

# Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity

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Innate immunity provides the first line of defence against invading pathogens and provides important cues for the development of adaptive immunity. Type-2 immunity—responsible for protective immune responses to helminth parasites<sup>1,2</sup> and the underlying cause of the pathogenesis of allergic asthma<sup>3,4</sup>—consists of responses dominated by the cardinal type-2 cytokines interleukin (IL)4, IL5 and IL13 (ref. 5). T cells are an important source of these cytokines in adaptive immune responses, but the innate cell sources remain to be comprehensively determined. Here, through the use of novel *Il13-eGFP* reporter mice, we present the identification and functional characterization of a new innate type-2 immune effector leukocyte that we have named the nuocyte. Nuocytes expand *in vivo* in response to the type-2-inducing cytokines IL25 and IL33, and represent the predominant early source of IL13 during helminth infection with *Nippostrongylus brasiliensis*. In the combined absence of IL25 and IL33 signalling, nuocytes fail to expand, resulting in a severe defect in worm expulsion that is rescued by the adoptive transfer of *in vitro* cultured wild-type, but not IL13-deficient, nuocytes. Thus, nuocytes represent a critically important innate effector cell in type-2 immunity.

Type-2 immunity evolved to respond to parasitic helminth infections, with type-2 cytokines orchestrating eosinophilia, goblet cell hyperplasia, mucus secretion, and IgE production<sup>5–7</sup>. These highly complex host responses involve the coordination of innate and adaptive immune cell types. Of the defined innate immune cells, basophils, eosinophils and mast cells are known sources of type-2 cytokines, but it is not clear whether they are essential for *N. brasiliensis* expulsion<sup>5,8–12</sup>.

To identify new cell types that may mediate type-2 immunity we investigated the cellular sources of IL13, a critical cytokine in the host response to helminth infection<sup>7,13</sup> and allergy<sup>6,14</sup>. To allow live imaging of enhanced green fluorescent protein (eGFP) as a surrogate for IL13 gene expression during the induction of type-2 responses we generated *Il13-eGFP* mice (Supplementary Fig. 1). Analysis of these mice showed very few constitutive eGFP<sup>+</sup> cells in naive mice (Supplementary Fig. 1). Administration of IL25 or IL33 to *Il13-eGFP* mice resulted in the detection of ~3% eGFP<sup>+</sup> cells in the mesenteric lymph nodes (MLNs) (Fig. 1a), at least 80% of which could not be assigned to known cell lineages (including T cells, B cells, natural killer T (NKT) cells, natural killer (NK) cells, dendritic cells, neutrophils, eosinophils, mast cells, basophils or macrophages) using a spectrum of cell surface markers (Fig. 1a, b and Supplementary Fig. 2a). Immunofluorescence showed highly increased numbers of eGFP<sup>+</sup> cells in the intestines (Fig. 1c) and spleens (Supplementary Fig. 2b) of both IL25- and IL33-treated *Il13-eGFP* mice, and these were confirmed to be non-T cells. The

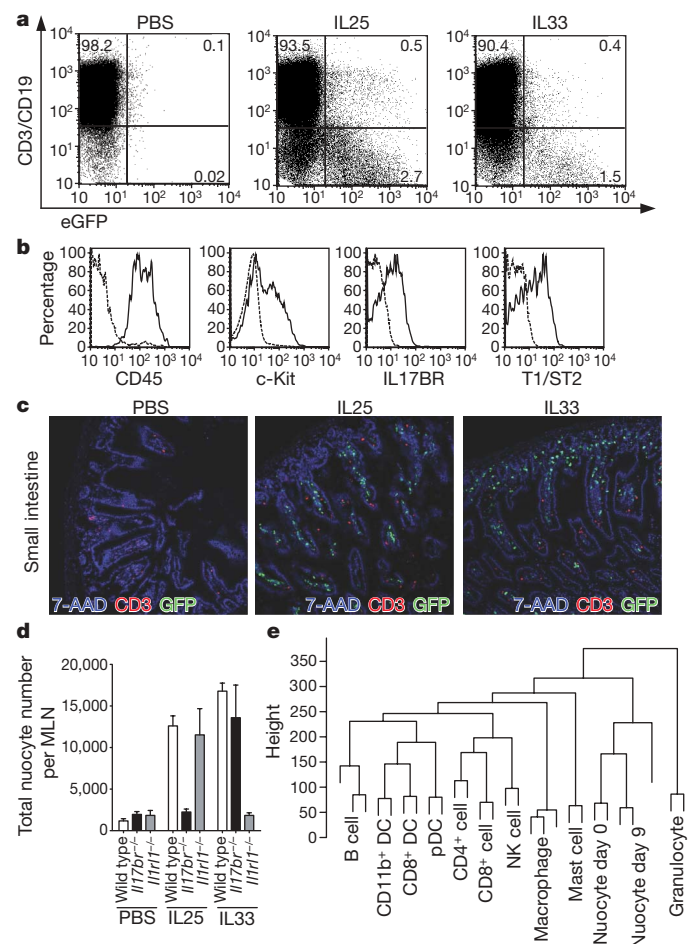


**Figure 1 | IL25 and IL33 induce IL13-producing nuocytes.** **a**, Detection of *Il13-eGFP*<sup>+</sup> non-B, non-T (NBNT) cells in MLNs of IL25- or IL33-treated mice. **b**, Cell surface marker expression of *Il13-eGFP*<sup>+</sup> NBNT cells in MLNs after IL25 administration. **c**, Immunofluorescence detection of IL13-eGFP<sup>+</sup> cells in the small intestine of mice treated with IL25 and IL33. 7-AAD, 7-amino-actinomycin. Data in **a–c** are representative of five experiments with >3 mice per group. **d**, Nuocyte number. Data are representative of two independent experiments with >4 mice per group. Data in bar charts are the mean and s.e.m. **e**, Cluster analysis for freshly isolated nuocytes (*ex vivo*) or day 9 *in vitro* expanded nuocytes (single data sets are shown for clarity). DC, dendritic cell; pDC, plasmacytoid dendritic cell.

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lineage<sup>-</sup>eGFP<sup>+</sup> cells were T1/ST2<sup>+</sup> (IL1RL1, a subunit of IL33R) and IL17BR<sup>+</sup> (also known as IL17RB and IL25R) (Fig. 1b), suggesting that they respond to IL33 and IL25 directly. Analysis of *Il17br*<sup>-/-</sup> (Supplementary Fig. 3) and *Il1rl1*<sup>-/-</sup> mice demonstrated that exogenous IL25 and IL33 act redundantly to induce these cells *in vivo* (Fig. 1d). These lineage<sup>-</sup>eGFP<sup>+</sup>T1/ST2<sup>+</sup>IL17BR<sup>+</sup> cells represent a new IL13-producing leukocyte population that we have named nuocytes owing to their high level of IL13 expression, and with nu being the 13th letter of the Greek alphabet. As discussed later, nuocytes can also be defined as lineage<sup>-</sup> cells expressing ICOS, T1/ST2, IL17BR and IL7Rα.

Although present in the spleen, MLNs and bone marrow of naive mice, nuocytes represent less than 0.2% of cells in each tissue, but increase significantly in these tissues (Supplementary Fig. 4), with the exception of bone marrow (data not shown), after intraperitoneal administration of IL25. In contrast, basophil numbers did not increase in the blood or spleen, and their IL4 production was unaffected by IL25 treatment (data not shown). Confirming that nuocytes were not T or B cells, mast cells, NKT cells or lymphoid tissue inducer cells, we detected IL25-driven nuocyte induction in *Rag2*<sup>-/-</sup> mice and nude mice, *Kit*<sup>W-sh/W-sh</sup> mice, *Ja18*<sup>-/-</sup> (also known as *Tcra*<sup>-/-</sup>) mice and *Rorγ*<sup>-/-</sup> (also known as *Rorc*<sup>-/-</sup>) mice, respectively (Supplementary Fig. 5a–d and data not shown). Furthermore, microarray analysis of highly purified nuocytes failed to show any significant gene expression similarity to known leukocyte lineages (Fig. 1e). However, it did show several cell surface markers (Supplementary Table 1), including the receptor for IL7 and the co-stimulatory molecule ICOS and major histocompatibility complex (MHC) class II, which were subsequently confirmed as being expressed on nuocytes by flow cytometry (Supplementary Fig. 2c).

Notably, nuocytes represent the predominant cell type expressing *Il13-eGFP* at 5 days post-infection (d.p.i.) with the helminth parasite *N. brasiliensis* (Fig. 2a and Supplementary Fig. 2d). To investigate the potential roles of IL25 and IL33 in regulating nuocytes during

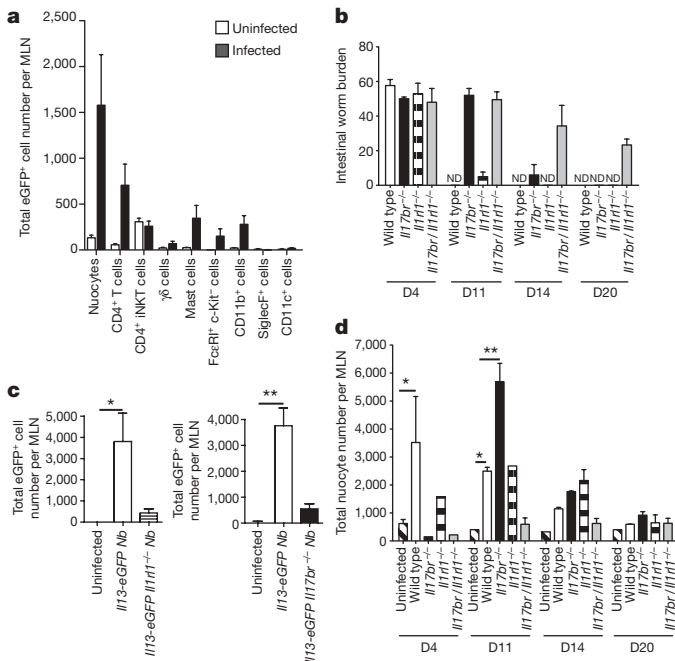


**Figure 2 | IL25 and IL33 have partially redundant roles for nuocyte induction and worm expulsion.** **a**, Quantification of *Il13-eGFP*<sup>+</sup> cells 5 d.p.i. with *N. brasiliensis*. Data are representative of two independent experiments with >5 mice per group. iNKT cells, invariant NKT cells. **b**, Intestinal worm burden of *N. brasiliensis*-infected mice. D, day; ND, none detected. **c**, Quantification of *Il13-eGFP*<sup>+</sup> cells in *N. brasiliensis* (*Nb*)-infected mice at 5 d.p.i. Data are representative of two independent experiments with >5 mice per group. **d**, Quantification of nuocytes in *N. brasiliensis*-infected mice. \**P* < 0.05, \*\**P* < 0.01. Data are representative of two independent experiments with >5 mice per group. Data in bar charts are mean and s.e.m.

helminth infection we infected *Il17br*<sup>-/-</sup>, *Il1rl1*<sup>-/-</sup> and combined *Il17br*<sup>-/-</sup> *Il1rl1*<sup>-/-</sup> mice with *N. brasiliensis*. *Il17br*<sup>-/-</sup> mice expelled their worms more slowly than wild types, but by day 14 even *Il17br*<sup>-/-</sup> mice had relatively few worms and a complete absence of worms by 20 d.p.i. (Fig. 2b). By contrast, *Il1rl1*<sup>-/-</sup> mice efficiently expelled their worm burden, with very few worms present at day 11 and complete absence by day 14 (Fig. 2b). Notably, the absence of both IL25 and IL33 signalling severely impaired worm expulsion from the *Il17br*<sup>-/-</sup> *Il1rl1*<sup>-/-</sup> mice, with significant worm burden persisting to 20 d.p.i. (Fig. 2b). Using *Il17br*<sup>-/-</sup> *Il13-eGFP* and *Il1rl1*<sup>-/-</sup> *Il13-eGFP* mice we found that the loss of either IL17BR or T1/ST2 resulted in a substantial fall in the numbers of eGFP<sup>+</sup> cells early in the response (Fig. 2c). Notably, the expansion of nuocytes in the various mouse strains correlated with the onset of worm expulsion. Thus, nuocytes arose more rapidly in *Il1rl1*<sup>-/-</sup> mice (although more slowly than in wild-type controls) than in *Il17br*<sup>-/-</sup> mice. Nuocytes failed to expand in the combined *Il17br*<sup>-/-</sup> *Il1rl1*<sup>-/-</sup> mice in either the MLNs (Fig. 2d) or peritoneal lavage (Supplementary

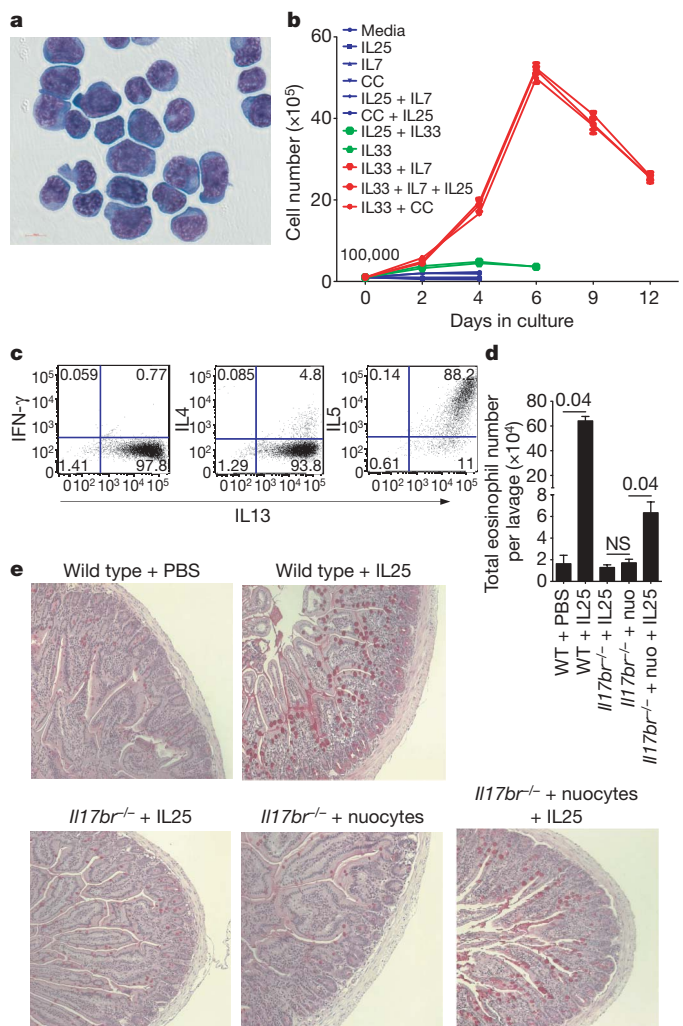


**Figure 3 | Adoptive transfer of cultured nuocytes into *Il17br*<sup>-/-</sup> mice restores an IL25-responsive phenotype.** **a**, Morphology of Giemsa-stained nuocytes. **b**, Quantification of nuocyte growth *in vitro*. CC, cytokine cocktail. **c**, Flow cytometric analysis of interferon (IFN)-γ, IL4, IL5 and IL13 intracellular staining of nuocytes after 7 days culture with IL7 and IL33. Data in **a–c** are representative of three independent experiments. **d**, Quantification of eosinophil infiltration of the peritoneal cavity after nuocyte (nuo) transfer. WT, wild type. Data in bar charts are mean and s.e.m. **e**, Transverse histological jejunum sections stained with periodic acid–Schiff (PAS) for goblet cells. Data are representative of two independent experiments with >5 mice per group. Original magnifications, ×100 (**a**) and ×40 (**e**).

Fig. 6a), even at 20 d.p.i. Although eosinophils and IgE levels were also reduced in the *Il17br*<sup>-/-</sup>, *Il1rl1*<sup>-/-</sup> and *Il17br*<sup>-/-</sup> *Il1rl1*<sup>-/-</sup> mice (Supplementary Fig. 6b, c), these have been shown by others to not be essential for worm expulsion<sup>5,15</sup>. In addition, we found no defect in basophil expansion in the *Il17br*<sup>-/-</sup> mice (data not shown and Supplementary Fig. 7b).

To address the functional importance of nuocytes in the immune response to helminth infection, we purified nuocytes to homogeneity (Fig. 3a) and determined conditions for their expansion *in vitro*. Nuocytes did not grow or differentiate in culture with a cytokine cocktail used previously to differentiate a basophil/mast cell progenitor *in vitro*<sup>16</sup> (Fig. 3b), or under conditions that readily generate mast cells from total bone marrow<sup>17</sup> (data not shown). By contrast, the inclusion of IL33 and IL7 in the cultures induced substantial nuocyte expansion (Fig. 3b). The addition of IL25 to IL33 plus IL7 culture conditions did not change the growth rate of nuocytes (Fig. 3b).

The cultured nuocytes maintained the expression of most cell surface markers, including high levels of CD45 (data not shown), ICOS, T1/ST2, IL17BR and IL7R $\alpha$  (Supplementary Fig. 8), and did

not differentiate into any of the currently known leukocyte lineages even after 15 days in culture (Fig. 1e and data not shown). All nuocytes expressed IL13, with more than 70% also secreting IL5, although less than 5% produced IL4 (Fig. 3c). Analyses of nuocyte culture supernatants also showed the substantial secretion of IL6, IL10 and GM-CSF (Supplementary Fig. 9).

Notably, adoptive transfer of nuocytes into *Il17br*<sup>-/-</sup> mice re-established many of the features of IL25-evoked type-2 immune responses (Fig. 3d, e) that are normally absent in these mice (Supplementary Fig. 3e–g). Crucially, the adoptive transfer of nuocytes did not induce any spontaneous inflammation in *Il17br*<sup>-/-</sup> recipients but restored their ability to respond to subsequent IL25 administration (Fig. 3d, e). Cellular infiltrate of the peritoneal lavage, characterized by eosinophilia (Fig. 3d), and intestinal goblet cell hypertrophy and hyperplasia, were restored in *Il17br*<sup>-/-</sup> mice that received nuocytes, as compared to controls (Fig. 3e).

To investigate whether nuocytes have a critical role in coordinating immunity to helminth infection, we sought to restore protective immunity to *Il17br*<sup>-/-</sup> mice in response to *N. brasiliensis* through

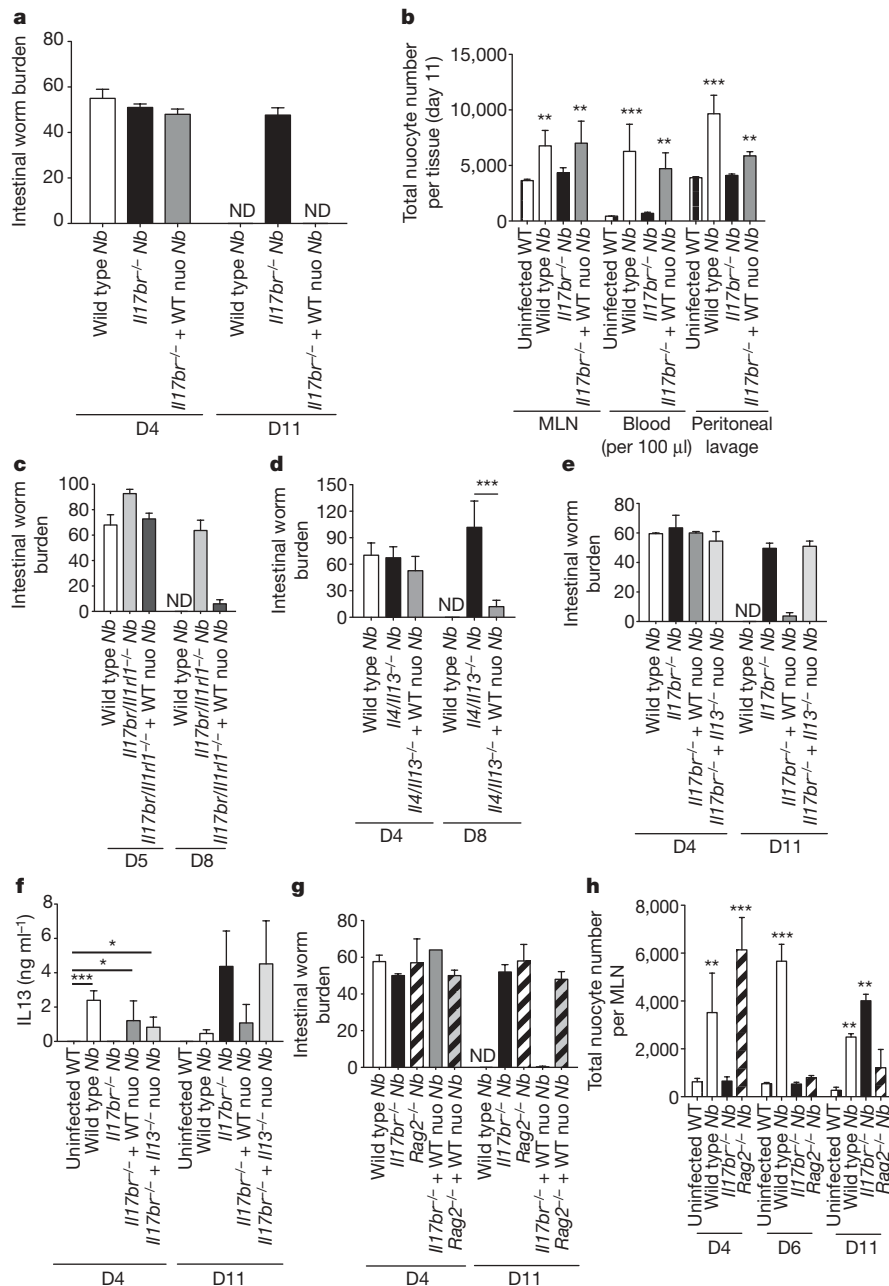


**Figure 4 | Adoptive transfer of wild-type nuocytes, but not IL13-deficient nuocytes, restores rapid worm expulsion in *N. brasiliensis*-infected *Il17br*<sup>-/-</sup> mice.** **a**, Intestinal worm burdens. **b**, Quantification of nuocyte numbers in tissues. Data are representative of three independent experiments with >6 mice per group. **c**, **d**, Intestinal worm burdens. Data are from single experiments with 6–7 mice per group. **e**, **f**, *N. brasiliensis* antigen-specific IL13 production. **g**, Intestinal worm burden. **h**, Quantification of nuocyte numbers. **e–h**, Data are representative of two independent experiments with >6 mice per group. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.005. Data in bar charts are mean and s.e.m.

the adoptive transfer of wild-type (IL25-responsive) nuocytes. Four days after infection, all infected animals had equivalent intestinal worm burdens (Fig. 4a), demonstrating that the transfer of nuocytes did not prevent the establishment of infection. Notably, most of the infected *Il17br*<sup>-/-</sup> animals that received nuocytes had completely expelled their worms by 11 d.p.i., similar to wild-type controls (Fig. 4a). This contrasted with the *Il17br*<sup>-/-</sup> mice that had not received nuocytes, and had burdens of greater than 50 worms at the same time point (Fig. 4a). The transfer of nuocytes also restored the numbers of nuocytes in the tissues at 11 d.p.i. (Fig. 4b), and restored the early eosinophil response at 6 d.p.i., but did not appreciably alter the levels of basophils at this time point (Supplementary Fig. 7a, b). Furthermore, adoptive transfer of wild-type nuocytes into the combined *Il17br*<sup>-/-</sup> *Il1rl1*<sup>-/-</sup> mice also resulted in the restoration of *N. brasiliensis* expulsion (Fig. 4c).

We have shown previously that IL13, among the type-2 cytokines, is essential for the rapid eradication of *N. brasiliensis*<sup>13,18</sup>. By transferring wild-type nuocytes into *Il4*<sup>-/-</sup> *Il13*<sup>-/-</sup> mice, we now show that even when nuocytes are the only IL13-secreting cells, they are capable of inducing worm expulsion (Fig. 4d). To test the importance of nuocyte-derived IL13 on the kinetics of *N. brasiliensis* expulsion further, nuocytes were prepared from *Il13*<sup>-/-</sup> animals and transferred into *N. brasiliensis*-infected *Il17br*<sup>-/-</sup> animals. In contrast to the wild-type nuocytes, IL13-deficient nuocytes failed to mediate worm expulsion, with mice continuing to contain high numbers of intestinal worms at 11 d.p.i. (Fig. 4e).

*In vitro* antigen re-stimulation of MLN cells from *N. brasiliensis*-infected *Il17br*<sup>-/-</sup> mice showed delayed T-cell-derived IL13 secretion that was absent at 4 d.p.i. However, the continuing presence of intestinal worms and the resulting antigen burden led to a robust cytokine response by 11 d.p.i. (Fig. 4f), as reported previously<sup>19</sup>. We also observed fewer IL13-eGFP<sup>+</sup> T cells in both *Il1rl1*<sup>-/-</sup> mice and *Il17br*<sup>-/-</sup> mice at 5 d.p.i. (data not shown). However, after adoptive transfer of wild-type or *Il13*<sup>-/-</sup> nuocytes into *Il17br*<sup>-/-</sup> mice, antigen-specific T-cell production of IL13 was restored (Fig. 4f). As expected, because *Il13*<sup>-/-</sup> nuocytes failed to induce worm expulsion, the duration of antigen-specific T-cell responses was prolonged (Fig. 4f). Thus, nuocytes are capable of enhancing T-cell cytokine production, and nuocytes augment T-cell responses independently of IL13.

Expulsion of *N. brasiliensis* is a T-cell-dependent process, but neither T-cell-derived IL4 or IL13 is necessary for worm expulsion<sup>15</sup>, indicating that an alternative cell source is responsible for the production of the IL13 necessary for worm expulsion. It was not surprising then that nuocytes were unable to induce worm expulsion in *Rag2*<sup>-/-</sup> mice (Fig. 4g). However, analysis of nuocyte numbers in the MLNs of *N. brasiliensis*-infected *Rag2*<sup>-/-</sup> mice showed that, despite rapid early nuocyte expansion by 4 d.p.i. (Fig. 4h), nuocyte numbers were not maintained in the absence of T cells, falling to uninfected levels as assessed on 6 and 11 d.p.i. (Fig. 4h), despite the continued presence of intestinal worms (Fig. 4g). This suggests that T cells (or possibly B cells, although B cells have been shown to be dispensable for worm expulsion<sup>15</sup>) mediate prolonged nuocyte expansion, migration or survival through an as yet unknown mechanism that requires further investigation. Our data demonstrate that a dialogue exists between T cells and nuocytes, and that this is necessary for robust *N. brasiliensis* expulsion.

Thus, nuocytes clearly provide a critical effector mechanism, through their provision of the IL13 required to induce helminth expulsion. The ability of nuocytes to expand rapidly in response to two potent initiators of type-2 immunity (IL25 and IL33), and in response to helminth infection, their presence at several immunosurveillance sites around the body, their capacity to secrete high levels of IL13 and IL5, and their ability to enhance T-cell responses, shows them to be highly specialized type-2 regulatory cells. The characterization of nuocytes will now allow assessment of their roles in other immune responses and disease pathologies including allergic asthma.

## METHODS SUMMARY

**Adoptive transfers.** Purified nuocytes were cultured for 2–5 days in RPMI supplemented with 10 ng ml<sup>-1</sup> IL33 and 10 ng ml<sup>-1</sup> IL7. Cells were washed and injected intravenously in PBS by the tail vein at 0.5 × 10<sup>6</sup> cells per mouse. Adoptive transfer was performed 4 h after infection with *N. brasiliensis*, or 12 h before the first intraperitoneal cytokine administration of IL25.

**Full Methods** and any associated references are available in the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

Received 1 December 2009; accepted 12 February 2010.

Published online 3 March 2010.

- Maizels, R. M., Pearce, E. J., Artis, D., Yazdanbakhsh, M. & Wynn, T. A. Regulation of pathogenesis and immunity in helminth infections. *J. Exp. Med.* **206**, 2059–2066 (2009).
- Perrigoue, J. G., Marshall, F. A. & Artis, D. On the hunt for helminths: innate immune cells in the recognition and response to helminth parasites. *Cell. Microbiol.* **10**, 1757–1764 (2008).
- Holgate, S. T. & Polosa, R. Treatment strategies for allergy and asthma. *Nature Rev. Immunol.* **8**, 218–230 (2008).
- Larché, M., Robinson, D. S. & Kay, A. B. The role of T lymphocytes in the pathogenesis of asthma. *J. Allergy Clin. Immunol.* **111**, 450–463 (2003).
- Fallon, P. G. et al. IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9, and IL-13. *Immunity* **17**, 7–17 (2002).
- Grünig, G. et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* **282**, 2261–2263 (1998).
- Urban, J. F. J. et al. IL-13, IL-4Rα, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis*. *Immunity* **8**, 255–264 (1998).
- Min, B. et al. Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J. Exp. Med.* **200**, 507–517 (2004).
- Ohnmacht, C. & Voehringer, D. Basophil effector function and homeostasis during helminth infection. *Blood* **113**, 2816–2825 (2009).
- Perrigoue, J. G. et al. MHC class II-dependent basophil-CD4<sup>+</sup> T cell interactions promote Th2 cytokine-dependent immunity. *Nature Immunol.* **10**, 697–705 (2009).
- Voehringer, D. The role of basophils in helminth infection. *Trends Parasitol.* **25**, 551–556 (2009).
- Yoshimoto, T. et al. Basophils contribute to Th2-IgE responses *in vivo* via IL-4 production and presentation of peptide-MHC class II complexes to CD4<sup>+</sup> T cells. *Nature Immunol.* **10**, 706–712 (2009).
- McKenzie, G. J., Bancroft, A., Grecnis, R. K. & McKenzie, A. N. A distinct role for interleukin-13 in Th2-cell-mediated immune responses. *Curr. Biol.* **8**, 339–342 (1998).
- Walter, D. M. et al. Critical role for IL-13 in the development of allergen-induced airway hyperreactivity. *J. Immunol.* **167**, 4668–4675 (2001).
- Voehringer, D., Reese, T. A., Huang, X., Shinkai, K. & Locksley, R. M. Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. *J. Exp. Med.* **203**, 1435–1446 (2006).
- Arinobu, Y. et al. Developmental checkpoints of the basophil/mast cell lineages in adult murine hematopoiesis. *Proc. Natl Acad. Sci. USA* **102**, 18105–18110 (2005).
- Haig, D. M. et al. Effects of stem cell factor (kit-ligand) and interleukin-3 on the growth and serine proteinase expression of rat bone-marrow-derived or serosal mast cells. *Blood* **83**, 72–83 (1994).
- McKenzie, G. J., Fallon, P. G., Emson, C. L., Grecnis, R. K. & McKenzie, A. N. Simultaneous disruption of interleukin (IL)-4 and IL-13 defines individual roles in T helper cell type 2-mediated responses. *J. Exp. Med.* **189**, 1565–1572 (1999).
- Fallon, P. G. et al. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J. Exp. Med.* **203**, 1105–1116 (2006).

**Supplementary Information** is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

**Acknowledgements** We thank members of the McKenzie laboratory for their comments on the manuscript. We thank D. Cousins for assistance with preliminary microarray analysis. R.J.F. was supported by Asthma UK. P.G.F. is supported by Science Foundation Ireland.

**Author Contributions** D.R.N., S.H.W. and A.B. performed experiments, interpreted data, provided intellectual input and wrote the paper; R.J.F. and T.K.A.L. performed the infection studies; M.D. performed cell isolation studies; C.B. and C.M.K. performed microarray studies and Luminex; P.G.F. provided reagents and intellectual input; R.P. and H.E.J. provided reagents and experimental assistance; A.N.J.M. conceived the study and wrote the paper.

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## METHODS

**Animals.** BALB/c mice were purchased from Charles River Laboratories as required. *Rag2*<sup>-/-</sup> mice<sup>20</sup>, on a BALB/c background, were provided by J. Langhorne. *Il13*<sup>-/-</sup> mice<sup>13</sup> and *Il4*<sup>-/-</sup> *Il13*<sup>-/-</sup> mice<sup>18</sup> were on a BALB/c background. *Ja18*<sup>-/-</sup> mice<sup>21</sup>, on a C57BL/6 background. *Ki1*<sup>W-sh/W-sh</sup> mice<sup>22</sup>, on a C57BL/6 background, were provided by C. Lawrence. In individual experiments all mice were matched for age, gender and background strain. Mice were maintained in specific pathogen-free conditions. All animal experiments undertaken in this study were done so with the approval of the UK Home Office.

***Il13-eGFP* mice.** The *Il13-eGFP* mice were generated by recombineering<sup>23,24</sup> (Supplementary Information). Neomycin-negative, Cre-recombinase-negative mice were backcrossed onto the BALB/c and C57BL/6 backgrounds. Genotyping of *Il13-eGFP* mice used PCR primers (forward, 5'-TCAACAGGCTAAGGCCACAAGCC-3'), (forward, 5'-CATGGTCCTGCTGGAGTTCGTG-3') and (reverse, 5'-GCTTCGCTGTCTCACTCACACAGG-3'), giving a wild-type product of 300 base pairs (bp) and a targeted product of 522 bp.

***Il17br*<sup>-/-</sup> mice.** The replacement vector was designed to replace exons 2 and 3 of the *Il17br* gene (a region encoding 56 amino acids of IL17BR) with a neomycin-resistance gene (Supplementary Information). Targeted BALB/c embryonic stem cell clones were used to generate the line on a BALB/c background. Genotyping was performed by PCR using primers 5'-TTGCTGATCTTGGCTGCATCGTGC-3', 5'-AGCAGGGCTTGCATCTGAATGCCT-3' and 5'-CTATCAGGACATAGCGTTGGCTACC-3' that give a product of 600 bp for the wild-type allele and 400 bp for the targeted allele.

**Generation of monoclonal anti-IL17BR antibody (clone D9.2).** *Il17br*<sup>-/-</sup> mice were immunised by intraperitoneal injection with mouse IL17BR/Fc fusion protein (R&D Systems) and monoclonal anti-IL17BR antibodies generated by standard protocols (Supplementary Information).

**IL25 and IL33 administration.** Four-hundred nanograms per dose of recombinant mouse IL25 or recombinant mouse IL33 (R&D Systems) in PBS was administered daily for 3 days intraperitoneally. Mice were euthanized 24 h later and tissues were collected for analysis. Control animals received PBS only.

**Fluorescence-activated cell analysis.** Mouse tissue cell suspensions at  $2 \times 10^8$  cells ml<sup>-1</sup> were incubated with purified anti-Fc receptor blocking antibody (anti-CD16/CD32) before addition of the specific antibodies. Cell surface markers were stained using a combination of fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, PE-Cy7-, allophycocyanin (APC)-conjugated and biotin-conjugated monoclonal antibodies (see Supplementary Information). In each experiment the appropriate isotype control monoclonal antibodies and single conjugate controls were also included. Samples were analysed using a Becton Dickinson FACScalibur flow cytometer running CellQuest acquisition and analysed using FlowJo software (version 8.8.3, Tree Star).

**Fluorescence-activated cell sorting of nuocytes.** Spleen cells prepared from IL25-treated mice were depleted of lineage<sup>+</sup> cells before cell sorting by incubation with biotin-conjugated anti-CD3, anti-CD19, anti-CD11b and anti-FcεRI antibodies before removal of antibody-bound cells by magnetic separation using Dynabeads (Invitrogen). Lineage-depleted cells were stained with PE-conjugated antibodies against CD4, CD8, B220 (also known as Ptpcr), TER-119 (Ly76) and CD11b (Itgam), a FITC-conjugated antibody against CD45, and an APC-conjugated antibody against ICOS. PE<sup>-</sup>, FITC<sup>+</sup>, APC<sup>+</sup> cells were collected using a Mo-flo cell sorter, and purity was checked by staining with lineage antibodies and antibodies against IL17BR and T1/ST2.

**Nuocyte cytokine/chemokine profile analysis.** Supernatants collected from day-7 cultured nuocytes ( $\sim 2 \times 10^6$  cells ml<sup>-1</sup>) were analysed by bioplex assay (Milliplex MAP mouse cytokine/chemokine 22-plex, Millipore). Supernatants were assayed according to manufacturer's instructions. Data were collected with the Bio-Plex 200 system, analysed in Excel and graphed with GraphPad Prism 4.0 software. Statistical significance was determined by one-way analysis of variance (ANOVA) with Tukey's post-hoc test.  $P < 0.05$  was considered significant.

**Helminth infection and antigen re-stimulation.** Mice were inoculated subcutaneously with 300 viable third-stage *N. brasiliensis* larvae. MLN cells were stimulated *in vitro* at  $2 \times 10^6$  cells ml<sup>-1</sup> with 50 µg ml<sup>-1</sup> of parasite antigens (*N. brasiliensis* excretory/secretory antigen) for 72 h. Supernatants were collected and analysed for IL13 by Quantikine ELISA (R&D Systems).

**Immunofluorescence on cryosections and confocal microscopy.** Cryosections were prepared (Supplementary Information) and incubated with conjugated antibodies: anti-mouse CD11b-Pacific Blue (clone M1/70, eBioscience), anti-mouse B220-Pacific Blue (clone RA3-6B2, eBioscience), anti-mouse CD4-biotin (clone RM4-5, Biolegend), anti-mouse CD3-Pacific Blue (clone eBio500A2, eBioscience), anti-mouse SIGN-R1-AlexaFluor 647 (clone eBio22D1, eBioscience), anti-GFP (rabbit IgG, Invitrogen). Sections were then rinsed and incubated with streptavidin conjugated with AlexaFluor 546 (Invitrogen) and/or anti-rabbit-AlexaFluor 488 (goat IgG, Invitrogen). 7-AAD (eBioscience) was included in the last incubation to stain nuclei. Finally, samples were mounted with Vectashield (Vector Labs). Images were taken on a Carl-Zeiss inverted microscope (LSM 710) and processed with ZEN 2008 (Carl-Zeiss).

**Microarray data analysis.** Mouse Genome 430 2.0 GeneChips were used to analyse the gene expression of freshly isolated or 9 days *in vitro* expanded nuocytes from wild-type mice. Raw expression data were initially imported into R and Bioconductor software for quality assessment using the affyQCReport package. Nuocyte data were combined with publically available immune cells data sets with the same GeneChips. These include mouse B cells ( $n = 3$ ), CD11b<sup>+</sup> DCs ( $n = 2$ ), CD8<sup>+</sup> DCs ( $n = 2$ ), pDCs ( $n = 2$ ), CD8<sup>+</sup> cells ( $n = 2$ ), NK cells ( $n = 2$ ) (all from the Gene Expression Omnibus (GEO) (<http://www.ncbi.nlm.nih.gov/projects/geo>) under accession GSE9810), CD4<sup>+</sup> cells ( $n = 2$ , GEO accessions GSM44979 and GSM44982), mast cells ( $n = 2$ , GEO accessions GSM258711 and 258712) and granulocytes ( $n = 2$ , GEO accessions GSM149595 and GSM149596). Data sets for macrophages ( $n = 3$ ) were obtained from the National Cancer Institute caArray (<http://caarray.nci.nih.gov/>). All data were imported into R and Bioconductor software (<http://bioconductor.org>) using affy package. Background correction, normalization, perfect match correction and summarization of the data were performed with the methods: rma, quantiles, pmonly and medianpolish respectively, using the affy function `expresso`. The statistical properties of all the arrays after the pre-processing step were examined and confirmed to be very similar. Cluster analysis was then performed using the clustering algorithm divisive analysis clustering (Diana), which is a function in the cluster package from bioconductor.

**Statistical analysis.** Graph Pad Prism was used to calculate the s.d. between experimental samples when each experimental group contained an equal number of data sets. In the case where different numbers of data sets existed in each experimental group the s.e.m. was used. When data were normally distributed and when two independent variables were being analysed, a two-way ANOVA with Bonferroni post-analysis was performed. In all other instances statistical differences between groups were calculated using Student's *t*-test, with  $P < 0.05$  considered significant.

20. Shinkai, Y. *et al.* RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* **68**, 855–867 (1992).
21. Cui, J. *et al.* Requirement for Vα14 NKT cells in IL-12-mediated rejection of tumors. *Science* **278**, 1623–1626 (1997).
22. Tono, T. *et al.* *c-kit* gene was not transcribed in cultured mast cells of mast cell-deficient *W<sup>sh</sup>/W<sup>sh</sup>* mice that have a normal number of erythrocytes and a normal *c-kit* coding region. *Blood* **80**, 1448–1453 (1992).
23. Liu, P., Jenkins, N. A. & Copeland, N. G. A highly efficient recombineering-based method for generating conditional knockout mutations. *Genome Res.* **13**, 476–484 (2003).
24. Warming, S., Costantino, N., Court, D. L., Jenkins, N. A. & Copeland, N. G. Simple and highly efficient BAC recombineering using galK selection. *Nucleic Acids Res.* **33**, e36 (2005).

## SUPPLEMENTARY INFORMATION

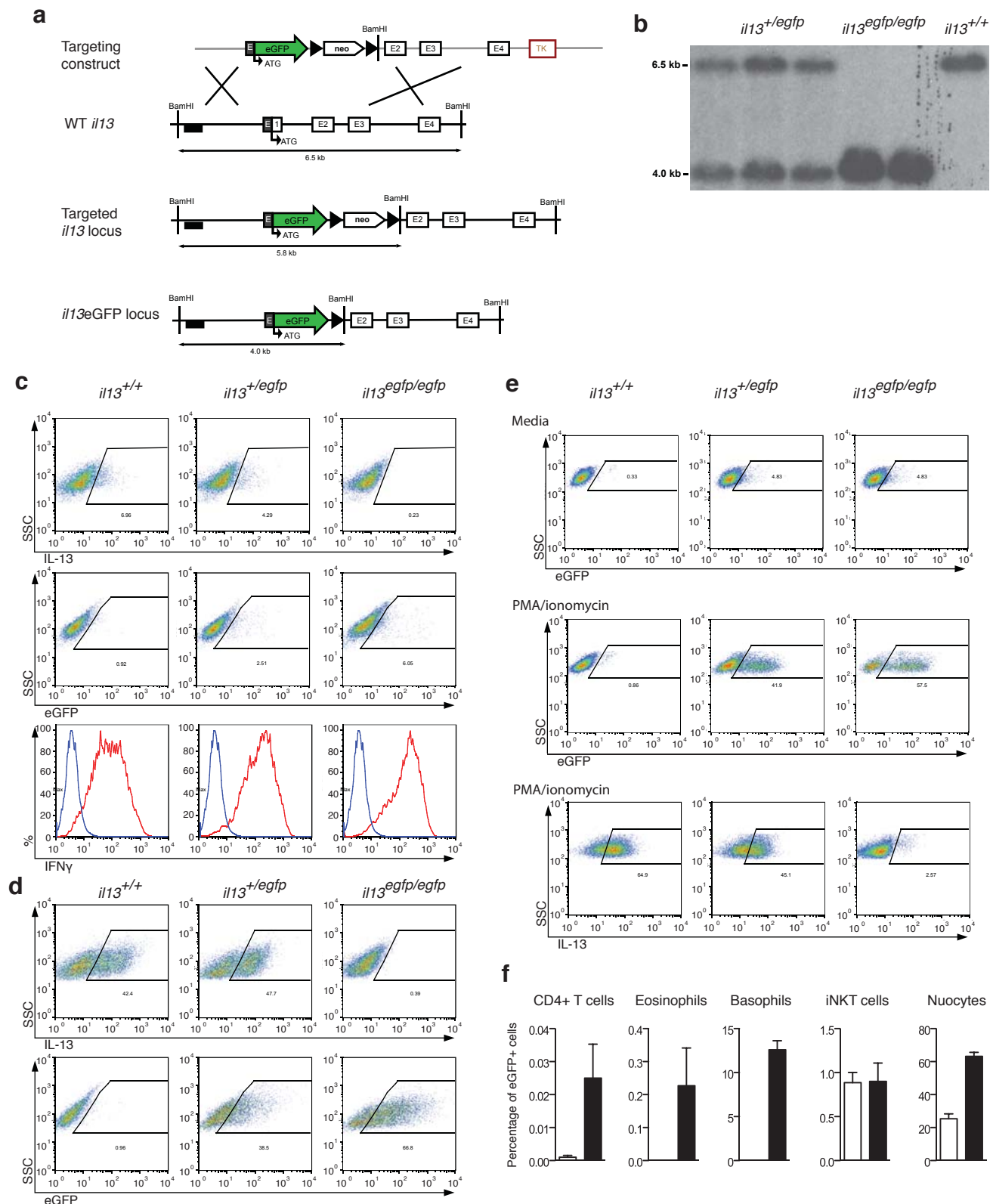


**Figure S1. Generation and characterisation of *Il13eGFP*<sup>+</sup> mice.** (a) Targeting strategy to insert the eGFP gene at the start codon of the *il13* gene. (b) Southern blot analysis of BamHI-digested genomic DNA from wildtype and heterozygote or homozygote *Il13eGFP* mice. (c-e) IL-13 production in cultured T-cells and mast cells. CD4<sup>+</sup> splenocytes from mice of the indicated genotypes were cultured under Th1 (c) or Th2 (d) conditions. Dot plots of gated live cells show eGFP or IL-13 production (intracellular staining), and histograms show interferon gamma production (in red, blue is isotype control). (e) Bone marrow derived mast cells. Dot plots show eGFP or IL-13 (intracellular staining) production. Data in (c) – (e) are representative of two independent experiments with two mice per group. (f) eGFP<sup>+</sup> cells as percentage of total in different populations from infected (black bars) and uninfected (white bars) *Il13eGFP* heterozygous animals. CD4<sup>+</sup> T cells, iNKT cells and Nuocytes are from the mLN at 5 days post infection (d.p.i.), eosinophils are from peritoneal lavage at 8 d.p.i. and basophils are from spleen at 8 d.p.i.

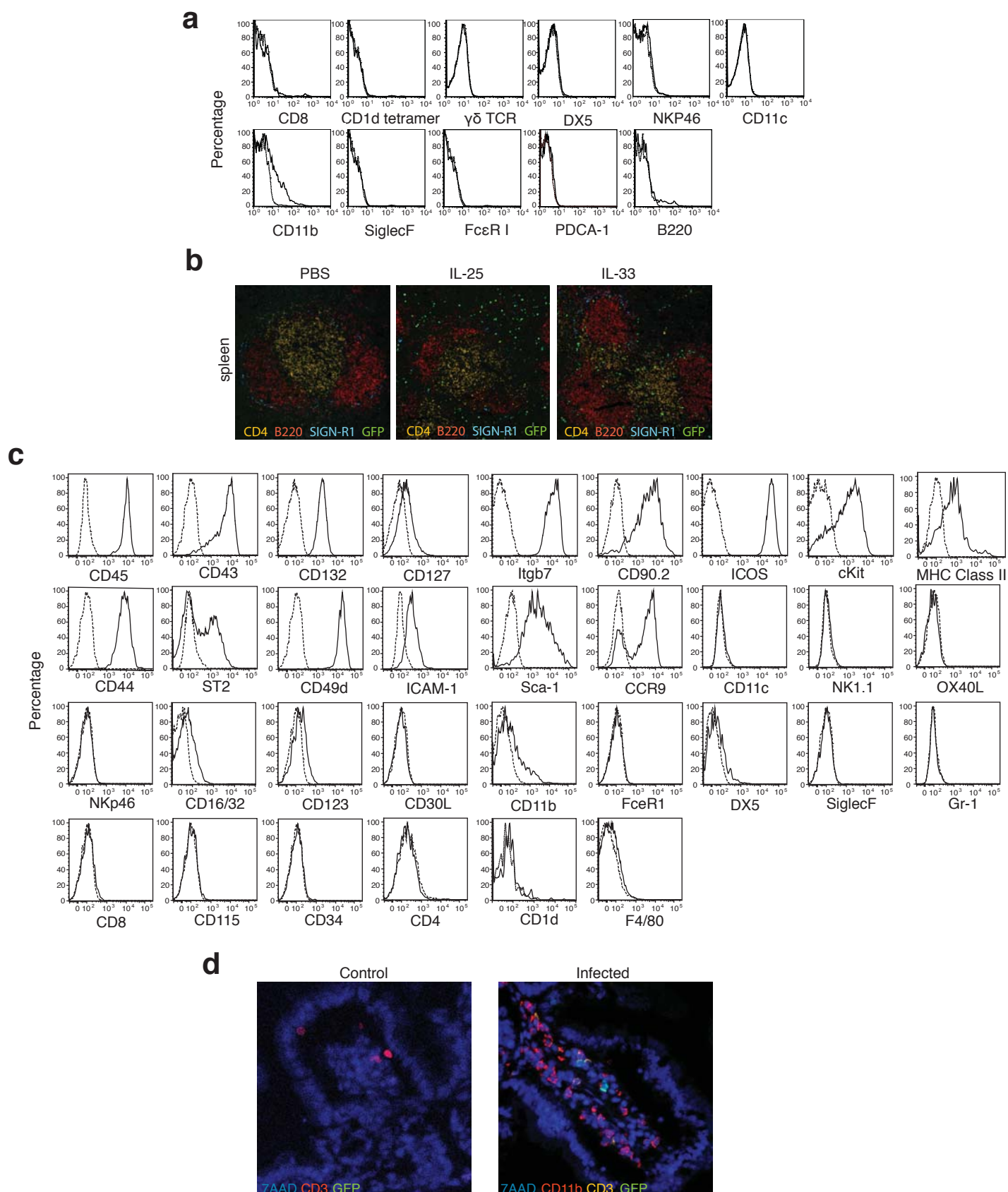


**Figure S2. Phenotypic characterisation of nuocytes.** (a) Cell surface marker expression of IL13eGFP<sup>+</sup> cells in the MLN following IL-25 administration. (b) Detection of NBNT IL13eGFP<sup>+</sup> cells in the spleen of IL-25 and IL-33 treated mice. (c) Flow cytometry analysis of cell surface markers expressed by nuocytes in spleen following three daily i.p. doses of IL-25. Specific antibodies (solid line) or isotype control (dashed line). Results shown in (a) – (c) are representative of at least 3 independent experiments with >3 mice per group. (d) Identification of NBNT IL13eGFP<sup>+</sup> cells in the small intestine 9 days p.i. with *N. brasiliensis*. Results are from two experiments with 2 mice per group.

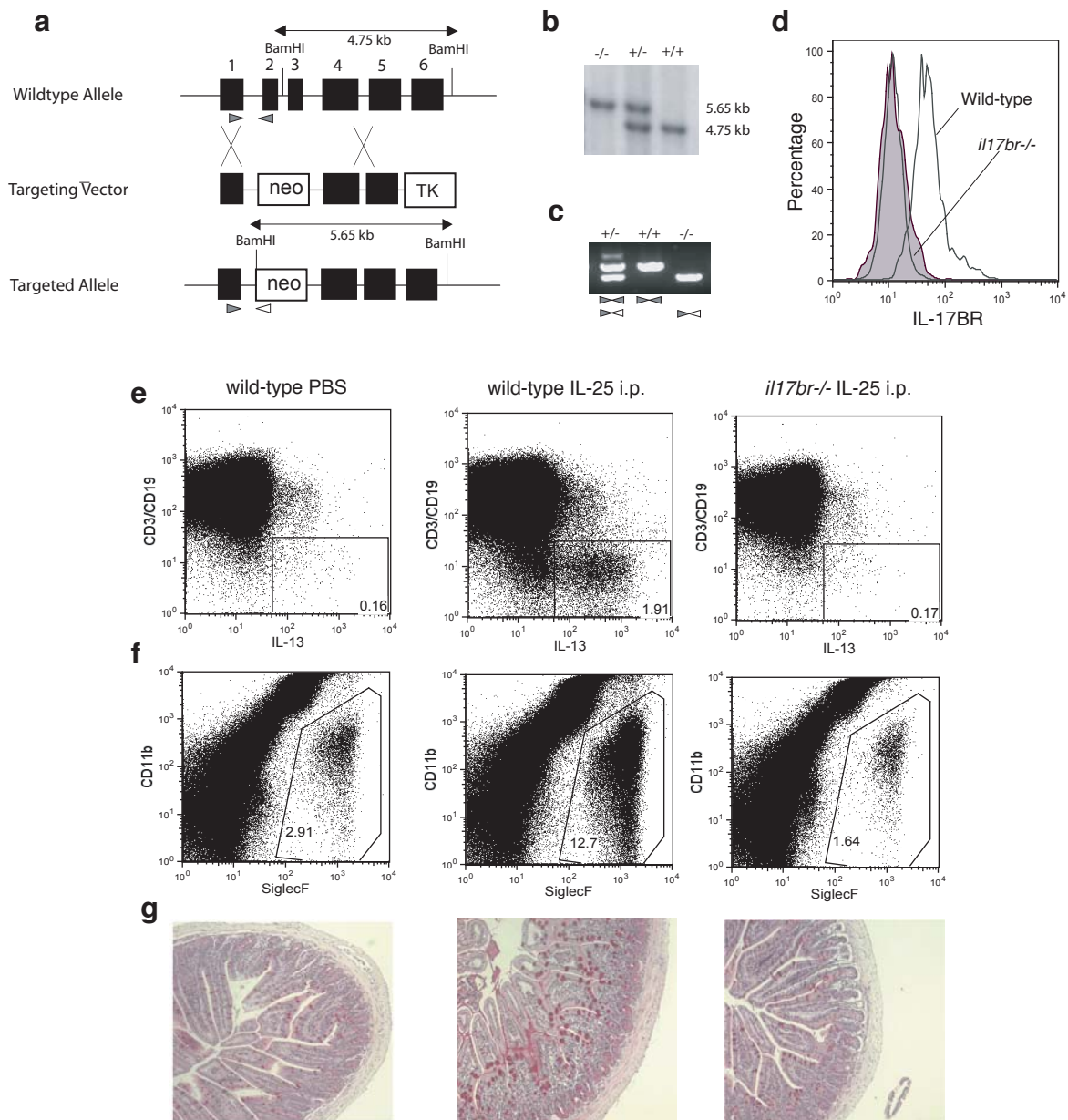


**Figure S3. Generation and characterisation of *il17br*<sup>-/-</sup> mice and anti-IL-17BR antibodies.** (a) Targeting strategy for *il17br*. Neo; neomycin cassette. TK; thymidine kinase cassette. Triangles represent locations of PCR genotyping primers. (b) Southern blot of BamHI-digested genomic DNA from wild-type (+/+), heterozygote (+/-) and homozygote targeted (-/-) mice. (c) PCR genotyping of the wild-type (+/+), heterozygote (+/-) and homozygote targeted (-/-) *il17br* alleles. (d) Flow cytometric analysis of IL-17BR expression on in vitro differentiated Th2 cells from wildtype or *il17br*<sup>-/-</sup> animals. The shaded line represents isotype-control staining. (e) Intracellular IL-13 staining in NBNT-cells in the mLN. (f) Eosinophil (SiglecF<sup>high</sup>, CD11b<sup>int</sup>) influx into the peritoneal cavity. (g) PAS staining of intestinal sections. Mucus and goblet cells stain pink. Data in (e) – (g) are representative of three independent experiments with >4 mice per group given three i.p. administrations of IL-25.

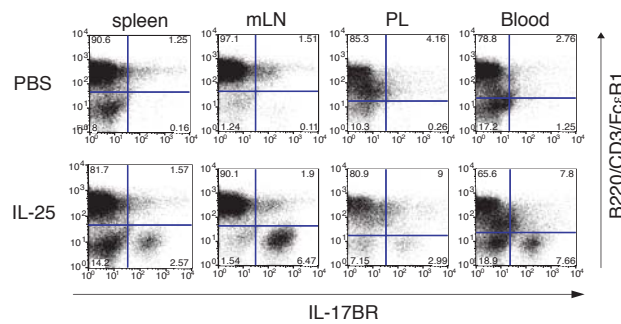


**Figure S4. Tissue distribution of nuocytes following four daily administrations i.p. of IL-25.** PL: peritoneal lavage. Data are representative of at least three independent experiments with >4 mice per group.

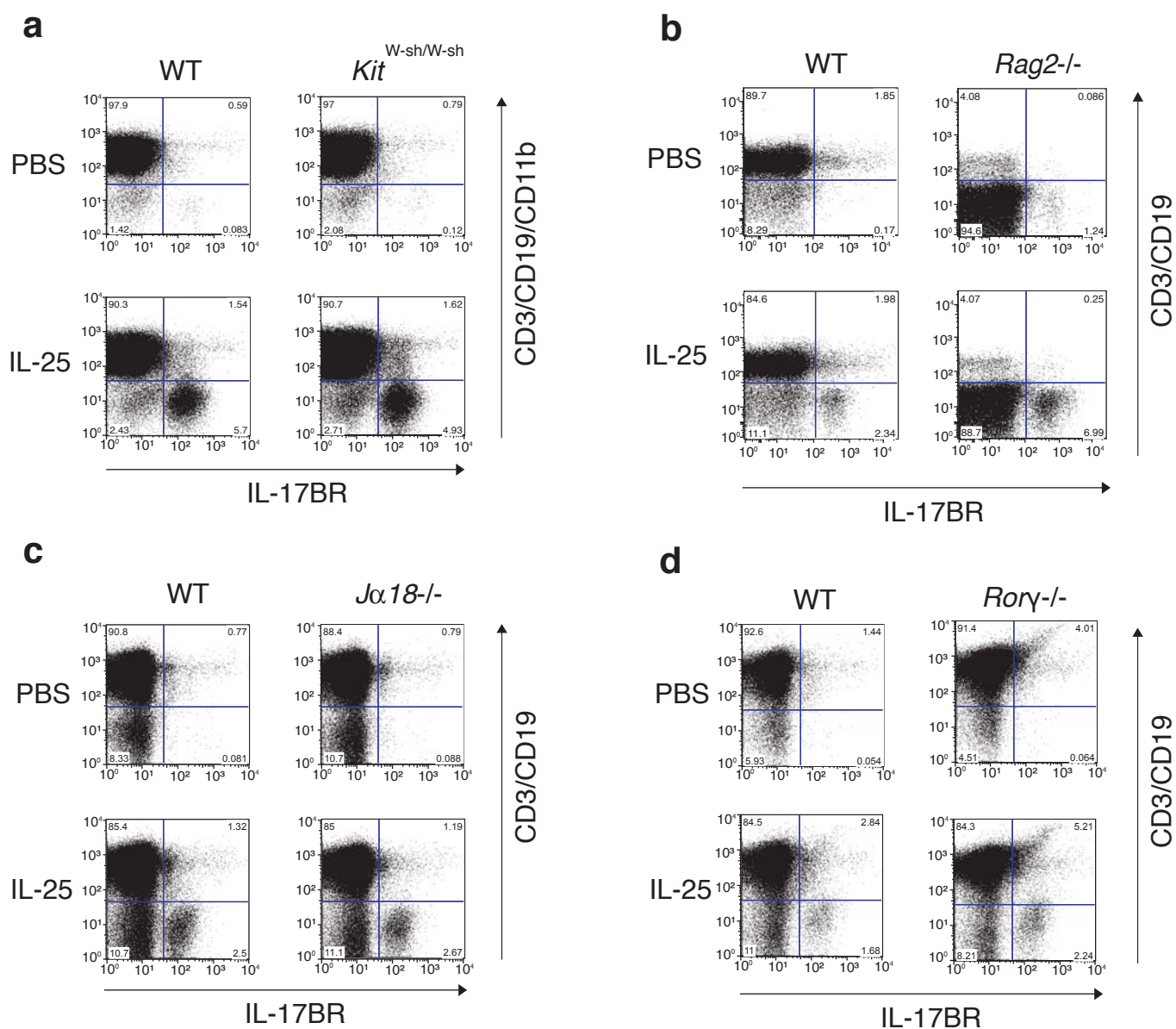


**Figure S5. Nuocytes have a distinct developmental requirement from mast cells, B/T cells and NKT cells.** Flow cytometric analysis of mice given 3 daily i.p. injections of IL-25 **(a)** Splenic nuocytes in mast cell-deficient *Kit* mutant (*Kit*<sup>W-sh/W-sh</sup>) mice. **(b)** Splenic nuocytes in T and B lymphocyte-deficient *Rag*<sup>-/-</sup> mice. **(c)** Splenic nuocytes in NKT cell-deficient *Jα18*<sup>-/-</sup> mice. **(d)** Splenic nuocytes in LT $\alpha$ -deficient *Rorγ*<sup>-/-</sup> mice. Results shown are representative of at least two independent experiments with >3 mice per group.

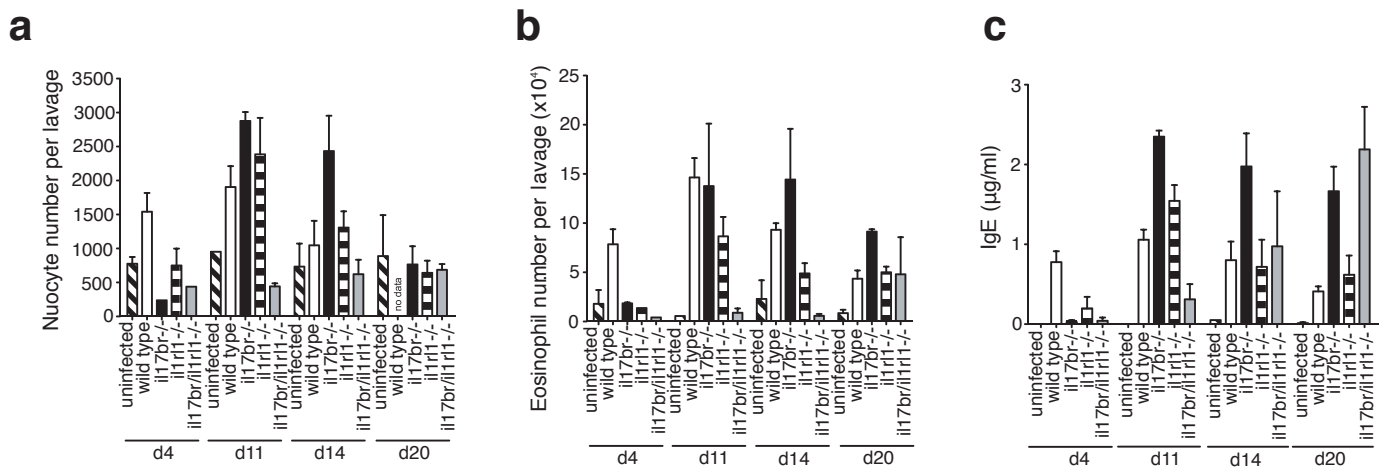


**Figure S6. IL-25 and IL-33 have redundant functions in *N. brasiliensis* infection.** (a) Quantification of nuocytes in the peritoneal lavage. (b) Quantification of eosinophils in the peritoneal lavage. (c) Quantification of total IgE in the serum. Data are representative of two independent experiments with >6 mice per group.

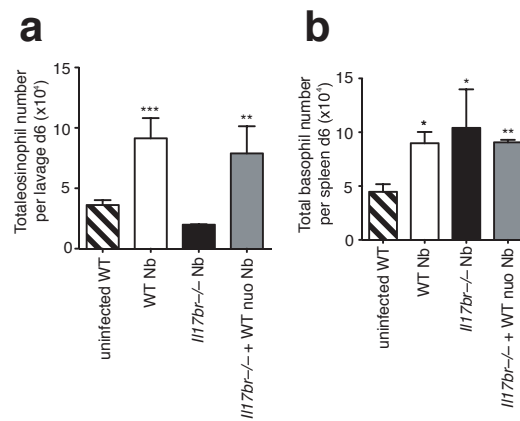


**Figure S7. Eosinophil and basophil numbers following *N. brasiliensis* infection.** (a) Quantification of peritoneal lavage eosinophil numbers 6 days p.i. (b) Quantification of basophil numbers in the spleen 6 days p.i. Data are representative of two independent experiments with > 6 mice per group.

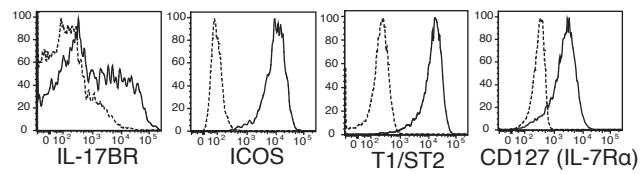


**Figure S8. Flow cytometric analysis of nuocytes after 7 day culture with IL-7 and IL-33.** Data are representative of three independent experiments.



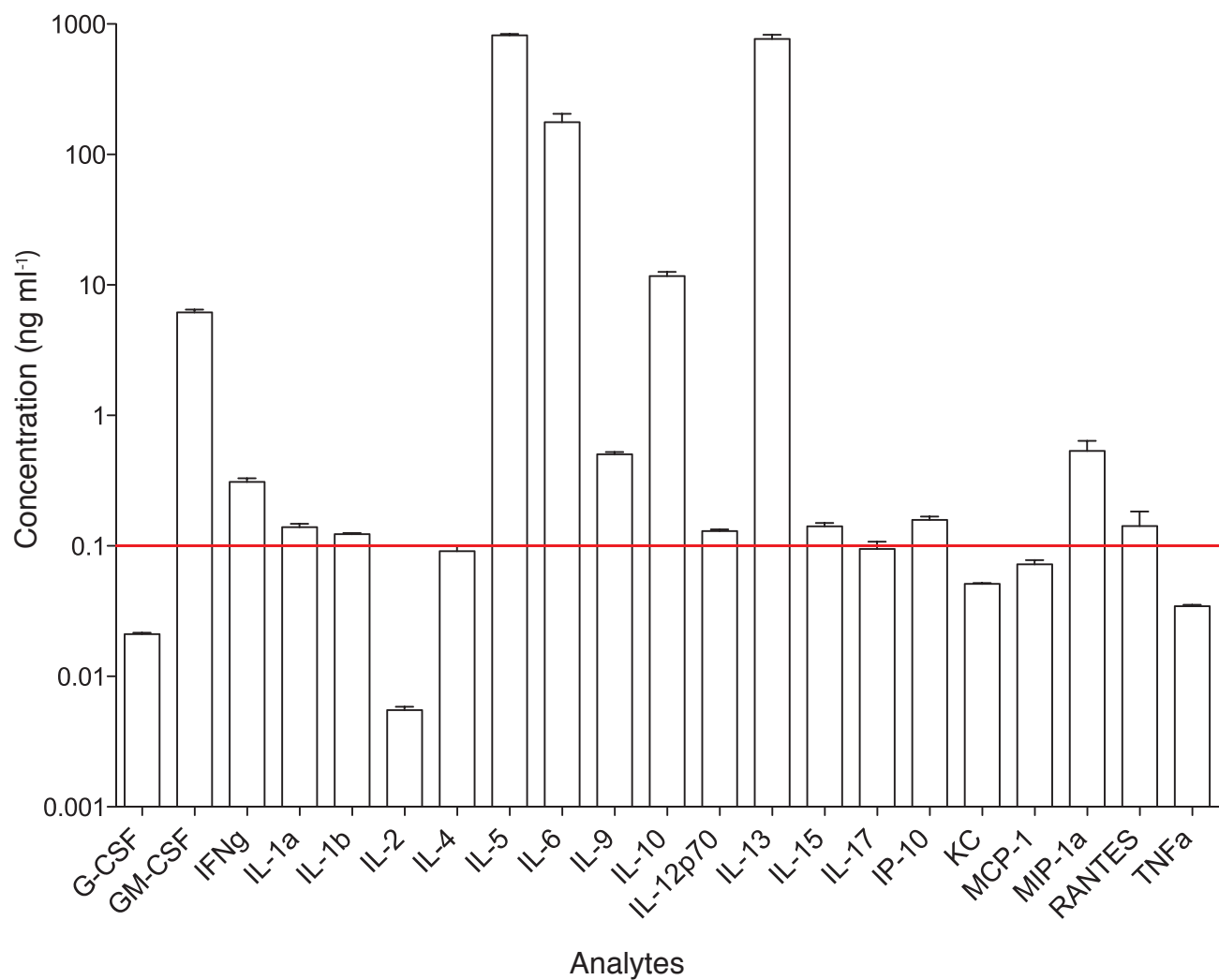


**Figure S9. Cytokine/chemokine profile from nuocyte culture supernatant.** Data are the mean  $\pm$  SEM of 5 separate experiments.

Table 1

Affymetrix gene expression profiles of isolated nuocytes

|                              | Probeset ID  | Gene name      | Expression signal |          |
|------------------------------|--------------|----------------|-------------------|----------|
|                              |              |                | nuocyte1          | nuocyte2 |
| Chemokine receptors          | 1448710_at   | <i>Cxcr4</i>   | 1924              | 2120     |
|                              | 1422812_at   | <i>Cxcr6</i>   | 2504              | 1898     |
|                              | 1421920_a_at | <i>Ccr9</i>    | 8422              | 8422     |
| Cytokine receptors           | 1425145_at   | <i>Il1rl1</i>  | 2740              | 2539     |
|                              | 1420692_at   | <i>Il2ra</i>   | 247               | 280      |
|                              | 1448759_at   | <i>Il2rb</i>   | 1499              | 1552     |
|                              | 1416296_at   | <i>Il2rg</i>   | 5185              | 4905     |
|                              | 1448575_at   | <i>Il7r</i>    | 3259              | 2957     |
|                              | 1419455_at   | <i>Il10rb</i>  | 729               | 639      |
|                              | 1418166_at   | <i>Il12rb1</i> | 298               | 338      |
|                              | 1420905_at   | <i>Il17ra</i>  | 4360              | 4068     |
|                              | 1420678_a_at | <i>Il17rb</i>  | 5673              | 5078     |
|                              | 1449508_at   | <i>Il27ra</i>  | 3743              | 3343     |
| Adhesion molecules           | 1439713_at   | <i>Itga1</i>   | 399               | 313      |
|                              | 1427615_at   | <i>Itga4</i>   | 294               | 377      |
|                              | 1449216_at   | <i>Itgae</i>   | 826               | 755      |
|                              | 1452784_at   | <i>Itgav</i>   | 2837              | 2916     |
|                              | 1450678_at   | <i>Itgb2</i>   | 2557              | 2195     |
|                              | 1455257_at   | <i>Itgb3</i>   | 6657              | 6252     |
|                              | 1418741_at   | <i>Itgb7</i>   | 9675              | 9090     |
|                              | 1424067_at   | <i>Icam1</i>   | 541               | 969      |
|                              | 1448862_at   | <i>Icam2</i>   | 1795              | 1846     |
| Other cell surface molecules | 1420788_at   | <i>Klrg1</i>   | 10960             | 10513    |
|                              | 1436598_at   | <i>Icos</i>    | 8719              | 8306     |
|                              | 1423135_at   | <i>Thy1</i>    | 2020              | 1965     |
|                              | 1453304_s_at | <i>Ly6e</i>    | 1314              | 1468     |
|                              | 1452514_a_at | <i>Kit</i>     | 352               | 333      |
|                              | 1423760_at   | <i>Cd44</i>    | 1746              | 2020     |

Gene expression profiles of selected cell surface molecules for isolated nuocytes were analysed as described in Methods. Values are shown as Affymetrix expression signal intensity for two nuocyte samples.