

IL25 elicits a multipotent progenitor cell population that promotes T_H2 cytokine responses

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CD4⁺ T helper 2 (T_H2) cells secrete interleukin (IL)4, IL5 and IL13, and are required for immunity to gastrointestinal helminth infections¹. However, T_H2 cells also promote chronic inflammation associated with asthma and allergic disorders². The non-haematopoietic-cell-derived cytokines thymic stromal lymphopoietin, IL33 and IL25 (also known as IL17E) have been implicated in inducing T_H2 cell-dependent inflammation at mucosal sites^{3–6}, but how these cytokines influence innate immune responses remains poorly defined. Here we show that IL25, a member of the IL17 cytokine family, promotes the accumulation of a lineage-negative (Lin[−]) multipotent progenitor (MPP) cell population in the gut-associated lymphoid tissue that promotes T_H2 cytokine responses. The IL25-elicited cell population, termed MPP^{type2} cells, was defined by the expression of Sca-1 (also known as Ly6a) and intermediate expression of c-Kit (c-Kit^{int}), and exhibited multipotent capacity, giving rise to cells of monocyte/macrophage and granulocyte lineages both *in vitro* and *in vivo*. Progeny of MPP^{type2} cells were competent antigen presenting cells, and adoptive transfer of MPP^{type2} cells could promote T_H2 cytokine responses and confer protective immunity to helminth infection in normally susceptible *IL25*^{−/−} mice. The ability of IL25 to induce the emergence of an MPP^{type2} cell population identifies a link between the IL17 cytokine family and extramedullary haematopoiesis, and suggests a previously unrecognized innate immune pathway that promotes T_H2 cytokine responses at mucosal sites.

Epithelial cell-derived thymic stromal lymphopoietin (TSLP), IL33 and IL25 promote the development of mucosal T_H2 cytokine responses through the induction of IL4 in distinct innate immune cell lineages^{7–12}. TSLP elicits major histocompatibility complex (MHC) class II⁺ basophils that promote T_H2 cytokine responses, whereas IL33 treatment can activate basophils¹³, eosinophils¹⁴ and natural helper cells (NHCs)¹⁵. In contrast, IL25 is thought to promote IL4 production in either natural killer T (NKT) cells or an undefined non-B, non-T (NBNT) c-Kit⁺ cell population^{7,14,16}. On the basis of c-Kit expression, IL25-elicited NBNT cells were suggested to be mast cells or mast cell precursor populations^{14,16}. Haematopoietic stem cells (HSCs) are known to express c-Kit, circulate through peripheral tissues and differentiate in response to microbial signals^{17,18}. However, whether IL25 influences the population expansion or differentiation of peripheral HSCs and whether these cells can influence CD4⁺ T_H2 cell responses have not been examined.

After administration of IL25 to IL4-eGFP (enhanced green fluorescent protein) reporter mice¹⁹, a 1.8-fold increase in the total cell numbers was observed in the mesenteric lymph nodes (MLNs), with equivalent increases in the total numbers of CD4⁺ and CD8⁺ T cells,

B cells and macrophages (Fig. 1a). In contrast, IL25-treatment resulted in a 56-fold increase in an NBNT c-Kit⁺ cell population (Fig. 1b), indicating that the NBNT c-Kit⁺ cells had the greatest relative population expansion after IL25 administration. The IL25-mediated population expansion of the NBNT c-Kit⁺ cells was associated with increased expression of *Il4*, *Il5* and *Il13* messenger RNA in the large intestine (Supplementary Fig. 1a), increased levels of serum IgE (Supplementary Fig. 1b), and increased mucin production in the intestine (Supplementary Fig. 1c)⁵.

Analysis of the IL25-elicited cells showed that in comparison to c-Kit⁺ mast cells, this cell population exhibited intermediate expression of c-Kit (c-Kit^{int}) (Supplementary Fig. 2a). Delivery of IL25 elicited increased frequencies of c-Kit^{int} cells in *W-sash* (*W^{sh}*) mice (Supplementary Fig. 2b), which lack classical mast cell populations²⁰ and induced equivalent expression of *Il13* mRNA and mucin responses in wild-type and *W^{sh}* mice (Supplementary Fig. 2c, d), indicating that IL25 promotes T_H2 cytokine responses independently of mast cells.

Compared to control-treated animals (Supplementary Fig. 3a–c), administration of IL25 increased the frequency of c-Kit^{int} cells in all compartments of the gut-associated lymphoid tissue (GALT) examined, including the MLNs (Fig. 1c), the Peyer's patches (Fig. 1d) and the caecal patch (Fig. 1e). However, IL25 did not elicit this population in the spleen or bone marrow (data not shown), suggesting that IL25-responsive cells may be located in the GALT. Furthermore, analysis of IL25-elicited c-Kit^{int} cells in the GALT showed two distinct cell populations distinguished by expression of IL4-eGFP (Fig. 1c–e, right), indicating that the IL25-elicited c-Kit^{int} cells are a heterogeneous population.

Previous studies reported increased expression of IL25 and increased frequencies of a c-Kit⁺ cell population after exposure to the helminth parasite *Nippostrongylus brasiliensis*^{16,21}. Compared to uninfected controls (Supplementary Fig. 3d), increased frequencies and absolute numbers of c-Kit^{int} cells were observed in the MLNs of wild-type mice after infection with *N. brasiliensis* (Fig. 1f, g). Mice lacking expression of either *Il17rb* or *Il17ra* failed to exhibit IL25-elicited population expansion of the c-Kit^{int} cells (Supplementary Fig. 4a) or the development of *Il13* mRNA and mucin responses (Supplementary Fig. 4b, c), indicating that both IL17RB and IL17RA are required for the IL25-mediated induction of this cell population. Furthermore, the total number of c-Kit^{int} cells induced after *N. brasiliensis* infection were reduced after the administration of an anti-IL25 monoclonal antibody (infected plus control IgG: 58,981 ± 4,975 (mean ± s.e.m.); infected plus anti-IL25 monoclonal antibody: 26,109 ± 3,039).

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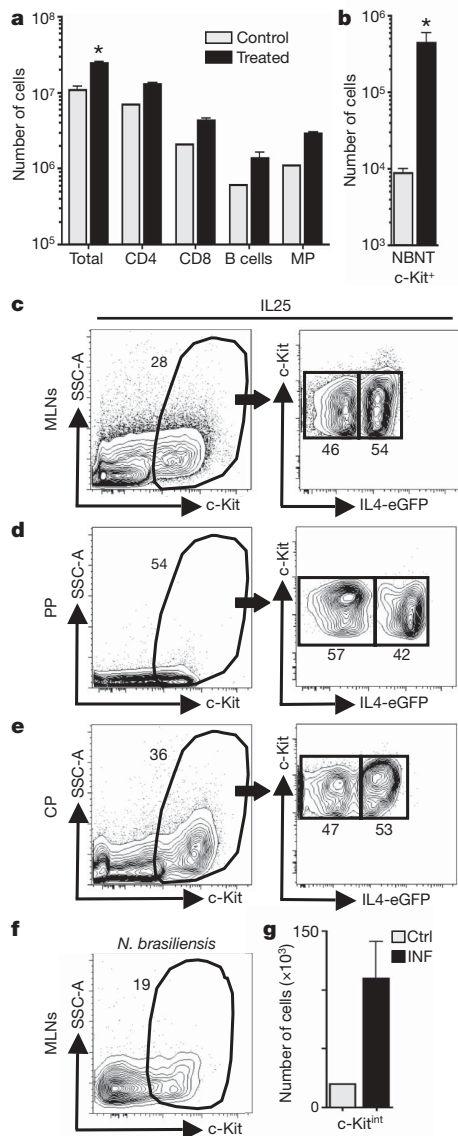


Figure 1 | IL25 elicits a c-Kit^{int}-GFP⁻ and c-Kit^{int}-GFP⁺ cell population in the GALT. **a, b**, Cell numbers from IL25-treated IL4-eGFP reporter mice. Numbers of total MLN cells or of T cells (CD4⁺ or CD8⁺), B cells (CD19⁺) and macrophages (MP; CD11b⁺ MHC class II⁺) (**a**) and total numbers of NBNT c-Kit⁺ cells (**b**) are shown. **c–e**, The frequency of c-Kit^{int} cells in MLNs (**c**), Peyer's patches (PP) (**d**) and caecal patch (CP) (**e**) was assessed by flow cytometry. SSC-A, side scatter area. **f, g**, Frequencies (**f**) and total numbers (**g**) of c-Kit^{int} cells in control (Ctrl) or *N. brasiliensis*-infected (INF) mice. Plots shown are gated on live, CD4⁻ CD8⁻ CD11b⁻ (also known as Itgam or Mac1) CD11c⁻ (Itgax) and B220⁻ (Ptpcr) cells or as indicated. **P* < 0.05. Error bars indicate s.e.m. Data in **a–e** are representative of more than five independent experiments (control, *n* = 16; IL25-treated, *n* = 34). Data in **f** and **g** are representative of at least two independent experiments (control, *n* = 2; infected, *n* = 6).

To test whether IL25-elicited c-Kit^{int} cells influenced the development of antigen-specific or protective T_H2 cytokine responses *in vivo*, carboxyfluorescein succinimidyl ester (CFSE)-labelled ovalbumin (OVA)-specific T-cell receptor (TCR) transgenic T cells were transferred alone or in combination with IL25-elicited c-Kit^{int} cells into naive congenic recipients. As early as 48 h after OVA immunization, donor CD4⁺ T cells began to proliferate (Fig. 2a, left) and accumulated at the site of immunization (Fig. 2b). In mice that also received IL25-elicited c-Kit^{int} cells, the OVA-specific CD4⁺ T-cell proliferation was augmented (Fig. 2a, right) and there was a significant increase in the accumulation of antigen-specific cells (Fig. 2b). Furthermore, MLN cells isolated from recipients of both OVA-specific

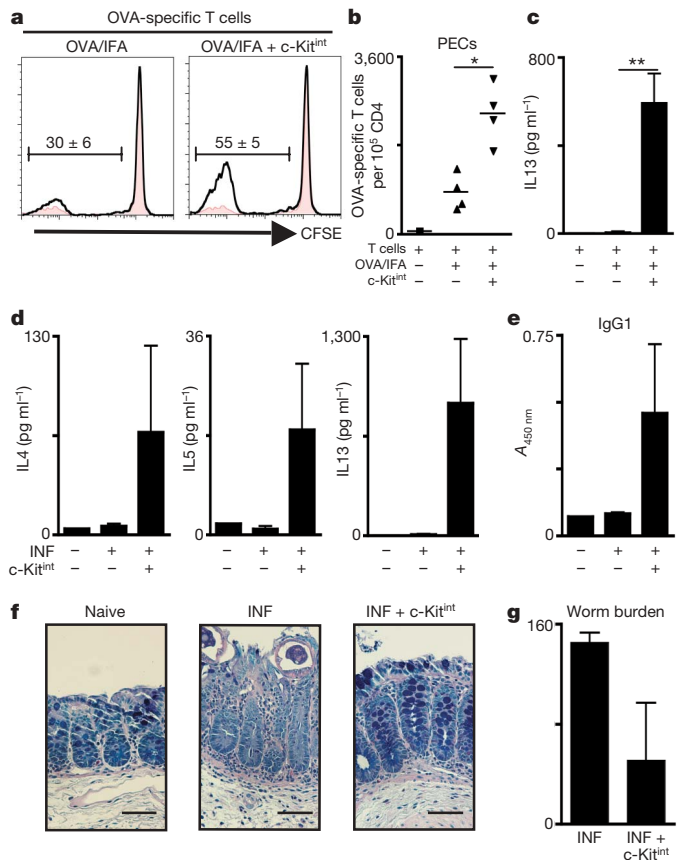


Figure 2 | IL25-elicited c-Kit^{int} cells promote T_H2 cytokine-dependent responses *in vivo*. **a–c**, CFSE-labelled CD45.2 OVA-specific CD4⁺ T cells were adoptively transferred by intravenous (i.v.) injection into CD45.1 congenic recipients, and mice were immunized by intraperitoneal (i.p.) injection with OVA/IFA in the presence or absence of IL25-elicited c-Kit^{int} cells. **a**, Proliferation of donor CD45.2 cells in recipient mice receiving T cells alone (red shaded histogram) or T cells and OVA/IFA immunization ± c-Kit^{int} cells (black histograms) was measured by flow cytometry. **b**, The frequency of donor OVA-specific CD4⁺ cells per total CD4⁺ T cells isolated from the peritoneum. PECs, peritoneal exudate cells. **c**, IL13 production from anti-CD3/CD28-stimulated MLN cells was measured by ELISA. Data in **a–c** are representative of two independent experiments (*n* = 7). **P* < 0.05, ***P* < 0.01. **d–g**, Adoptive transfer of IL25-elicited c-Kit^{int} cells protects *T. muris*-infected IL25^{-/-} mice. Cytokine production by anti-CD3/CD28-stimulated MLN cells (**d**), *T. muris*-specific serum IgG1 antibody titres (**e**), intestinal mucin responses (**f**), and number of worms from *T. muris*-infected mice (**g**) were assessed at day 20 after infection. Scale bars, 50 μm. Data in **c–e** and **g** are mean ± s.e.m.; horizontal bars in **b** denote the mean.

CD4⁺ T cells and IL25-elicited c-Kit^{int} cells secreted increased levels of IL13 compared to controls (Fig. 2c). *Trichuris muris*-infected IL25^{-/-} mice produced low levels of T_H2 cytokines and parasite-specific IgG1 (Fig. 2d, e), impaired mucin responses (Fig. 2f), and were susceptible to infection (Fig. 2g)²². However, adoptive transfer of IL25-elicited c-Kit^{int} cells from wild-type mice into infected IL25^{-/-} mice resulted in increased production of IL4, IL5 and IL13 and parasite-specific IgG1 (Fig. 2d, e), increased mucin responses (Fig. 2f), and host-protective immunity (Fig. 2g). Taken together, these data indicate that IL25-elicited c-Kit^{int} cells could promote antigen-specific T_H2 cytokine responses and protective immunity to helminth infection.

IL25-elicited c-Kit^{int}-GFP⁻ or c-Kit^{int}-GFP⁺ cells lacked expression of CD4 (Supplementary Fig. 5a), indicating that they were distinct from the CD4⁺ NKT cell populations⁷, and did not express other surface markers associated with CD4⁺ T cells (Supplementary Fig. 5a). Furthermore, the IL25-elicited c-Kit^{int} populations did not express B-cell-, basophil- or eosinophil-associated surface markers (Supplementary Fig. 5b, c). Most IL25-elicited

c-Kit^{int}-GFP⁻ cells were T1/ST2^{-/-} (also known as IL1RL1, a subunit of IL33R) and IL7R α ⁻ whereas IL25-elicited c-Kit^{int}-GFP⁺ cells were T1/ST2^{-/-} and heterogeneous for expression of IL7R α (Supplementary Fig. 5d), suggesting that both populations are distinct from NHCs¹⁵. Consistent with this, both c-Kit^{int} populations expressed little or no mRNA encoding *Gata3*, *Junb*, *Maf*, *Stat6* and *Il1rl1* (Supplementary Fig. 5e). Delivery of IL25 resulted in increased frequencies of c-Kit^{int} cells in the peritoneum and mesentery (Supplementary Fig. 6a, b). However, although IL25 treatment increased the cellularity in the mesentery, no changes were observed in the frequency of NHCs or in their expression of CD44 or Thy1.2 (Thy1) (Supplementary Fig. 6c). Taken together, these data indicate that IL25-elicited c-Kit^{int} cells are a unique population and are not T- or B-lymphocytes, NKT cells, basophils, eosinophils, mast cells or NHCs.

HSCs and MPPs express c-Kit and Sca-1 and are characterized as Lin⁻ (refs 23, 24). Although HSCs are primarily localized in the bone marrow, they can circulate in the periphery^{25–28} and have been implicated in immunosurveillance^{17,18}. IL25-elicited c-Kit^{int}-GFP⁻ and c-Kit^{int}-GFP⁺ populations were Lin⁻ (Supplementary Fig. 7), and most of the IL25-elicited c-Kit^{int}-GFP⁻ and c-Kit^{int}-GFP⁺ cells expressed Sca-1, were CD150⁻ (also known as SLAMF1), and showed heterogeneous expression of CD34 (Fig. 3a, b). Therefore, the IL25-elicited cell populations had a surface phenotype consistent with being a MPP-like cell. Although the administration of IL25 induced MPP-like cells in the GALT, the frequencies of MPPs, and of short-term and long-term HSCs in the bone marrow were unchanged after IL25 treatment (Supplementary Fig. 8a, b).

To assess the capacity of the c-Kit^{int} MPP-like cell population to exhibit multipotent potential, IL25-elicited c-Kit^{int}-GFP⁻ or c-Kit^{int}-GFP⁺ cells were sorted and cultured *in vitro* in the presence of SCF (also known as Kitl) and IL3 (Fig. 3c–f). Unfractionated bone marrow cells from naive mice differentiated into a CD11b⁺ macrophage-like population (Supplementary Fig. 9a, orange gate) and a CD11b⁻ granulocyte population that could be identified as mast cells or basophils based on expression of c-Kit and Fc ϵ RI in addition to cell morphology (Supplementary Fig. 9a, b). Sorted IL25-elicited c-Kit^{int}-GFP⁺ cells gave rise to a CD11b⁻ c-Kit⁺ Fc ϵ RI⁺ mast cell population (Fig. 3c, red gate), but failed to give rise to CD11b⁺ progeny. Consistent with this, the progeny of c-Kit^{int}-GFP⁺ cells were morphologically similar to mast cells (Fig. 3d). IL25-elicited c-Kit^{int}-GFP⁻ input cells also gave rise to a CD11b⁻ mast cell population (Fig. 3e, red gate). In addition, the c-Kit^{int}-GFP⁻ input cells gave rise to c-Kit⁺ Fc ϵ RI⁺ basophils (Fig. 3e, blue gate) and CD11b⁺ macrophages (Fig. 3e, orange gate). The multipotent potential of the c-Kit^{int}-GFP⁻ cell population was confirmed by cell morphology (Fig. 3f). c-Kit^{int}-GFP⁻ cells cultured in the presence of SCF and IL3 or using OP9 stromal cells²⁹ also gave rise to a CD11b⁺ M-CSFR⁺ Ly6C⁺ MHC class II⁺ cell population (Supplementary Fig. 10a, b), consistent with myeloid potential. IL25-elicited c-Kit^{int}-GFP⁻ cells were isolated from wild-type CD45.2 donor mice and adoptively transferred into naive CD45.1 congenic recipients. Six days after transfer, c-Kit^{int}-GFP⁻ donor cells differentiated into CD11b⁺ cells as well as CD11b⁻ c-Kit⁺ Fc ϵ RI⁺ cells (Supplementary Fig. 11). Collectively, these data indicate that the c-Kit^{int}-GFP⁻ cells may represent a previously unrecognized IL25-responsive MPP-like cell population in the GALT that selectively has multipotent potential *in vitro* and *in vivo*.

IL25-elicited c-Kit^{int} cell populations were sorted (Fig. 4a, left) and cultured *in vitro* in the presence of SCF and IL3 for 8 days. Most of the progeny derived from c-Kit^{int}-GFP⁺ cells were IL4-eGFP⁺ MHC class II⁻, whereas the c-Kit^{int}-GFP⁻-derived progeny contained IL4-eGFP⁺ and MHC class II⁺ populations (Fig. 4a, right). To test whether these cells could influence T-cell proliferation and/or differentiation, progeny from sorted c-Kit^{int}-GFP⁻ or c-Kit^{int}-GFP⁺ cells were pulsed with OVA and co-cultured with CFSE-labelled OVA-specific TCR transgenic CD4⁺ T cells. In the absence of antigen,

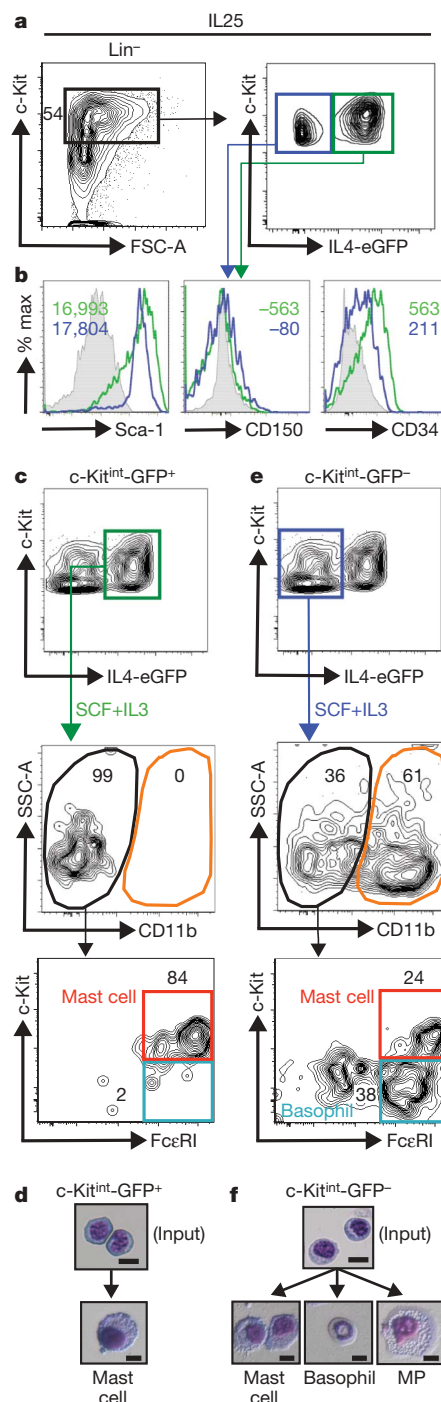


Figure 3 | IL25-elicited c-Kit^{int} cells show multipotent capacity.

a, b, Frequencies of c-Kit^{int} cells in the MLNs of IL25-treated IL4-eGFP reporter mice (**a**) and expression of HSC markers by c-Kit^{int}-GFP⁻ (blue histograms) or c-Kit^{int}-GFP⁺ (green histograms) cells from IL25-treated IL4-eGFP reporter mice (**b**). Number indicates the mean fluorescent intensity. Plots shown are gated on live, lineage⁻ cells (CD3 ϵ , CD8 α , CD8 β , TCR β , TCR $\gamma\delta$, B220, CD19, CD11b, CD11c, Gr-1 (also known as Ly6g), NK1.1 (Kl1rb1c) and Ter119 (Ly76)) or as indicated. FSC-A, forward scatter area. Data in **a** and **b** are representative of two independent experiments (control, $n = 4$; IL25-treated, $n = 7$). **c, e**, Flow cytometric analysis of myeloid cell and granulocyte differentiation of FACS-purified Lin⁻ c-Kit^{int}-GFP⁺ (**c**) or c-Kit^{int}-GFP⁻ (**e**) cells from IL25-treated IL4-eGFP reporter mice after *in vitro* culture in SCF and IL3. **d, f**, Cytopsin preparation of progeny derived from IL25-elicited c-Kit^{int}-GFP⁺ cells (**d**) or c-Kit^{int}-GFP⁻ cells (**f**). MP, macrophage. Scale bars, 10 μ m. Data in **c–f** are representative of at least three independent experiments.

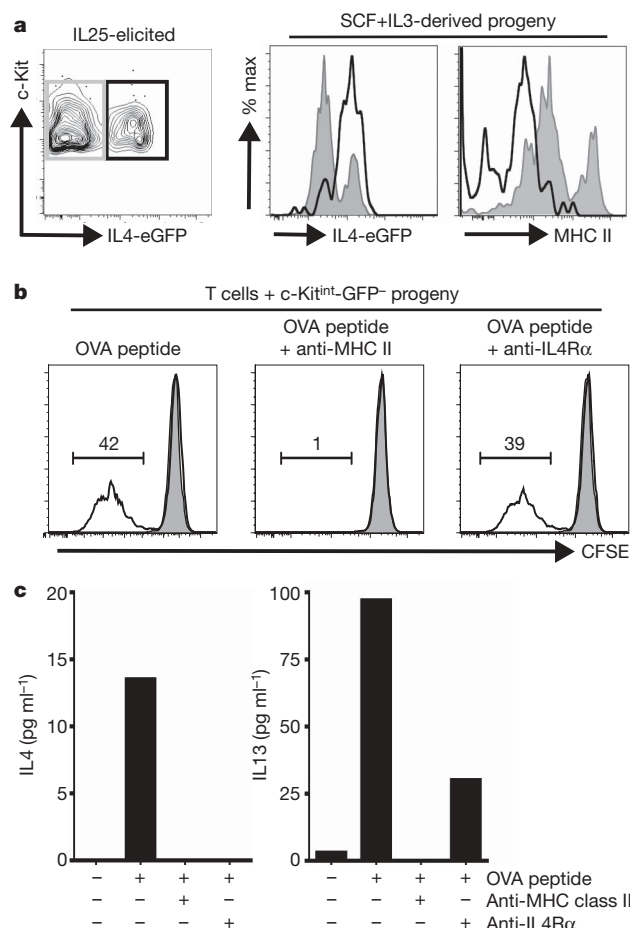


Figure 4 | Progeny from IL25-elicited c-Kit^{int}-GFP⁻ cells promote T_H2 cell differentiation. **a**, FACS-purified IL25-elicited c-Kit^{int}-GFP⁻ cells were cultured for 8 days in SCF and IL3 and the resulting progeny from c-Kit^{int}-GFP⁺ (black histogram) or c-Kit^{int}-GFP⁻ (grey shaded histogram) cells were assessed for expression of IL4-eGFP and MHC class II. Results in **a** are representative of three independent experiments. **b**, CFSE-dilution by OVA-specific CD4⁺ CD62L^{hi} CD44^{lo} T cells after 4 days co-culture with progeny from FACS-purified IL25-elicited c-Kit^{int}-GFP⁻ cells in the presence of OVA peptide with or without addition of monoclonal antibody against MHC class II or IL4Rα. Grey histogram indicates CFSE-dilution by OVA-specific CD4⁺ T cells cultured in medium alone. **c**, IL4 and IL13 protein levels in cell-free supernatants from **b** were assayed by ELISA. Results in **b** and **c** are representative of at least two independent experiments.

T cells showed minimal proliferation and T_H2 cytokine production (1% CFSE^{dim}, Fig. 4b, shaded histograms, c). Antigen-pulsed c-Kit^{int}-GFP⁺-derived progeny cells failed to induce T-cell proliferation (Supplementary Fig. 12, black histogram) or the production of T_H2 cytokines (data not shown). In contrast, antigen-pulsed progeny derived from c-Kit^{int}-GFP⁻ cells induced MHC class II-dependent T-cell proliferation and production of IL4 and IL13 (Fig. 4b, c). Inclusion of an anti-IL4Rα monoclonal antibody did not affect T-cell proliferation (Fig. 4b), but resulted in decreased production of IL4 and IL13 (Fig. 4c), indicating that both MHC class II and IL4R signalling are required for the c-Kit^{int}-GFP⁻-derived progeny to influence T_H2 cell differentiation. No IFN-γ was detected in any culture conditions (data not shown). These results indicate that IL25-elicited c-Kit^{int} cells contain a population of progenitors with multipotent capacity, termed MPP^{type2} cells, the progeny of which act to promote CD4⁺ T_H2 cell differentiation.

Collectively, these findings indicate that in addition to a c-Kit^{int}-GFP⁺ cell population that differentiates into mast cells, IL25 elicits a MPP-like cell population that can differentiate into monocyte/macrophage and granulocyte lineages (Supplementary Fig. 13).

Coupled with reports that peripheral HSCs express Toll-like receptors and can respond to microbial stimulation^{17,18}, these findings indicate a previously unrecognized pathway in which peripheral MPP^{type2} cells can promote type 2 inflammation at mucosal sites, and suggest an evolutionarily conserved pathway between the IL17 cytokine family, extramedullary haematopoiesis and adaptive immunity.

METHODS SUMMARY

Mice were treated by intraperitoneal (i.p.) injection with PBS or 0.4 μg recombinant IL25 daily for 4 days, or infected with *N. brasiliensis*. MLNs from IL4-eGFP reporter mice were separated from the mesentery, homogenized and stained with anti-mouse fluorochrome-conjugated monoclonal antibodies against combined lineage markers and c-Kit, FcεRIα, T1/ST2 and IL7Rα. IL25-elicited c-Kit^{int} cells were sorted using a FACSaria and transferred into wild-type mice immunized with OVA in incomplete Freund's adjuvant (OVA/IFA) or into *T. muris*-infected IL25^{-/-} mice, and T_H2 cytokine responses were measured. IL25-elicited c-Kit^{int}-GFP⁺ and c-Kit^{int}-GFP⁻ cell populations were either sorted and transferred into CD45.1 congenic recipients to assess *in vivo* differentiation, or incubated *in vitro* in the presence of SCF (50 ng ml⁻¹) and IL3 (10 ng ml⁻¹). Progeny were analysed by cytochrome or flow cytometry for surface expression of lineage-specific markers. Some progeny were co-cultured with CFSE-labelled OVA-specific TCR transgenic CD4⁺ T cells in the presence of OVA peptide with or without blocking antibodies against MHC class II or IL4Rα. CFSE dilution in CD4⁺ T cells was assessed by flow cytometry, and cell-free supernatants were analysed for secretion of IL4 and IL13 by sandwich ELISA.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Mice. BALB/c, C57BL/6 and W^{sh} mice were obtained from Jackson Laboratory and IL4-eGFP reporter mice were obtained from M. Mohrs. $IL25^{-/-}$ mice were provided by R. A. Kastelein. $IL17ra^{-/-}$ and $IL17rb^{-/-}$ mice were provided by J. E. Tocker and A. L. Budelsky. Animals were bred and housed in specific pathogen-free conditions at the University of Pennsylvania. All experiments were performed under Institutional Animal Care and Use Committee (IACUC) approved protocols and in accordance with the guidelines of the IACUC of the University of Pennsylvania. All mice used were 4–12 weeks of age. Mice were treated intraperitoneally with PBS or recombinant IL25 (0.4 μ g; R&D Systems) daily for 4 days.

Flow cytometry, cell sorting and CD4⁺ T-cell co-culture. MLNs from IL4-eGFP reporter mice were separated from the mesentery, homogenized by passing through a 70- μ m nylon mesh filter, and stained with anti-mouse fluorochrome-conjugated monoclonal antibodies against CD3 ϵ , CD4, CD8, TCR β , TCR $\gamma\delta$, B220, CD19, CD11b, CD11c, MHC class II, Gr-1, NK1.1, Ter119, Fc ϵ RI α , c-Kit, Sca-1, CD150, CD34, CD62L (also known as Sell), CD69, IL7R α (CD127), CD45.2, CD49b and CCR3 (eBioscience and BD Bioscience). T1/ST2 staining was performed using T1/ST2 biotinylated monoclonal antibody (MD Biosciences) and phycoerythrin (PE)- or eFluor450-conjugated streptavidin (eBioscience). Peritoneal exudate cells ('peritoneum') were collected using peritoneal lavage with injection of 10 ml PBS and aspirated using the same syringe. Mesentery was processed as previously described¹⁵. Cells were run on a BD FACSCanto II using DiVa software (BD Bioscience) and analysed with FlowJo software (version 8.7.1; Tree Star). IL25-elicited c-Kit^{int} (GFP⁺ or GFP⁻) cell populations were sorted using a FACSARIA (BD Bioscience), and for *in vitro* differentiation studies were incubated in the presence of SCF (50 ng ml⁻¹; R&D systems) and IL3 (10 ng ml⁻¹; R&D systems). After *in vitro* culture, progeny were assessed for expression of CD11b, MHC class II, CD115 (also known as Csf1r and M-CSFR), Ly6C (also known as Ly6c1), Fc ϵ RI α and c-Kit (eBioscience) by flow cytometry as described earlier. Naive OVA-specific CD4⁺ T cells were isolated from DO11.10 mice as previously described¹⁴. T cells were stained with fluorochrome-conjugated monoclonal antibodies against CD4, CD62L and CD44 (eBioscience), re-suspended in 2% FBS in HBSS with 2 mM EDTA (Gibco) with 4',6-diamidino-2-phenylindole (DAPI) (1 μ g ml⁻¹; Molecular Probes), and naive T cells were sorted on the basis of live cells (DAPI⁻) CD4⁺ CD62L^{hi} CD44^{lo} using a FACSARIA (BD Bioscience). T cells were labelled with CFSE (Molecular Probes) and co-cultured in the presence of OVA peptide (1 μ g ml⁻¹) and blocking antibodies to MHC class II (5 μ g ml⁻¹; M5/114; eBioscience) or IL4R α (5 μ g ml⁻¹; mIL4R-M1; BD Bioscience) in complete medium (DMEM Iscove's with 10% (v/v) heat-inactivated FBS, 2 mM glutamine, 100 U ml⁻¹ penicillin, 100 μ g ml⁻¹ streptomycin, 25 mM HEPES and 50 μ M β -mercaptoethanol). Cell-free supernatants were assessed for cytokine production by standard sandwich ELISA (eBioscience) after 4 h stimulation with 50 ng ml⁻¹ phorbol myristate acetate, 750 ng ml⁻¹ ionomycin (Sigma Aldrich).

OVA immunization, helminth infections and adoptive transfers. CFSE-labelled OVA-specific CD45.2 CD4⁺ T cells ($3\text{--}5 \times 10^6$) were transferred intravenously into CD45.1 congenic recipient mice, and 24 h later were immunized by

i.p. injection with 100 μ g OVA emulsified in IFA, with one cohort receiving 5×10^4 IL25-elicited c-Kit^{int} cells intraperitoneally. Proliferation of OVA-specific CD4⁺ T cells in the spleen was assessed 48 h after immunization, and cytokine production was measured by ELISA. *T. muris* infections were performed as previously described²². *T. muris*-infected $IL25^{-/-}$ mice were left untreated or given 5×10^4 IL25-elicited c-Kit^{int} cells at day 10 after infection. Worm counts were performed at day 20 after infection. MLN cells were collected at necropsy, plated in medium alone or polyclonally stimulated with 1 μ g ml⁻¹ each of anti-CD3 and anti-CD28 (eBioscience). After 48 h, cell-free supernatants were assessed for cytokine production by sandwich ELISA (eBioscience). *T. muris*-specific IgG1 antibody titres were analysed by ELISA as described previously²². Total serum IgE was measured using the OptEIA IgE ELISA kit according to the manufacturer's instructions (BD Biosciences). For *N. brasiliensis* infections, wild-type mice were infected subcutaneously with 500 infective third-stage larvae (L3) and treated with 0.5 mg of anti-IL25 or control IgG (from J.E.T. and A.L.B.) on days 0, 2, 4, 6 and 8. MLN cells from infected mice were assessed at day 10 after infection for the induction of c-Kit^{int} cells. For *in vivo* differentiation assays, 3×10^4 CD45.2 IL25-elicited c-Kit^{int}-GFP⁻ cells were FACS-purified and transferred intraperitoneally into CD45.1 congenic recipient mice. Recipient mice were treated four times with 1 μ g each of SCF and IL3, and differentiation of donor cells was assessed on day 6 after transfer.

HSC differentiation assays. FACS-purified cell populations were plated onto semi-confluent OP9 stromal cells (American Type Culture Collection (ATCC), CRL-2749), as previously described³⁰. Monolayers were irradiated (30 Gy) before co-culture with FACS-purified populations. Cells were incubated in the presence of SCF (50 ng ml⁻¹; R&D systems) and IL3 (10 ng ml⁻¹; R&D systems).

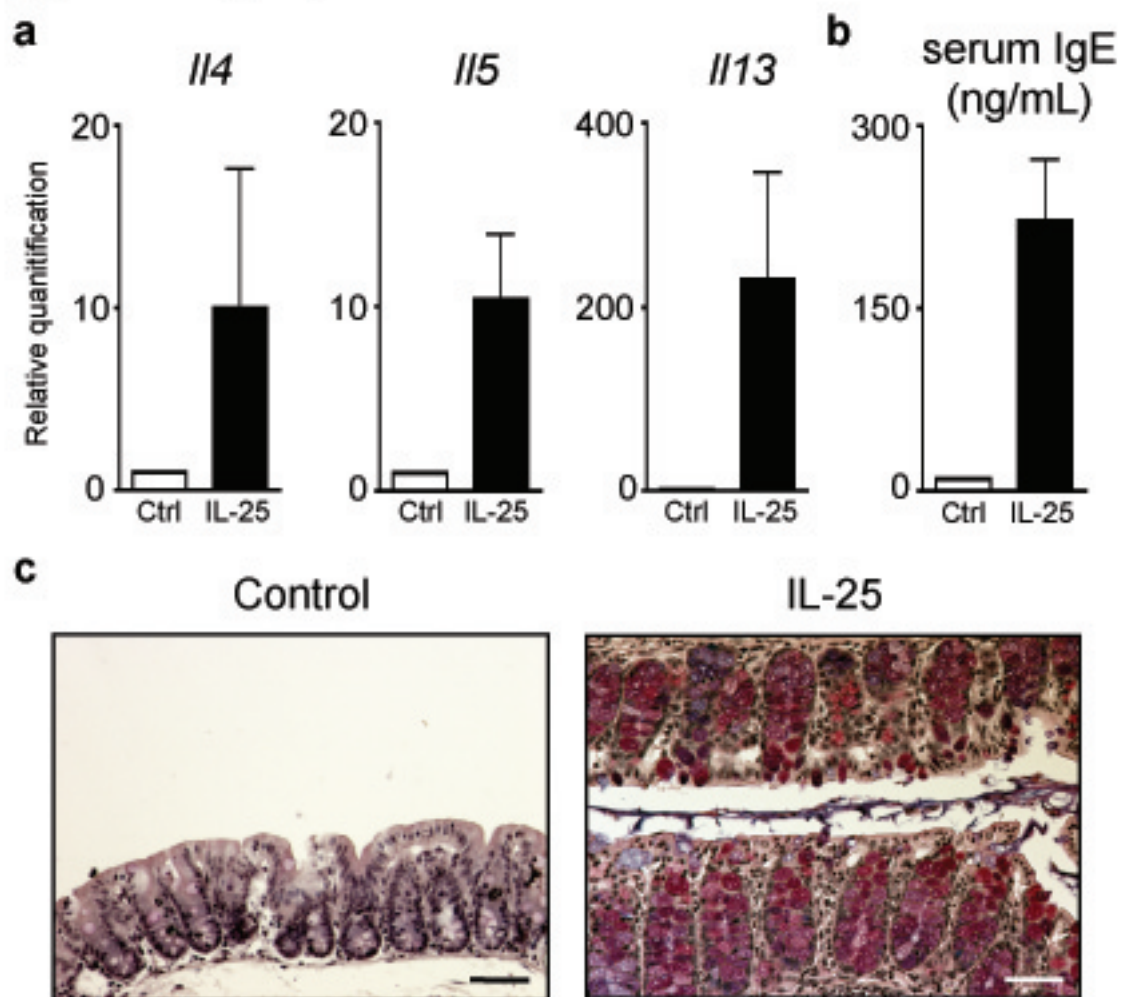
Histology and cytospin preparation. Colon sections were fixed in 4% (v/v) paraformaldehyde and embedded in paraffin wax. Four-micrometre sections were stained with periodic acid-Schiff (PAS) and Alcian blue. Purified cell populations were subjected to cytospin (Cytospin 3, Thermo Fisher Scientific) and stained by Diff-quick for analysis of cellular morphology.

Real-time PCR. RNA from colonic tissues of mice was isolated by TRIzol extraction (Invitrogen) or collected from sorted cell populations using an RNeasy Mini kit (Qiagen). Whole tissues were homogenized with a tissue homogenizer (TissueLyzer; Qiagen) and complementary DNA was prepared with SuperScript Reverse Transcriptase (Invitrogen). Quantitative real-time PCR analysis used commercial QuantiTect primer sets for *Il4*, *Il5*, *Il13*, *Gata3*, *Maf*, *Junb*, *Stat6* and *Il1rl1* (Qiagen) and SYBR Green chemistry (Applied Biosystems). All reactions were run on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). Target genes were normalized to endogenous β -actin levels and relative quantification of samples were compared to controls.

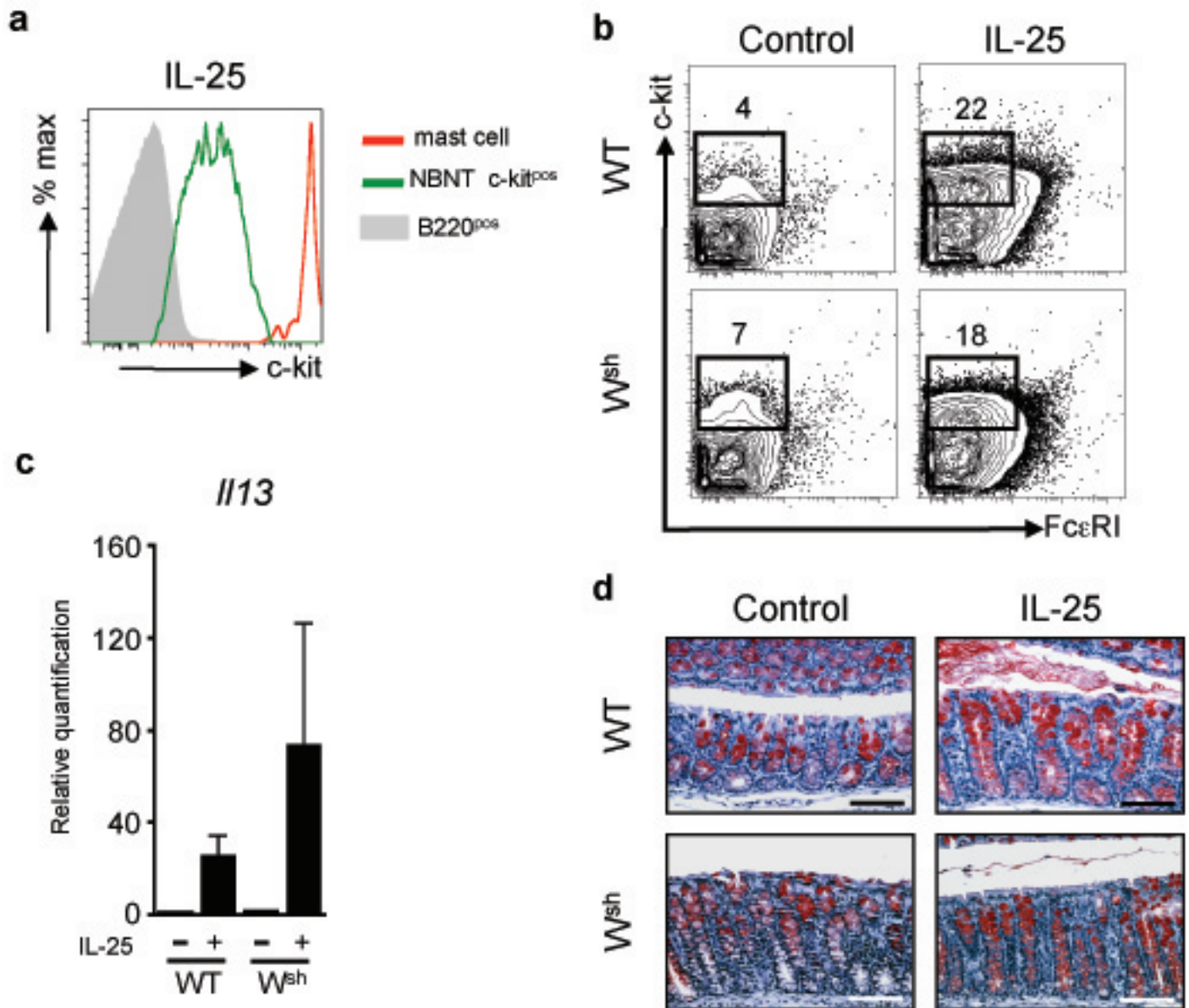
Statistical analysis. Results are shown as means $\pm$ s.e.m. for individual animals. Statistical significance was determined by Student's *t*-test. Results were considered significant at $P < 0.05$.

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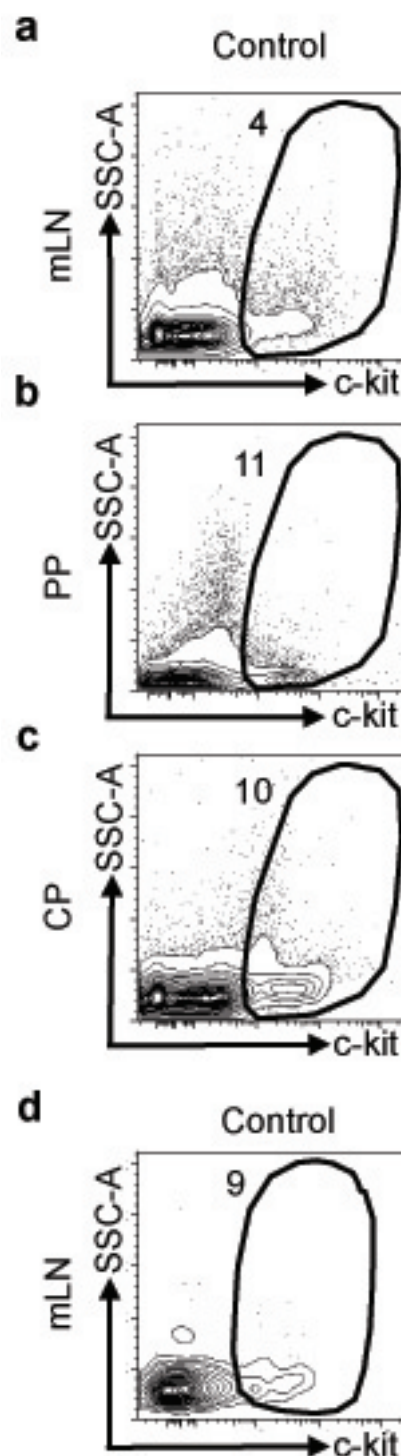
SUPPLEMENTARY INFORMATION



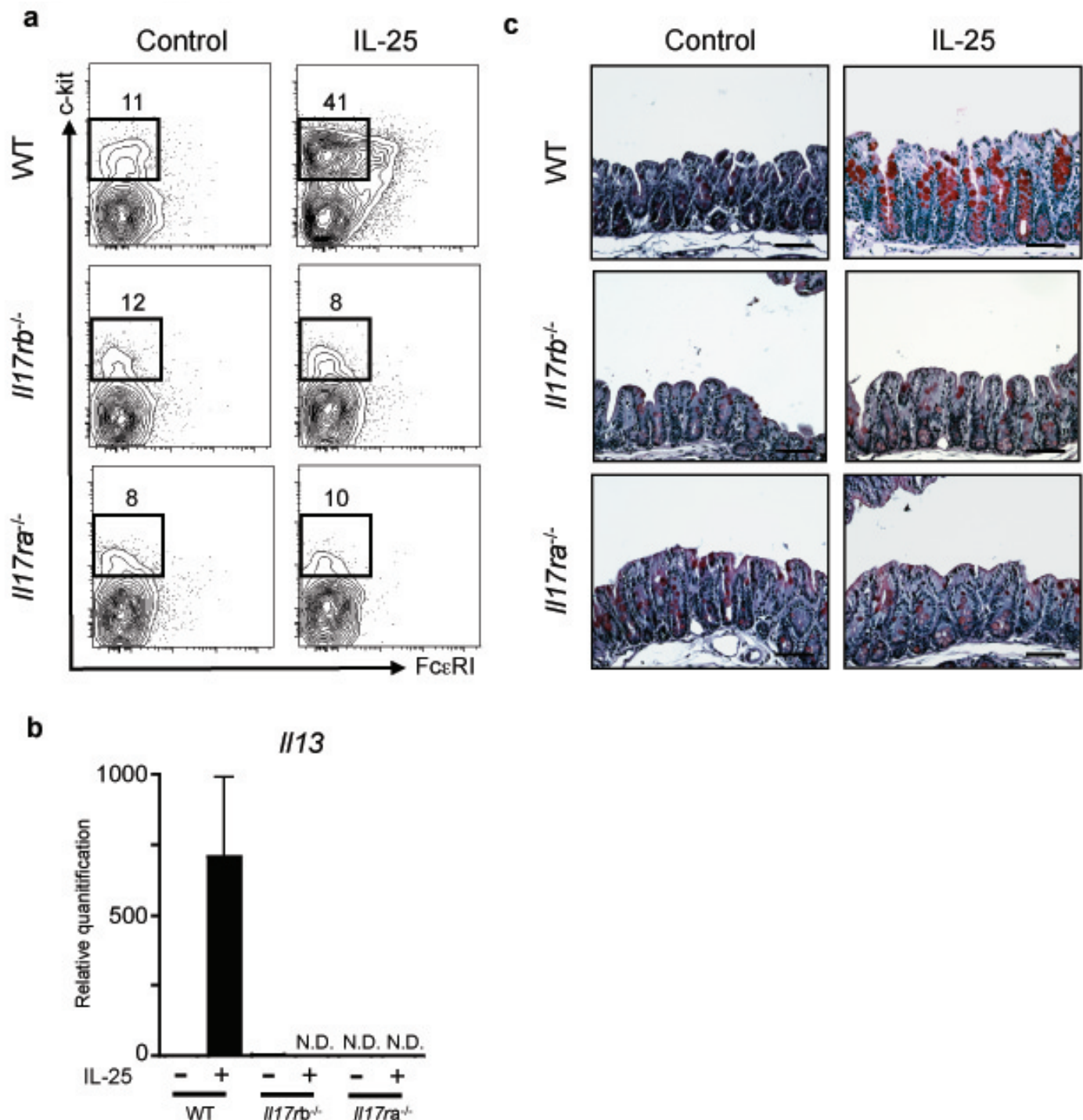
Supplementary Figure 1. IL-25 promotes type 2 inflammation *in vivo*. **a-c**, IL-4/eGFP reporter mice were treated daily with recombinant IL-25 for 4 days. Real-time PCR analysis of *IL4*, *IL5* and *IL13* mRNA expression from proximal colon sections of control or IL-25-treated IL-4/eGFP reporter mice (**a**). Total serum IgE levels measured by ELISA (**b**). Mucin responses analyzed by Periodic acid-Schiff/Alcian blue staining of proximal colon sections from control or IL-25-treated IL-4/eGFP reporter mice (**c**). Scale bar, 50 μ m. Error bars indicate s.e.m. Data in **a-c** are representative of three independent experiments (control, n=4; IL-25-treated, n=7).



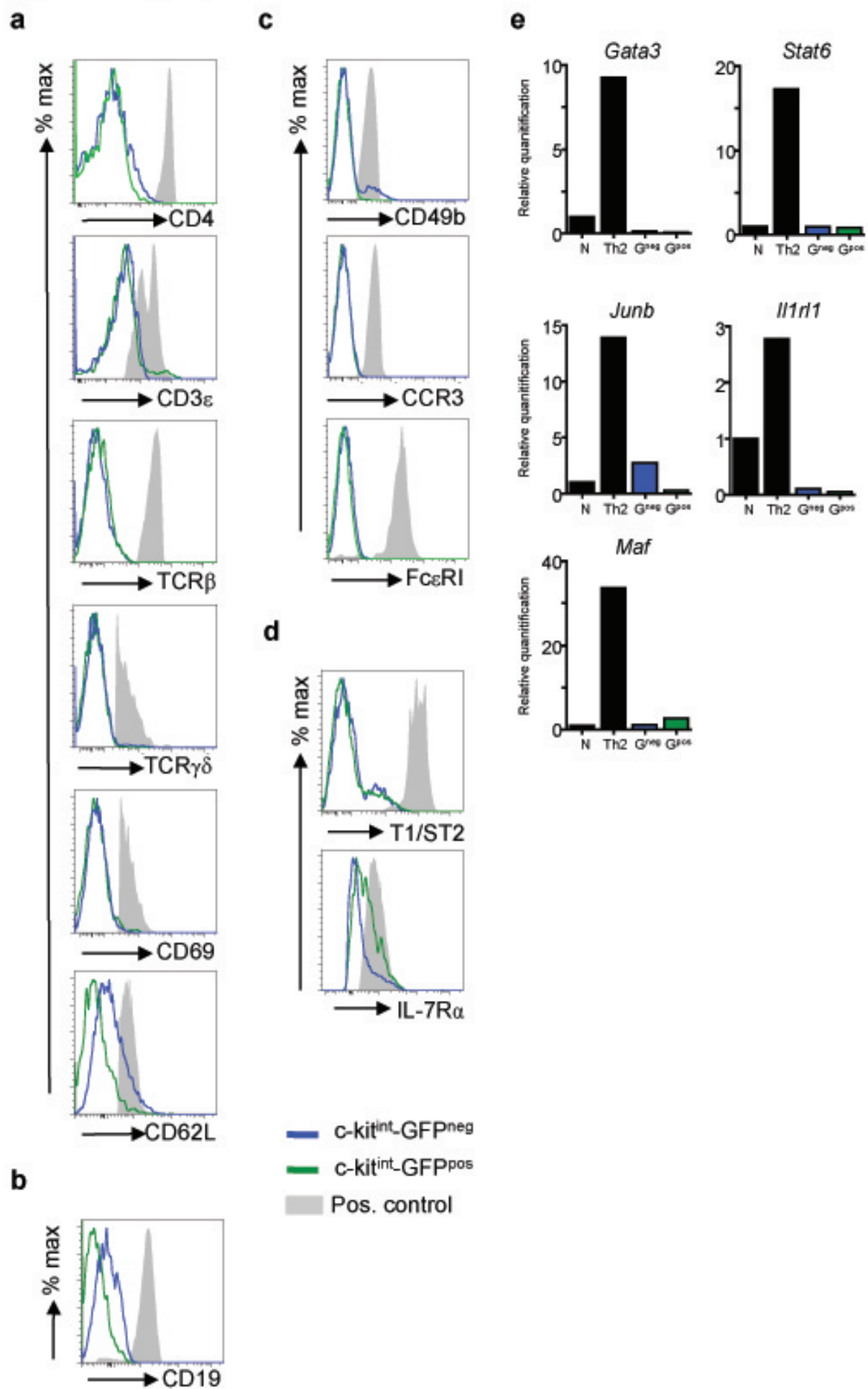
Supplementary Figure 2. IL-25-induced $c\text{-kit}^{\text{int}}$ cells do not represent a classical mast cell population. **a**, Expression of $c\text{-kit}$ by mature mast cells (from peritoneum, red line), NBNT $c\text{-kit}^{\text{pos}}$ cells (from mLN, green line) and B220 $^{\text{pos}}$ cells (from mLN, shaded line) from IL-25-treated IL-4/eGFP reporter mice. **b-d**, Recombinant IL-25 was administered daily to C57BL/6 (WT) or W^{sh} mice for 4 days. Mesenteric lymph node cells were analyzed for $c\text{-kit}$ and FcεRI surface expression by flow cytometry (**b**). Plots shown are gated to exclude CD4, CD8, B220, CD11b, and CD11c positive cells. Numbers are frequencies of $c\text{-kit}^{\text{int}}$ FcεRI $^{\text{neg}}$ cells of total NBNT population. Real time-PCR analysis of *I13* mRNA from proximal colon sections (**c**). Mucin responses analyzed by Periodic acid-Schiff/Alcian blue staining of proximal colon sections from control or IL-25-treated mice (**d**). Scale bar, 100 μm . Error bars indicate s.e.m. Data in (**a**) are representative of 10 independent experiments (control, $n=16$; IL-25-treated, $n=34$). Data in **b-d** are representative of at least two independent experiments (control, $n=2$; IL-25-treated, $n=6-8$).



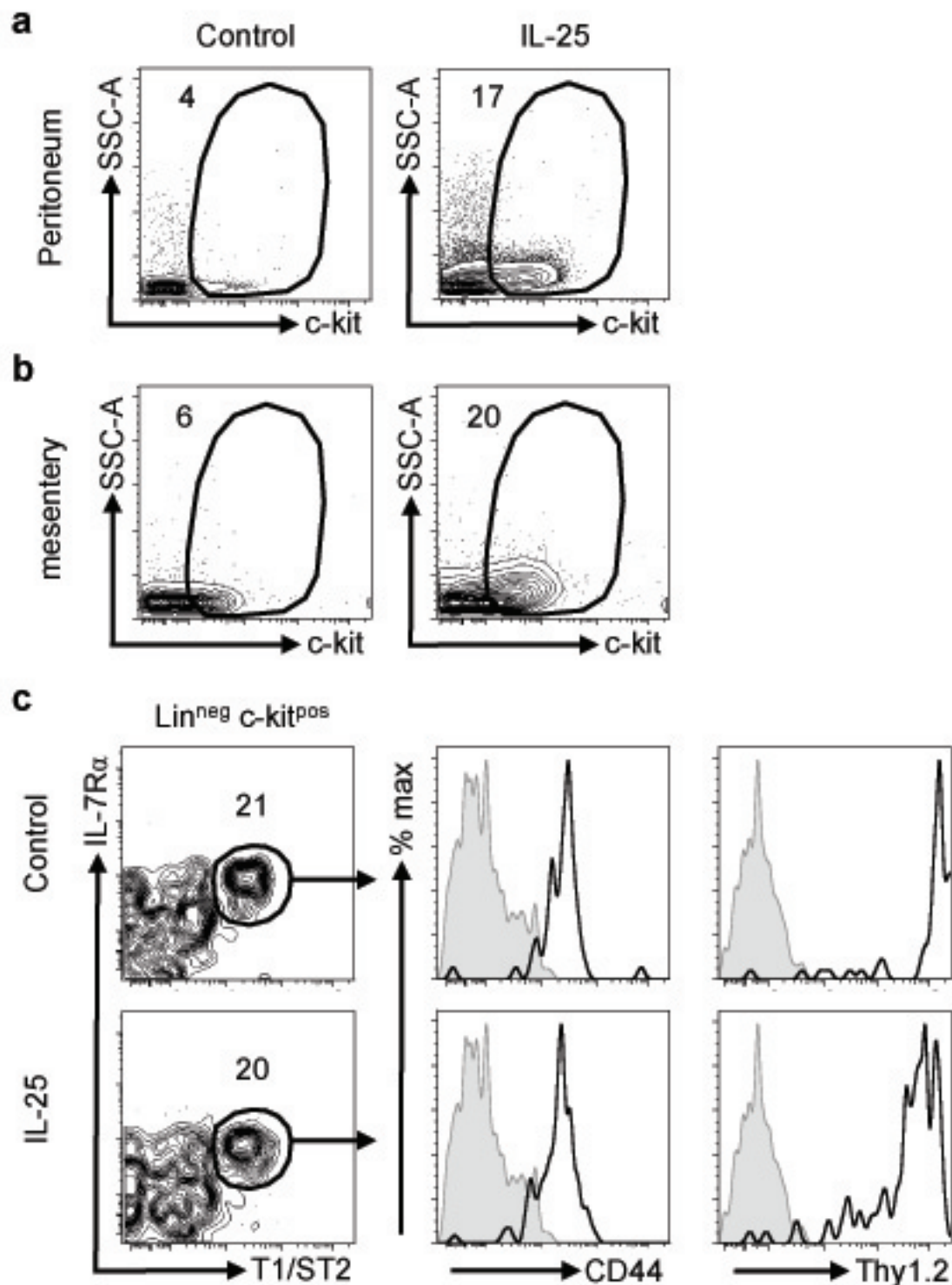
Supplementary Figure 3. NBNT c-kit^{int} cell population in the GALT of control mice. **a-c**, Frequency of c-kit^{int} cells in mLNs (**a**), Peyer's patches (PP) (**b**) and cecal patch (CP) (**c**) from control IL-4/eGFP reporter mice was assessed by flow cytometry. **d**, Frequency of c-kit^{int} cells in uninfected control (Ctrl) WT Balb/c mice. Plots shown are gated on live, CD4^{neg} CD8^{neg} CD11b^{neg} CD11c^{neg} and B220^{neg} cells. Data in **a-c** are representative of five independent experiments. Data in (**d**) are representative of at least two independent experiments.



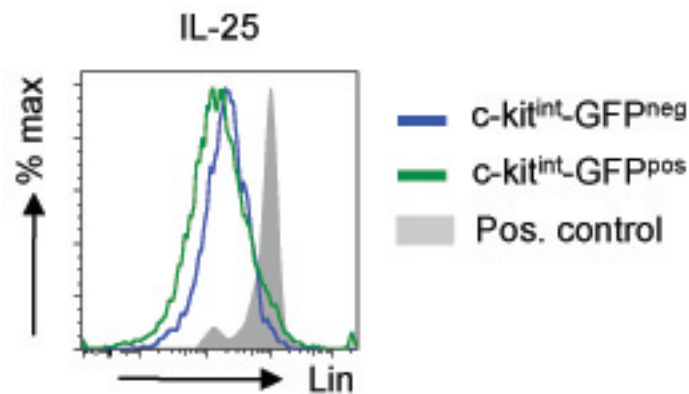
Supplementary Figure 4. IL-17RB and IL-17RA are required for IL-25-mediated induction of the c-kit^{int} cell population and Th2 cytokine responses. **a-c**, C57BL/6 (WT), *Il17rb*^{-/-}, and *Il17ra*^{-/-} mice were treated daily with recombinant IL-25 for 4 days. Mesenteric LN cells were analyzed for c-kit and FcεRI expression (**a**). Plots shown are gated to exclude CD4, CD8, B220, CD11b, and CD11c positive cells. Real-time PCR analysis of intestinal *Il13* mRNA expression (**b**). Mucin responses analyzed by Periodic acid-Schiff/Alcian blue staining of proximal colon sections from control or IL-25-treated mice (**c**). Scale bar, 100 μm. Error bars indicate s.e.m. Not detected, N.D. Data in **a-c** are representative of at least two independent experiments (control, n=2; IL-25-treated, n=6).



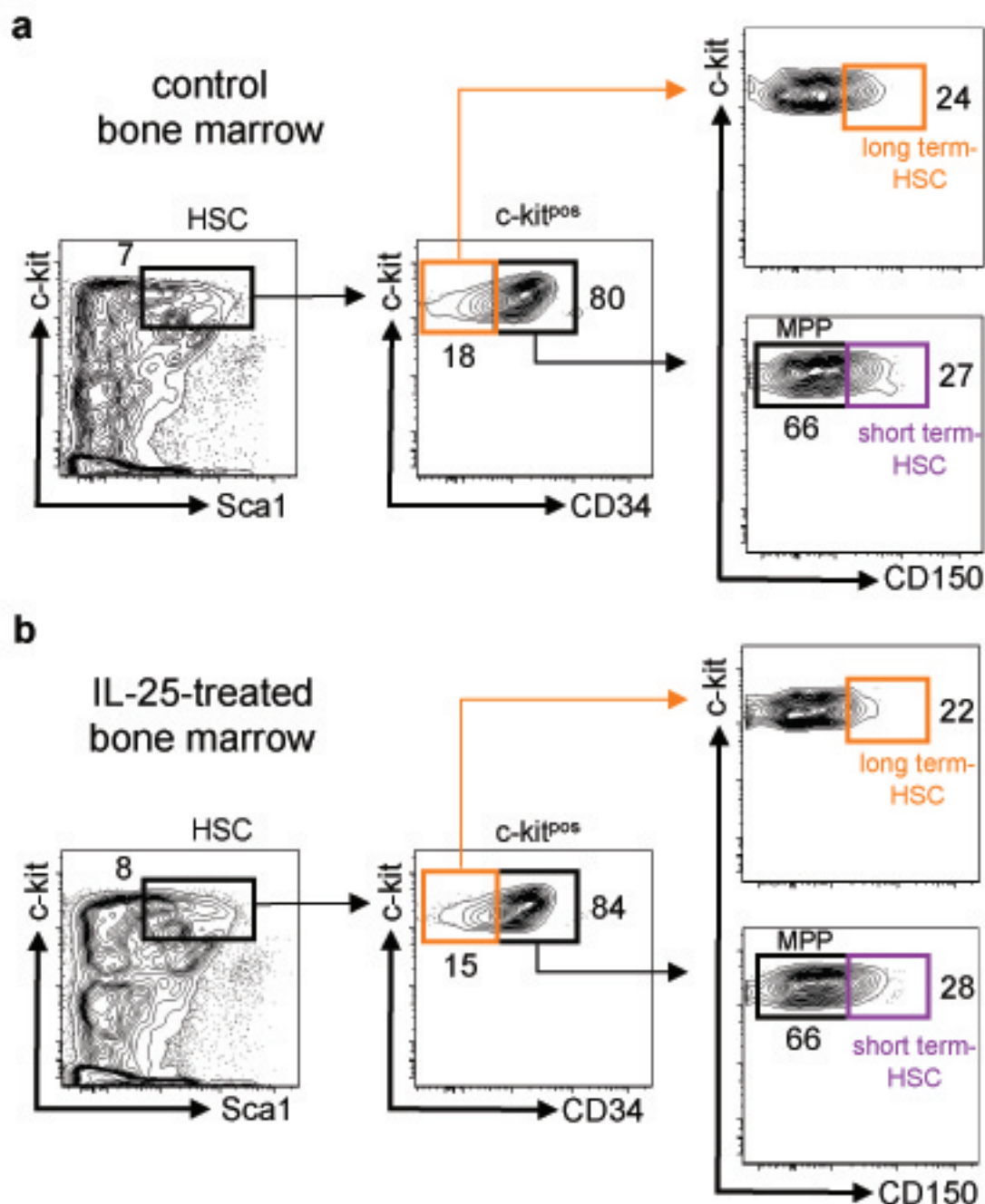
Supplementary Figure 5. IL-25-induced c-kit^{int} cells do not represent NKT cell, basophil, eosinophil, mast cell or natural helper cell populations. a-d, Expression of surface markers on c-kit^{int}-GFP^{neg} (blue line) or c-kit^{int}-GFP^{pos} (green line) cells from IL-25-treated mice analyzed by flow cytometry. Shaded histograms indicate the positive control for each respective surface marker (**a**, gated on CD4^{pos} T cells expressing each respective marker; **b**, gated on B cells; **c**, CD49b, gated on CD49^{pos} FcεRI^{pos} basophils; CCR3, gated on c-kit^{neg} CCR3^{pos} eosinophils; FcεRI, gated on c-kit^{pos} FcεRI^{pos} mast cells; **d**, IL-7Rα, gated on CD4^{pos} T cells; T1/ST2, gated on c-kit^{pos} mast cells). IL-25-elicited c-kit^{int} cells in (**a** and **b**) were FACS-purified prior to surface staining. Positive controls in (**a** and **b**) were derived from whole splenocytes stained *ex vivo*. Cells in (**c** and **d**) were surface stained *ex vivo*. Plots shown in (**c**) are gated on live CD4^{neg} CD8^{neg} B220^{neg} CD11b^{neg} and CD11c^{neg} cells. *Gata3*, *Junb*, *Maf*, *Stat6* and *Il1rl1* mRNA expression by naïve CD4^{pos} T cells (N), IL-4/eGFP^{pos} CD4^{pos} T cells from helminth-infected mice (Th2) or IL-25-elicited c-kit^{int}-GFP^{neg} or c-kit^{int}-GFP^{pos} cells was measured by real-time PCR (**e**). Data are representative of at least two independent experiments.



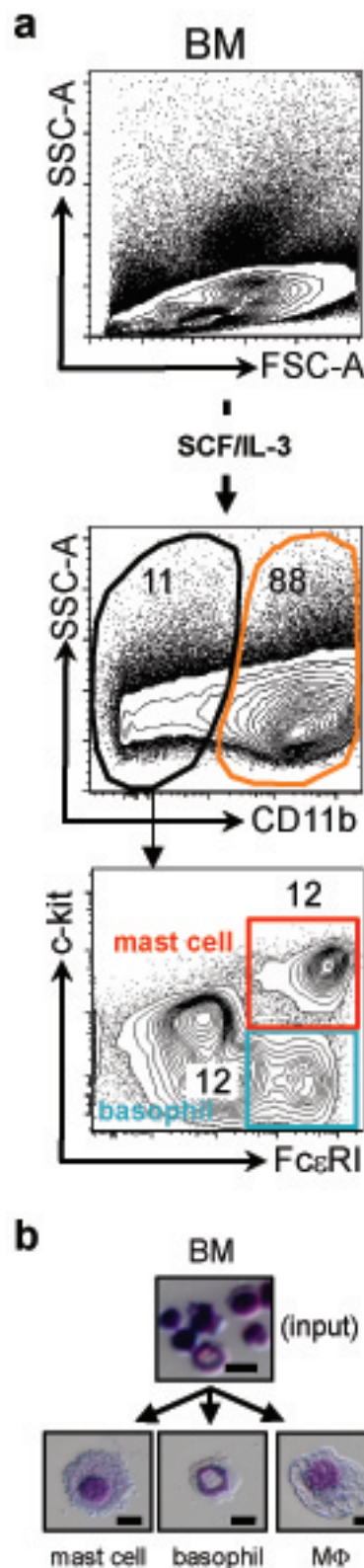
Supplementary Figure 6. IL-25 elicits c-kit^{int} cell population in the mesentery, but does not affect natural helper cells. **a-c**, WT mice were treated with IL-25 daily for 4 days and induction of c-kit^{int} cells in the peritoneum (**a**) and mesentery (**b**) of control or IL-25-treated mice was assessed by flow cytometry. Plots shown are gated on live CD3 ϵ ^{neg} CD4^{neg} CD19^{neg} CD11b^{neg} cell populations and in (**a**) gated to exclude Fc ϵ RI α ^{pos} mast cells. Frequencies of natural helper cells in the mesentery of control or IL-25-treated mice were assessed by flow cytometry (**c**). Data in **a** and **b** are representative of at least two independent experiments. (control, n=7; IL-25-treated, n=7). Data in (**c**) are representative of two control-treated and three IL-25-treated mice.



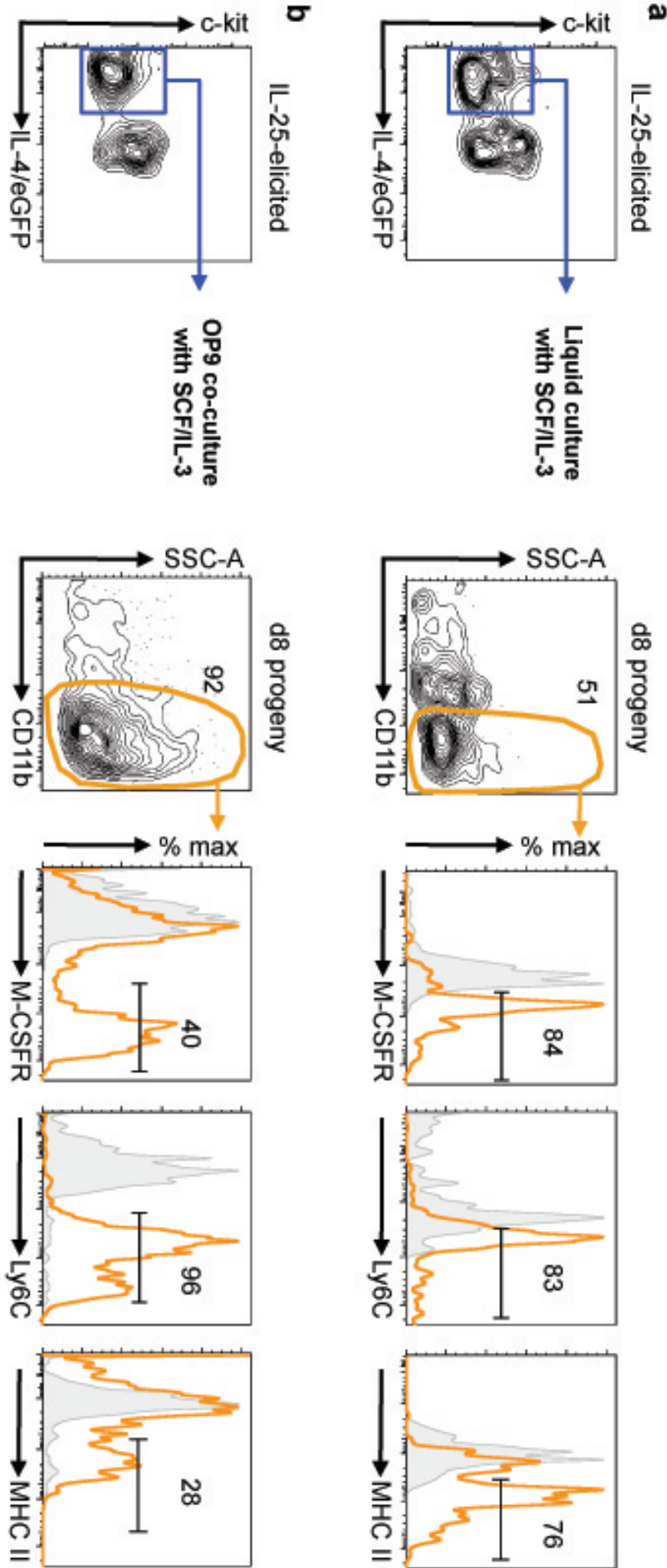
Supplementary Figure 7. IL-25-elicited c-kit^{int} cells are Lin^{neg/lo}. Expression of combined lineage markers (CD3 ϵ , CD8 α , CD8 β , TCR β , TCR $\gamma\delta$, B220, CD19, CD11b, CD11c, Gr-1, NK1.1 and Ter119) by c-kit^{int}-GFP^{neg} (blue line) and c-kit^{int}-GFP^{pos} (green line) cells from mesenteric lymph nodes of IL-25-treated IL-4/eGFP reporter mice. Plot shown is gated on live cells. Data are representative of two independent experiments. (n=4-6 per group).



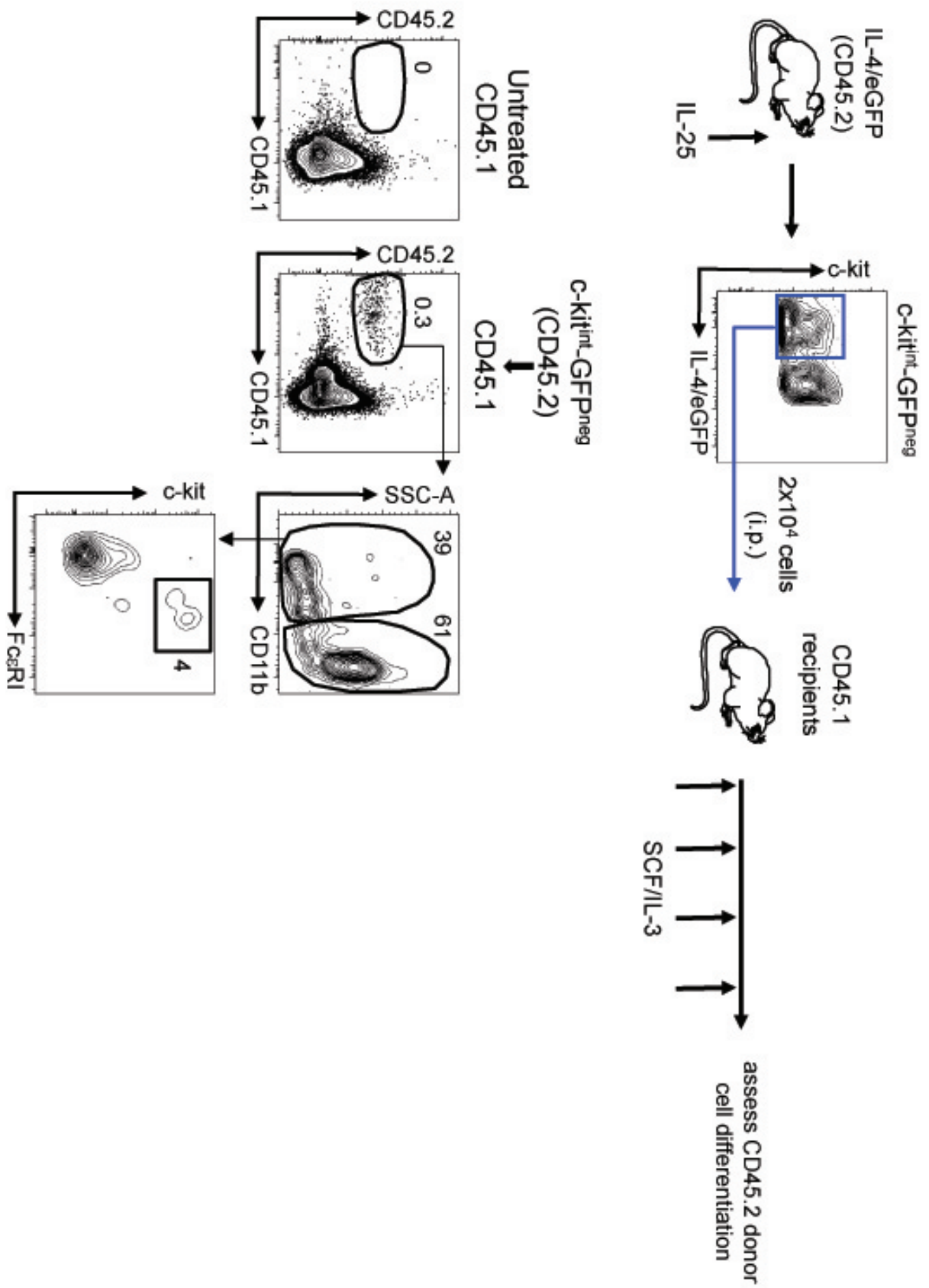
Supplementary Figure 8. IL-25 does not affect the frequencies of bone marrow-resident hematopoietic stem cells (HSCs). **a, b,** Expression of HSC markers on bone marrow-resident cells of control (**a**) or IL-25-treated (**b**) IL-4/eGFP reporter mice was analyzed by flow cytometry. Plots shown are gated on live lineage^{neg} cells (CD3 ϵ , CD8 α , CD8 β , TCR β , TCR $\gamma\delta$, B220, CD19, CD11b, CD11c, Gr-1, NK1.1 and Ter119). Data are representative of at least two independent experiments. (control, n=4; IL-25-treated, n=6).



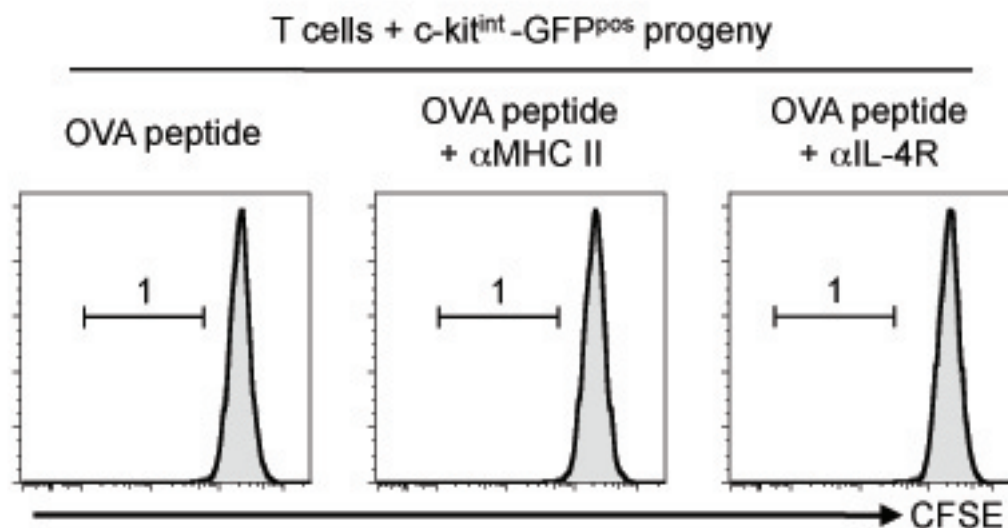
Supplementary Figure 9. Myeloid and granulocyte differentiation of unfractionated bone marrow. **a, b,** Analysis of myeloid and granulocyte cell differentiation of unfractionated bone marrow by flow cytometry (**a**) or cytopsin preparation (**b**) following culture *in vitro*. Scale bar, 20 μ m. Data are representative of at least three independent experiments.



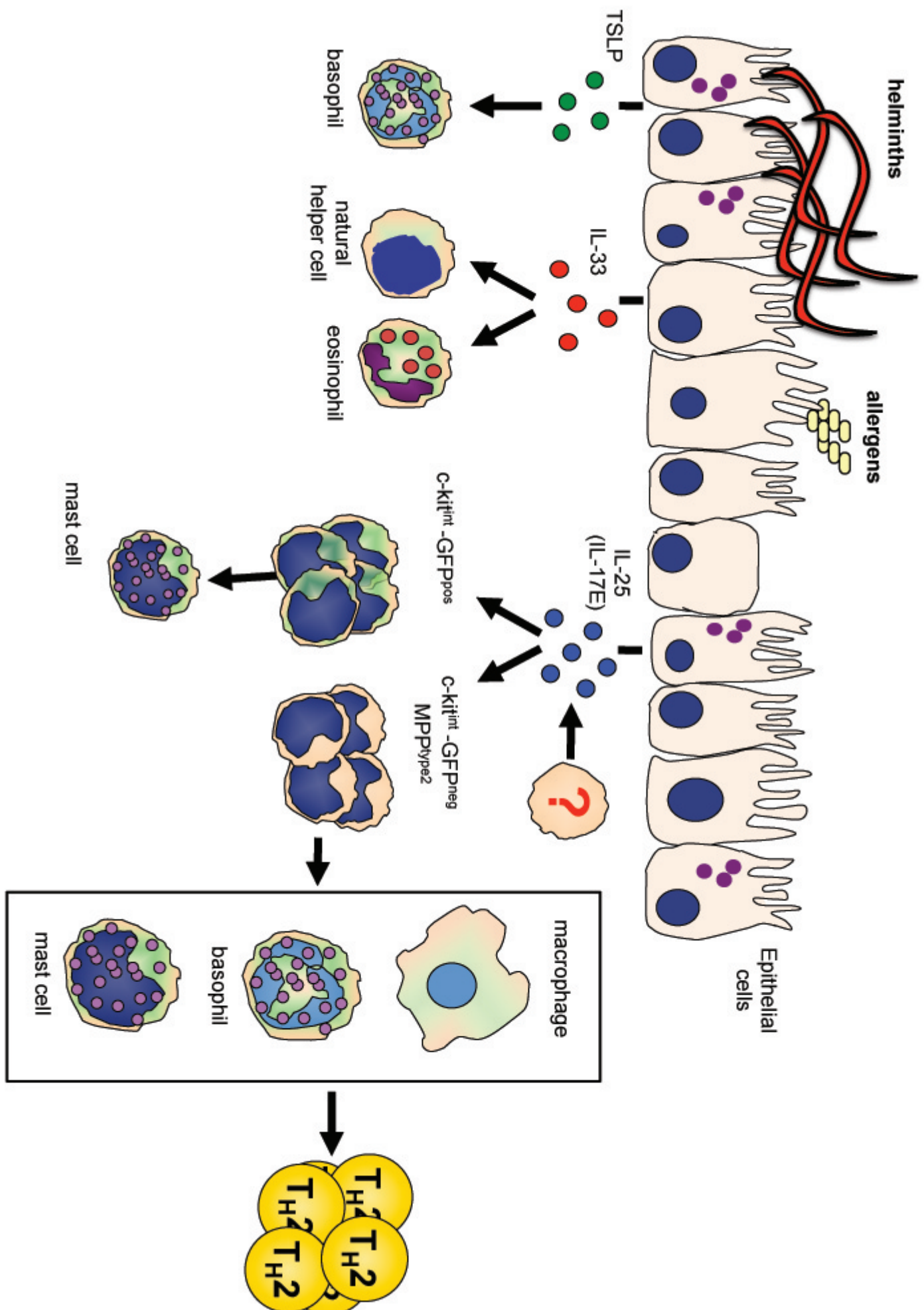
Supplementary Figure 10. IL-25-elicited $c\text{-kit}^{\text{int}}$ -GFP^{neg} cells possess monocyte/macrophage potential. **a, b,** $c\text{-kit}^{\text{int}}$ -GFP^{neg} cell populations from the mLN of IL-25-treated IL-4/eGFP mice were cultured in liquid culture (**a**) or co-cultured on OP9 stromal cells (**b**) in the presence of SCF (50 ng mL⁻¹) and IL-3 (10 ng mL⁻¹). Cultures were assessed for surface expression of monocyte/macrophage markers at day 8 by flow cytometry. Plots shown are gated on live cells or as indicated. Numbers indicate the percentage of gated region of total population. Data in (**a**) and (**b**) are representative of three independent experiments.



Supplementary Figure 11. IL-25-elicited c-kit^{int}-GFP^{neg} cells display multi-potent potential *in vivo*. CD45.1 congenic recipients were left untreated or given 2×10^4 c-kit^{int}-GFP^{neg} cells i.p. from IL-25-treated CD45.2 mice. Recipient mice were treated 4 times with 1 μ g each of SCF and IL-3 and differentiation of donor cells isolated from the peritoneal cavity was assessed on day 6 post-transfer. Plots shown are gated on live cells or as indicated. Data are representative of at least two independent experiments. (n=4).



Supplementary Figure 12. Progeny from IL-25-elicited c-kit^{int}-GFP^{pos} cells do not promote Th2 cell differentiation. FACS-purified IL-25-elicited c-kit^{int}-GFP^{pos} cells were cultured for 8 days in SCF and IL-3 and the resultant progeny were co-cultured for 4 days with CFSE-labeled OVA-specific CD4^{pos} CD62L^{hi} CD44^{lo} T cells in the presence of OVA peptide with or without addition of mAb against MHC class II or IL-4R α . Plots are gated on CD4^{pos} T cells. Gray histogram indicates CFSE-dilution by OVA-specific CD4^{pos} T cells cultured in medium alone. Results are representative of at least two independent experiments.



Supplementary Figure 13. IL-25-elicited c-kit^{int} cells possess multi-potent potential and promote Th2 cell differentiation. Schematic of one mechanism whereby IL-25 (IL-17E) promotes Th2 cytokine responses *in vivo*. Mucosal epithelial cells have been shown to express TSLP, IL-33, and IL-25. While TSLP and IL-33 have been shown to elicit or activate basophils, eosinophils or natural helper cells, IL-25 elicits c-kit^{int}-GFP^{pos} and c-kit^{int}-GFP^{neg} cell populations. While the c-kit^{int}-GFP^{pos} cells give rise to mast cells, the c-kit^{int}-GFP^{neg} cell population possesses multi-potent capacity, being able to differentiate into monocyte/macrophages, mast cells and basophils that collectively promote CD4^{pos} Th2 cell differentiation.