Bcl11b is essential for group 2 innate lymphoid cell development

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Group 2 innate lymphoid cells (ILC2s) are often found associated with mucosal surfaces where they contribute to protective immunity, inappropriate allergic responses, and tissue repair. Although we know they develop from a common lymphoid progenitor in the bone marrow (BM), the specific lineage path and transcriptional regulators that are involved are only starting to emerge. After ILC2 gene expression analysis we investigated the role of Bcl11b, a factor previously linked to T cell commitment, in ILC2 development. Using combined Bcl11b-tom and Id2-gfp reporter mice, we show that Bcl11b is expressed in ILC2 precursors in the BM and maintained in mature ILC2s. In vivo deletion of Bcl11b, by conditional tamoxifen-induced depletion or by Bcl11b−/− fetal liver chimera reconstitution, demonstrates that ILC2s are wholly dependent on Bcl11b for their development. Notably, in the absence of Bcl11b there is a concomitant expansion of the RORγt+ ILC3 population, suggesting that Bcl11b may negatively regulate this lineage. Using Nippostrongylus brasiliensis infection, we reveal that the absence of Bcl11b leads to impaired worm expulsion, caused by a deficit in ILC2s, whereas Citrobacter rodentium infection is cleared efficiently. These data clearly establish Bcl11b as a new factor in the differentiation of ILC2s.

The recently identified innate lymphoid cell (ILC) family consists of IFN-γ–secreting group 1 ILCs (ILC1s), type 2 cytokine-producing ILC2s, and IL-22– and/or IL-17–positive ILC3s (Walker et al., 2013). ILC1s and ILC3s play critical roles in protective immunity against bacteria, intracellular protozoan parasites (ILC1), and fungi (ILC3) and in autoimmune disorders (McKenzie et al., 2014). In contrast, ILC2s associate with immune responses to parasitic worms, allergy, and wound repair (McKenzie et al., 2014).

These functionally diverse cytokine-producing cells arise from a common lymphoid progenitor under the control of specific transcriptional regulators (Diefenbach et al., 2014). These include upstream factors such as initiator of DNA binding 2 (Id2), Notch, GATA-binding protein 3 (GATA3), nuclear factor interleukin-3 (Nfil3), T cell factor 1 (TCF1), and promyelocytic leukemia zinc finger (PLZF) that restrict the differentiation of a common helper ILC progenitor (CHILP) from the common lymphoid progenitor (Diefenbach et al., 2014). Downstream, lineage-specific transcription factors are necessary for lineage commitment: ILC1s require the T-box transcription factor T-bet (Tbx21), ILC2s require GATA3 and transcription factor retinoic acid receptor–related orphan nuclear receptor α (RORα), and ILC3s require RORγt (Diefenbach et al., 2014). Notably, the majority of factors identified in these pathways play roles in the specification of the progenitor cells and lie proximal to the division of the functional ILC subsets.

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Abbreviations used: CHILP, common helper ILC progenitor; FL, fetal liver; ILC, innate lymphoid cell; ILC2p, ILC2 precursor; MLN, mesenteric LN; NKp, NK-like precursor; p.i., postinfection; PLZF, promyelocytic leukemia zinc finger; siLP, small intestine lamina propria.
Although they comprise only a small proportion of the hematopoietic compartment, ILC2s (often at the mucosal borders) are a critical innate cellular source of type 2 cytokines, secreting copious IL-5 and IL-13, but also IL-9, IL-4, and GM-CSF (Moro et al., 2010; Neill et al., 2010; Price et al., 2010). These effector cytokines potently induce eosinophilia, goblet cell hyperplasia and mucus production, mastocytosis, alternatively activated macrophages, and muscle contractility and contribute to tissue repair (McKenzie et al., 2014). Thus, ILC2s that expand in response to the type 2 initiator cytokines IL–25, IL–33, and TSLP play a key role in protective immunity against parasitic helminth infection (Moro et al., 2010; Neill et al., 2010; Price et al., 2010), contribute to inappropriate allergic inflammation (Barlow et al., 2011; Chang et al., 2011; Mjösberg et al., 2011), and are associated with metabolic homeostasis, obesity, and dietary stress (Hams et al., 2013; Molofsky et al., 2013; Stanya et al., 2013).

ILC2s can be derived from a PLZF$^+$ fraction of CHILPs, and conditional deletion of GATA3 in Id2$^+$ cells or germine deletion has demonstrated that GATA3 is required both for the development of all ILCs and for the later restriction of the ILC2 lineage (Hoyler et al., 2012; Yagi et al., 2014). In contrast, RORα-deficient mice have apparently normal CHILP and ILC3 production, but BM progenitors from these mice fail to develop into ILC2s, both in vivo and in vitro (Halin et al., 2012; Wong et al., 2012). In addition to RORα and GATA3, the transcription factor B cell leukemia/lymphoma 11b (Bcl11b) was also reported to be up-regulated in ILC2s, indicating that Bcl11b is crucial for maintaining T cell compartmentalization (Li et al., 2010b). CD4$^+$ (Fig. 1 a) and CD8$^+$ T cells (not depicted) from the mesenteric LNs (MLNs) were strongly positive for Bcl11b-tom, whereas B cells and myeloid cells were negative (not depicted). ILC2s (phenotypic analysis and gating strategies in Figs. S1 and S2), isolated from ILC2-associated immunological sites in naive mice, also expressed Bcl11b-tom similar to T cells (Fig. 1, a–e). Bcl11b-tom expression was reduced slightly in ILC2s after in vivo stimulation with IL-25 and IL-33 (Fig. 1, c and d); the reason for this is unclear. Thus, mature ILC2s express high amounts of Bcl11b in the peripheral lymphoid tissues. Bcl11b-tom expression was also detected in ILC2-like cells in the BM, suggesting that it may be present in ILC2 precursors (ILC2ps; Fig. 1 e). Notably, Bcl11b−tom was also expressed heterogeneously in a proportion of non-LTii ILC3s from the small intestine lamina propria (siLP; Fig. 1 f and Fig. S2 a).

Characterization of a Bcl11b-expressing ILC2ps

Because mature ILC2s arise from ILC2ps in the BM, we sought to identify whether ILC2ps express Bcl11b. We identified BM-derived ILC2ps (ICOS$^+$) using mice in which Id2 expression, which marks early NK progenitors (Carotta et al., 2011) and is required for the development of all ILC subsets, is reported by the expression of an IRES green fluorescent protein (Id2$^{IRES^+}$; Jackson et al., 2011), in combination with surface markers to exclude NK-like precursors (NKps; CD244; CD244). Thus, the lin$^-Id2-gfp^+$ BM cell population was subdivided by ICOS and/or CD244 into four subsets (Fig. S2 b). To assess their lineage commitment, these cells were purified and plated onto OP9 or OP9–Delta-like ligand 1 (DL1) cell monolayers in the presence of ILC2-promoting IL-7 and IL-33 or NK-promoting IL-7 and IL-15. We demarcated ILC2ps (lin$^-Id2-gfp^-ICOS^-CD244^-$; Fig. 2, a and c) and NKps (lin$^-Id2-gfp^-ICOS^-CD244^+$; Fig. 2, b and c). In some instances, cultures gave rise to additional populations of cells (CD3$^+$, CD19$^+$, CD11b$^+$, CD11c$^+$, Gr−$^+$, NK1.1$^-$, NKp46$^-$) that could not be ascribed to any particular lineage. Subsequent analysis of intercrossed Id2$^{IRES^+}$/ and Bcl11b$^{IRES^+}$/ mice demonstrated that expression of Bcl11b−tom was almost mutually exclusive in the ILC2p and NKp populations, being expressed only by the ILC2ps, both upon direct isolation from the BM and after culture on OP9–DL1 feeder cells to produce ILC2s (Fig. 2, d and e).

To confirm the progenitor status of the Bcl11b$^+$ BM cells, we isolated Id2-gfp$^-Bcl11b^-ICOS^-$ or Id2-gfp$^-Bcl11b^-CD244^-$ BM precursor subsets and transferred these into Rag2$^{−/−}$ recipients (Fig. S2, c and d). Only Id2-gfp$^-Bcl11b^-$ precursors gave rise to ILC2s upon adoptive transfer (Fig. 2 f). Transfer of the Id2-gfp$^-Bcl11b^-$ precursors gave rise to NK cells (Fig. 2 g). Comparison of the Id2-gfp$^-Bcl11b^-$ precursors with the previously reported Lin$^-Sca1^+Id2^{hi}$ (LSI) precursor (Fig. S2, e and f; Hoyler et al., 2012) indicated that they are very similar (Fig. 2 h) and give rise to phenotypically identical Gata3$^+$ KLRG1$^+$ ILC2s upon adoptive transfer into Rag2$^{−/−}$ recipients (Fig. 2, i and j). These transfers also generated a population of Gata3$^+$ ICOS$^+$ KLRG1$^+$ cells, the significance of which is unclear, but that may represent immature ILC2s (Fig. 2, i and j).
controls, tamoxifen treatment did not alter ILC2 numbers in the MLNs (Fig. 3 a and Fig. S3 a), although we did observe a reduction in the relative frequency of ILC2s (Fig. S3 a), which may reflect as yet not understood changes in multiple cell populations. Bcl11b deletion did not affect the frequency of BM ILC2p (Fig. 3 b) or Gata3 expression (Fig. 3 c). We next examined the effect of Bcl11b depletion in the context of ILC2 stimulation by IL-33. Conditional Bcl11b-deficient (Bcl11bflox/−) mice bearing an Id2-gfp allele, intercrossed with Rosa26Cre-ERT2 mice (Cre-ERT2; Bcl11bflox/− referred to hereafter as Bcl11bflox/−) mice (black), Bcl11b-tom expression in ILC2s from siLP is shown for comparison (red). (a–f) Numbers adjacent to boxed areas indicate the relative percentage of gated populations. Gating strategies and phenotypic analysis are presented in Figs. S1 and S2. Data represent at least two independent experiments with three mice per group.

Although the majority of ICOS+CD244+ cells were Bcl11b−, ICOS−CD244+ cells displayed bimodal expression of Bcl11b-tom (Fig. 2 k). Upon culture under ILC2- or NK-promoting conditions, the Bcl11b+ fraction gave rise exclusively to ILC2s, whereas the Bcl11b− subset gave rise to mixed progeny (Fig. 2, l and m), suggesting that they may represent a mixed population of uncommitted precursors. Finally, we examined the expression of Gata3 in populations delineated by Id2-gfp and Bcl11b-tom and found that Gata3 is up-regulated in Id2-expressing cells, perhaps before the induction of Bcl11b (Fig. 2 n). These results demonstrate that Bcl11b is expressed by a lineage-restricted ILC2p and that, in combination with Id2, expression of Bcl11b can be used to purify the ILC2ps.

Deletion of Bcl11b ablates ILC2s and increases ILC3s
We next sought to determine whether Bcl11b was functionally important for ILC2 development or maintenance. Tamoxifen was used to delete Bcl11b in conditional Bcl11b-deficient (Cre-ERT2; Bcl11bflox/−) mice (Li et al., 2010b), and ILC2 frequency was analyzed 2 wk later. Relative to controls, tamoxifen treatment did not alter ILC2 numbers in the MLNs (Fig. 3 a and Fig. S3 a), although we did observe a reduction in the relative frequency of ILC2s (Fig. S3 a), which may reflect as yet not understood changes in multiple cell populations. Bcl11b deletion did not affect the frequency of BM ILC2p (Fig. 3 b) or Gata3 expression (Fig. 3 c). We next examined the effect of Bcl11b depletion in the context of ILC2 stimulation by IL-33. Conditional Bcl11b-deficient (Bcl11bflox/−) mice bearing an Id2-gfp allele, intercrossed with Rosa26Cre-ERT2 mice (Cre-ERT2; Bcl11bflox/− referred to hereafter as Bcl11bflox/−), Bcl11b−/− mice, or controls (Cre-ERT2; Bcl11b+/+ referred to hereafter as Bcl11b+/+) were treated with tamoxifen. After IL-33 stimulation, there were considerably fewer ILC2s in the MLNs of the Bcl11bflox/− mice as compared with the Bcl11b+/+ controls, which did not reach statistical significance but was consistent between two similar experiments (Fig. 3 d and Fig. S3 b). Tamoxifen administration was accompanied by a fall in the numbers of T cells in the absence of Bcl11b (Fig. 3 e) and a reciprocal increase in NK1.1+ cell numbers (Fig. 3 f), as reported previously (Li et al., 2010b). These findings suggest that Bcl11b is not required for
the short-term maintenance of ILC2s in tissues. However, they raise the question of whether Bcl11b is required for ILC2 expansion in the tissues or for the continued generation of ILC2ps in the BM.

To address this question and the potential issues of dosing and off-target effects of tamoxifen, we also generated fetal liver (FL) chimeras in which irradiated CD45.2+ hosts were reconstituted with CD45.1+ BM, in combination with either CD45.2+ Bcl11b+/+Id2gfp/+ or Bcl11b+−/−Id2gfp/+ FL. After 6–8 wk, there was an absence of FL donor-derived double-positive thymocytes in mice receiving Bcl11b+−/− FL (Fig. 3 g), but no significant difference in the numbers of FL donor-derived Id2-gfp+ NK cells (Fig. 3 g). Strikingly, there was a total absence of mature FL donor-derived Id2-gfp+ ILC2s from the short-term maintenance of ILC2s in tissues. However, they raise the question of whether Bcl11b is required for ILC2 expansion in the tissues or for the continued generation of ILC2ps in the BM.

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Bcl11b−/− FL in the MLNs, as compared with wild type (Fig. 3 h and Fig. S3 c). Furthermore, Bcl11b−/−-derived ILC2s could not be induced by treatment with IL-33 (Fig. 3 h and Fig. S3 c) or IL-25 (not depicted). There were also no detectable FL donor-derived Id2-gfp+ ILC2ps in the BM (Fig. 3 i and Fig. S3 d). Notably, analysis of ILCs in the siLP and MLN showed that the Bcl11b−/− donor cells gave rise to a disproportionately large population of NCR+ ILC3s as compared with wild type (Fig. 3, j and k; and Fig. S3 c).

Thus, Bcl11b is essential for the development of ILC2ps and consequently mature peripheral ILC2s. The complete absence of ILC2s in these mice is striking because residual ILCs have been observed after deletion of many of the previously reported factors that are important for ILC development. Indeed, even in the absence of RORα a basal level of ILC2s can be detected in the peripheral tissues (Wong et al., 2012). This implies that Bcl11b may act upstream of RORα during ILC2 development, although genetic complementation systems would be required to confirm this relationship.

We noted that although the absence of Bcl11b resulted in the loss of ILC2s, its absence also caused a reciprocal increase in the numbers of ILC3s in the siLP. This suggests that Bcl11b may act cell-intrinsically to repress ILC3 differentiation and that deletion of Bcl11b results in de-repression of the ILC3 program, leading to their expansion, or that the absence of ILC2s results in an expansion of ILC3s through cell-extrinsic factors.

**Bcl11b-deficient progenitors fail to provide ILC2s during *Nippostrongylus brasiliensis* infection**

ILC2s play critical roles in immunity to infection with parasitic helminths, such as *N. brasiliensis*. Consequently, mice deficient in the ILC2-inducing cytokines IL-25 and IL-33 (Neill et al., 2010) or the transcription factor RORα (Wong et al., 2012; Oliphant et al., 2014) have impaired worm clearance. To confirm that the deletion of Bcl11b resulted in the absence of functional ILC2s in vivo, and not for example a loss of specific lineage markers, we determined whether Bcl11b deletion in ILC2s impaired the absence of functional ILC2s in the siLP, This implies that Bcl11b may act cell-intrinsically to repress ILC3 differentiation and that deletion of Bcl11b results in de-repression of the ILC3 program, leading to their expansion, or that the absence of ILC2s results in an expansion of ILC3s through cell-extrinsic factors.

**Figure 3.** ILC2s fail to develop in the absence of Bcl11b. (a–k) Flow cytometry was performed to enumerate the indicated cell populations in conditional Bcl11b-deficient mice (a–f) or chimeras generated using Bcl11b−/− FL cells (g–k). (a and b) MLN ILC2s (a) and BM ILC2ps (b) in Cre-ERT2;Bcl11bflx/flx mice, treated as indicated (Veh, vehicle control; Tx, tamoxifen). Shown is mean ± SEM from two similar experiments with three to four mice per group. (c) Intracellular Gata3 staining in ILC2s from the MLNs of Cre-ERT2;Bcl11bflx/flx mice, treated as indicated. (d–f) MLN ILC2s (d), splenic CD3+ (e), and splenic NK1.1+ cells (f) after PBS or IL-33 administration. (i–k) BM ILC2ps (lin−Id2-gfp+Sca-1+ST2+) in the MLN of mice reconstituted as in panel g, (i) Mean ± SEM from two similar experiments with two to three mice per group. (j) The indicated cell populations were enumerated in mice 6–8 wk after reconstitution with either Bcl11b+/+ or Bcl11b−/− CD45.2+ FL, mixed with CD45.1+ BM. Bars represent mean ± SEM of five mice per group. Data represent three independent experiments. (h) ILC2s (lin−Id2-gfp+ICOS+KLRG1+) in the MLN of mice reconstituted as in panel g, after PBS or IL-33 administration. (–h) BM ILC2ps (lin−Id2-gfp+Sca−1+ST2−; i) and NCR− ILC3s (lin−Id2-gfp+NKp46−; j) and MLN ILC2s and NCR− ILC3s (k) in mice reconstituted as in panel g; (h–i) mean ± SEM; (j) mean ± SD. P-values refer to Student’s t-test analysis: *, P < 0.05; **, P < 0.01; ***, P < 0.001.
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Rora^fluoxIl7rCre/+ BM plus Bcl11b^+/-Id2^lo/+ FL rescued the ILC2-deficient phenotype caused by the production of ILC2s from the Bcl11b^+/-Id2^lo/+ FL (Fig. 4 a), resulting in worm clearance similar to the control Rora^fluox/+ mice (Fig. 4 b). Strikingly, Bcl11b^-/-Id2^lo/- derived FL reconstitution was unable to reverse the ILC2 deficiency (Fig. 4 a) and failed to efficiently clear N. brasiliensis by day 7 p.i. (Fig. 4 b). Thus, deletion of Bcl11b results in a critical loss of functional ILC2s, without which mice are unable to clear worm infection efficiently.

Bcl11b deficiency does not impair ILC3 responses to Citrobacter rodentium

To investigate whether the absence of Bcl11b alters the functional capacity of ILC3s, we used the C. rodentium infection model. C. rodentium is a bacterial pathogen of mice that is closely related to the clinically important human enteropathogenic and enterohemorrhagic E. coli. Infection of wild-type mice with C. rodentium causes modest and transient weight loss and diarrhea, with clearance of infection within 21–28 d. In contrast, mice lacking ILC3s fail to control C. rodentium infection in the gut, as the result of insufficient IL-22 production, and succumb rapidly to infection (Satoh-Takayama et al., 2008; Sonnenberg et al., 2011). Irradiated mice were reconstituted with either Bcl11b^+/- or Bcl11b^-/- FL before being challenged with C. rodentium. We observed no statistically significant differences in the weights of Bcl11b^+/- or Bcl11b^-/- FL reconstituted mice 20+ d p.i. (Fig. 4 c), contrasting markedly with the dramatic weight loss in Rag2^-/- mice (Fig. 4 d). Both groups also cleared the majority of bacteria by day 21 p.i. (Fig. 4 e). Notably, the frequency of NCR^+ ILC3s was elevated in C. rodentium–infected Bcl11b^-/- FL-reconstituted mice as compared with controls (Fig. 4 f–h), whereas ILC2s were absent. Bcl11b deficiency also resulted in the modest expansion of a KLRG1loNKp46^+ population, the etiology of which is unclear (Fig. 4 f). These results recapitulate the increase in NCR^+ ILC3 numbers we observed in naive Bcl11b-deficient mice.

Specific downstream gene targets of Bcl11b in ILC2s remain to be identified, and ChIP-Seq is challenging because of the small numbers of ILC2s available and uncertainty as to the exact sequence of the endogenous Bcl11b-binding site. However, studies of the T cell differentiation pathway suggest that Bcl11b can act as both a repressor and activator of transcription depending on its association with binding partners.
(Liu et al., 2010) and on its state of phosphorylation or sumoylation (Avram and Califano, 2014).

It has been proposed that Bcl11b lies downstream of Notch signals that regulate its expression during T cell differentiation. We have reported previously that ILC2 development also requires Notch signals (Wong et al., 2012), and it is possible that these signals differentially regulate Bcl11b expression in ILC2s and T cells as compared with NK cells and ILC3s. Indeed, Bcl11b−/− DN2 thymocytes have been reported to express elevated levels of ILC3 and NK-inducing genes (including Id2, Il2rb, Nfil3, and Plzf), suggesting that these genes may be de-repressed in the absence of Bcl11b (Li et al., 2010a). However, it is not clear that a simple dominant master transcriptional regulator explains these differentiation pathways, and it is likely that the ILC and T cell transcription factor circuitry will share rheostats and resistors that are temporally and spatially regulated.

Collectively, these findings reveal a novel Bcl11b-mediated mechanism for the generation of ILC2s and demonstrate that this transcription factor is essential for ILC2 development and their functional role in helminth immunity. It is also noteworthy that mutations, duplications, and rearrangements in this transcription factor is essential for ILC2 development and tissues collected for analysis. Tamoxifen (1 mg per dose) was administered intraperitoneally in 100 µl vehicle, containing 90% sunflower oil (Sigma-Aldrich) and 10% ethanol. Conditional Bcl11b-deficient (Bcl11b−/−) mice were treated with six doses of tamoxifen and analyzed 3.5 wk later.

OP9-DL1 cultures. Cultures of ILC progenitor populations on OP9 and OP9-DL1 stroma (provided by J.C. Zúñiga-Pflücker, University of Toronto, Toronto, Ontario, Canada) were conducted as described previously (Wong et al., 2012).

Adaptive cell transfer. Putative ILC progenitor populations were FACS purified and collected into a 1:1 mixture of PBS and C57BL/6 mouse serum. Cells were injected directly into the tail vein of Rag2−/− mice recipient in a volume of 100 µl. Id2-gfp+ progeny were analyzed 3–6 wk after transfer.

Irradiation chimeras. Cell suspensions derived from embryonic day (E) 15.5 FLs were frozen in RPMI supplemented with 25% FCS and 10% DMSO and thawed immediately before reconstitution. For mixed chimeric experiments, lethally irradiated C57BL/6 (CD45.2+) recipients were reconstituted with Bcl11b−/− or Bcl11b−/− FL cells, either alone or as a 1:1 mixture (4–1 × 10^6 cells with BM derived from either B6SJL (CD45.1+) or IL7R−/−; Roraflox−/− mice.

Infection models. For N. brasiliensis infection, mice were inoculated subcutaneously with 500 viable third-stage larvae and euthanized 4–7 d.p.i. Tissues were collected for analysis, and small intestine worm burdens were enumerated using a dissection microscope (Wild M3Z; Leica). For C. rodentium infection, 100 µl LB broth containing 50 mg/ml nalidixic acid was inoculated with a single colony of C. rodentium (ICC180). Cultures were grown for 16 h at 37°C, with shaking, and then concentrated 10-fold in PBS. Mice were gavaged with 7 × 10^7 CFU C. rodentium in 100 µl PBS. Weights of mice were recorded over the infection time course, and fecal pellets were collected at the indicated time points to ascertain the bacterial load. Pellets were weighed and resuspended in 100 µl PBS per 0.01 g. Supernatants from homogenized pellets were serially diluted and plated onto LB agar plates supplemented with 50 µg/ml kanamycin. Colonies were enumerated after overnight incubation at 37°C.

Statistical analysis. Prism (GraphPad Software) was used to calculate the SEM when different numbers of datasets existed in each experimental group. Statistical differences between groups were calculated using Student’s t test, with P < 0.05 considered significant.

Online supplemental material. Flow cytometry gating strategies are described in Figs. S1–S3. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20142224/DC1.

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Figure S1. Flow cytometry gating strategies and surface phenotype of ILC2 populations. (a–c) Shown are gating strategies for the identification of ILC2s in MLNs from naive or IL-33–treated mice (a) and fat-associated lymphoid clusters (FALC; b) and siLP (c). (d and e) Gating strategies for the identification of ILC2ps in the BM (d) and ILC2s in lung (e) are shown. Histograms depict expression of the indicated surface markers, relative to a “fluorescence minus one” (FMO) control.
Figure S2. Flow cytometry gating strategies used to identify ILC and progenitor populations. (a) Gating strategies used to define ILCs in siLP are shown. (b) Gating strategy for the isolation of the indicated lin−Id2-gfp+ BM cell subsets. (c) Gating strategy used to sort ILC2ps (lin−Id2-gfp+ICOS+Bcl11b−CD244+) and NKps (lin−Id2-gfp+ICOS−Bcl11b−CD244+) for adoptive transfer (relevant to Fig. 2, f and g) is shown. (d) Plots describe how Id2-gfp+ progeny were identified in the spleen of Rag2−/−γc−/− recipients 3 wk after adoptive transfer of ILC2ps (left) or PBS injection (right). (e) Lin−Id2-gfp+ BM cells were gated as shown, NKps (Bcl11b−ICOS+) and ILC2ps (Bcl11b+ICOS+), for flow cytometric analysis of surface marker expression (relevant to Fig. 2 h). (f) Gating strategy used to sort ILC2p (lin−Flt3−Id2-gfp+ICOS−Bcl11b+) and LSI (lin−Sca-1−Id2-gfp+) cells for adoptive transfer (relevant to Fig. 2 i) is shown.
Figure S3. Flow cytometry gating strategies used for the analysis of Bcl11b-deficient mice. (a) Flow cytometric gating of ILC2s in the MLNs of Cre-ERT2; Bcl11bfl ox/fl ox mice 2 wk after the administration of tamoxifen (Tx) or vehicle control (Veh) is shown. (b) Flow cytometric gating of ILC2s in the MLNs of Cre-ERT2; Bcl11bfl ox/fl ox and Cre-ERT2; Bcl11b+/H11002 mice after the administration of tamoxifen and IL-33 is shown. (c) Gating of ILC2s (lin−/Id2-gfp+/ICOS+/KLRG1−) in the MLNs of reconstituted mice after PBS or IL-33 administration is shown. (d) Gating of ILC2ps (lin−/Id2-gfp+/Sca-1−/ST2+) in the BM of reconstituted mice is shown. (e) Gating of ILC2s (lin−/Id2-gfp+/KLRG1−) and NCR+ ILC3s (lin−/Id2-gfp+/NKp46+) in the sILP of reconstituted mice is shown.