45+ lymphocytes (e). Percentage (n = 3) of GATA-3+KLRG1+ cells (f), h, i−k, Overnight incubation of sort-purified intestinal ILC2s from Il13YFP+/+ mice in medium with or without NMU. FSC (n = 3) (i), histogram overlay of IL-13 YFP (j) and percentage (n = 3) of IL-13 YFP+ ILC2s (k). i, ILC2s from the small intestine were sort-purified and incubated in medium without or with NMU overnight in vitro. Contour plots show intracellular flow cytometry analysis for IL-5 and IL-13. Error bars, mean ± s.d. Data are representative of two (b−d, g, h) or three independent experiments (e, f, i−l) with similar results. Gating in a is representative for cytokine assays used in the whole study.
Extended Data Figure 5 | See next page for caption.
Extended Data Figure 5  |  **Nmu** stimulates ILC2s in vivo.** a, PBS or NMU was injected daily in C57BL/6 mice. After two days, ILC3s from the small intestine were analysed by flow cytometry for Ki67. Plots are gated on Lin−CD45+ RORγt+ lymphocytes. Percentage (n = 3) of Ki67+ cells. b, PBS or NMU was injected daily for two days in CD45.1+ Nmur1+/−/CD45.2− Nmur1−/− mixed bone marrow chimaeras. One day later, ILC2s from the small intestine were analysed by flow cytometry for KLRG1 and Ki67. Plots are gated on Lin−CD127+ KLRG1+ lymphocytes and either CD45.1 or CD45.2. c, PBS or NMU (100 μg) was injected in CD45.1+ Nmur1+/−/CD45.2− Nmur1−/− mixed bone marrow chimaeras. One day later, ILC2s from the small intestine were analysed by flow cytometry for KLRG1 and Ki67 expression. Percentage (n = 3) of Ki67+ cells among all KLRG1+ cells. d, PBS or NMU was injected once in IIL3+/- mice. FSC (n = 3) of Lin−CD45−CD127−CD25− KLRG1+ LPLs one day after injection. e, C57BL/6 mice were infected with T. muris (n = 11) or left untreated (n = 6). On day 18, Nmu expression was determined by qPCR in a piece of the proximal colon. f, C57BL/6 mice were infected with H. polygyrus (n = 18). Control C57BL/6 (n = 4) mice were left untreated. On day 18, Nmu expression was determined by qPCR in a piece of the duodenum. g, Nmur1+/− or Nmur1+/+ mice were infected with N. brasiliensis. Control Nmur1+/− mice were left untreated. On day 7, mice (n = 6) were analysed. Histogram overlay shows expression of Nmur1 (FDG) on ILC2s from the small intestine and are gated on Lin−CD45− KLRG1+ lymphocytes. h, Nmur1+/− or Nmur1+/+ mice were infected with N. brasiliensis. Control mice were left untreated. On day 7, mice were analysed and the percentage of Nmur1+ (FDG) determined by flow cytometry in the indicated subsets. Percentage (n = 6 intestinal subsets) or 8 (lung subsets) for infected Nmur1+/+ and n = 3 for control mice) of Nmur1+ (FDG) cells. Plots are gated on CD45+ cells and FcγRI+CD49b−c-Kit− for basophils (BF), FcγRI+CD49b−c-Kit+ for mast cells, CD11b+ F4/80− for MΦ, CD3+ CD5+ for T cells and Lin− KLRG1− for ILC2s. j, Nmur1+/+ mice were infected with N. brasiliensis. On day 14, mice were analysed and the percentage (n = 7 or 9 (lung)) of Nmur1+ (FDG) CD3−CD5+ T cells or Lin− KLRG1+ ILC2s was determined by flow cytometry. k, I. BelGFP mice were infected with N. brasiliensis. On day 14, CD4+ T cells (gated on CD3+CD5+ lymphocytes) were sorted-purified in IL-4-positive and -negative populations based on GFP expression (k) and Nmur1 expression was determined by qPCR (I) (n = 3 (ILC2), n = 4 (IL-4+CD4+ lung) or n = 5). m, PBS or NMU was injected daily for two days in C57BL/6 or Cpa3+/- mice. One day later, ILC2s from the small intestine were analysed by flow cytometry for KLRG1 and Ki67 expression. Plots are gated on Lin−CD45+ GATA-3+ KLRG1+ cells (m). Percentage (n = 5 or 3 (PBS)) of Ki67+ KLRG1+ cells among all KLRG1+ cells (n). o, PBS or NMU was injected daily for two days in BALB/c or ΔdblGATA1 mice. One day later, ILC2s from the small intestine were analysed by flow cytometry for KLRG1 and Ki67 expression. Percentage (n = 6 or 5 (PBS ΔdblGATA1)) of Ki67+ KLRG1+ cells among all KLRG1+ cells. p, Bone marrow chimaeras reconstituted with Nmur1+ or Nmur1−/− bone marrow were infected subcutaneously with N. brasiliensis. On day 7, worm burden (n = 15 (Nmur1+/+), n = 14 (Nmur1−/−)) in the small intestine was quantified. q, Rag2−/−Il2rg−/− mice were reconstituted with ILC2 precursors from Nmur1+ or Nmur1−/− mice. After reconstitution, mice were infected with N. brasiliensis and NMU (20 μg) was injected i.p. on day 2, 4 and 6. Plots show flow cytometry analysis of cells from the lung and are gated on CD45+CD11c− cells. Error bars, mean ± s.d. Data are representative of two (b, c, k-o, q) or three independent experiments (a, e, h) with similar results. The data are pooled from two (d) or three (f, g, i) independent experiments. The data in j and p are representative of the indicated number of biological replicates per group.
ILC2 are required for NMU-induced lung inflammation. a, PBS or NMU was intranasally administered to C57BL/6 mice daily for four days. One day later, ILC2s from the lung were analysed by flow cytometry. b, PBS or NMU was delivered intranasally to C57BL/6 mice daily for five days. Three days later, eosinophil infiltration was determined in BAL by flow cytometry. c, PBS or NMU was intranasally administered to Rag2−/− or Rag2−/−Il2rg−/− mice daily for four days. One day later, ILC2s and eosinophils from the lung were analysed by flow cytometry. d, PBS or NMU was intranasally administered daily for four days to Rag2−/−Il2rg−/− mice or Rag2−/−Il2rg−/− that were reconstituted with ILC2 progenitors. One day later, ILC2s and eosinophils from the lung were analysed by flow cytometry. e, PBS or NMU was intranasally administered to Nmur1+/+ or Nmur1−/− mice daily for four days. One day later, ILC2s and eosinophils from the lung were analysed by flow cytometry. Plots are gated on Lin−CD45+GATA-3+CD25+ lymphocytes. Data are representative of two (d, e) or three independent experiments (a–c) with similar results.
Life Sciences Reporting Summary

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1. Sample size
   Describe how sample size was determined.
   No statistical methods were used to predetermine sample size. We chose to use 4 NSG mice per experimental group so that technical replicate tumours could be available for barcode data analysis.

2. Data exclusions
   Describe any data exclusions.
   Mice which did not harbour tumours at the pre-determined endpoint (6 months post-injection) were not used for immunohistochemistry or barcode sequencing analysis.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   Attempts to replicate our drug assays have been successful (n = 3 independent experiments). We did not attempt to replicate in vivo barcoding experiments due to limiting numbers for uncultured GBM cells.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   NSG mice from separate litters were randomized to vehicle control or TMZ treatment groups in order to control for age.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   For xenograft studies, investigators were not blinded during group allocation and data analysis. Investigators were blinded during data collection for in vitro limiting dilution analyses (LDA).

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

7. Software

Describe the software used to analyze the data in this study. Custom code used in this study are available upon reasonable request from B.D.S. Other software are listed within the relevant methods descriptions.

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

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. The lentiviral barcode library used in this study is available upon reasonable request from C.J.E. The primary GBM cells are unavailable due to limiting quantities and cell viability. The primary GBM cultures are available upon reasonable request from P.B.D. All other materials are commercially available.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species). All antibodies in this study are described in the methods section under "histopathology and immunohistochemistry".

10. Eukaryotic cell lines

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

b. Describe the method of cell line authentication used.

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

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.

Animals and human research participants

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study. Female NSG mice (Mus musculus) of age 1-3 months were used for all xenograft studies.

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants. GBM cells were processed following surgical resection from patients that were either untreated (Primary GBMs) or Temozolomide treated (Secondary GBM sample GBM-742).