SUPPLEMENTARY INFORMATION

# RORγt$^+$ innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota

Shinichiro Sawa[1,2], Matthias Lochner[1,2,3], Naoko Satoh-Takayama[4,5], Sophie Dulauroy[1,2], Marion Bérard [6], Melanie Kleinschek[7], Daniel Cua[7], James P. Di Santo[4,5] and Gérard Eberl[1,2]

[1]Institut Pasteur, Lymphoid Tissue Development Unit, 75724 Paris, France

[2]CNRS, URA1961, 75724 Paris, France

[3]Institute of Infection Immunology, TWINCORE, Centre for Experimental and Clinical Infection Research; a joint venture between the Medical School Hannover (MHH) and the Helmholtz Centre for Infection Research (HZI), Hannover, Germany

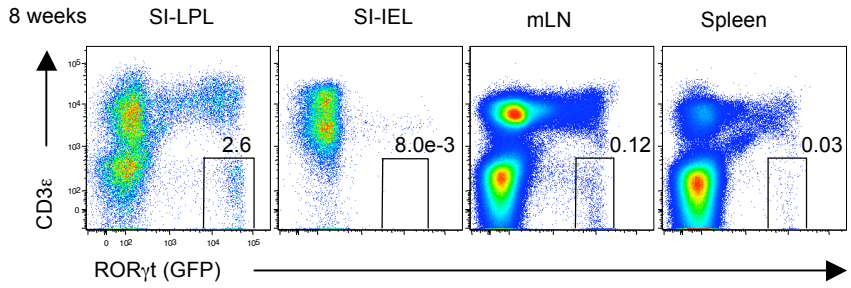
[4]Institut Pasteur, Innate Immunity Unit, 75724 Paris, France

[5]Inserm, U668, 75724 Paris, France

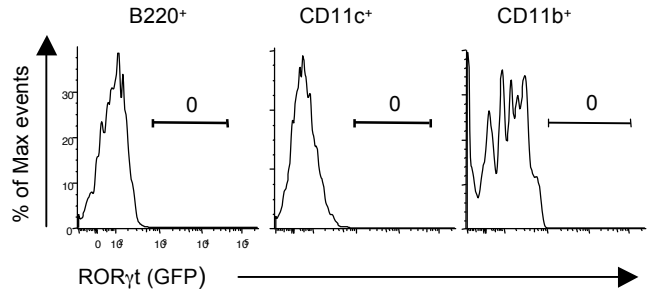
[6]Institut Pasteur, Animalerie Centrale, 25 rue du Dr Roux, 75724 Paris, France

[7]Merck Research Laboratories, DNAX Discovery Research, 94304 Palo Alto, USA

a

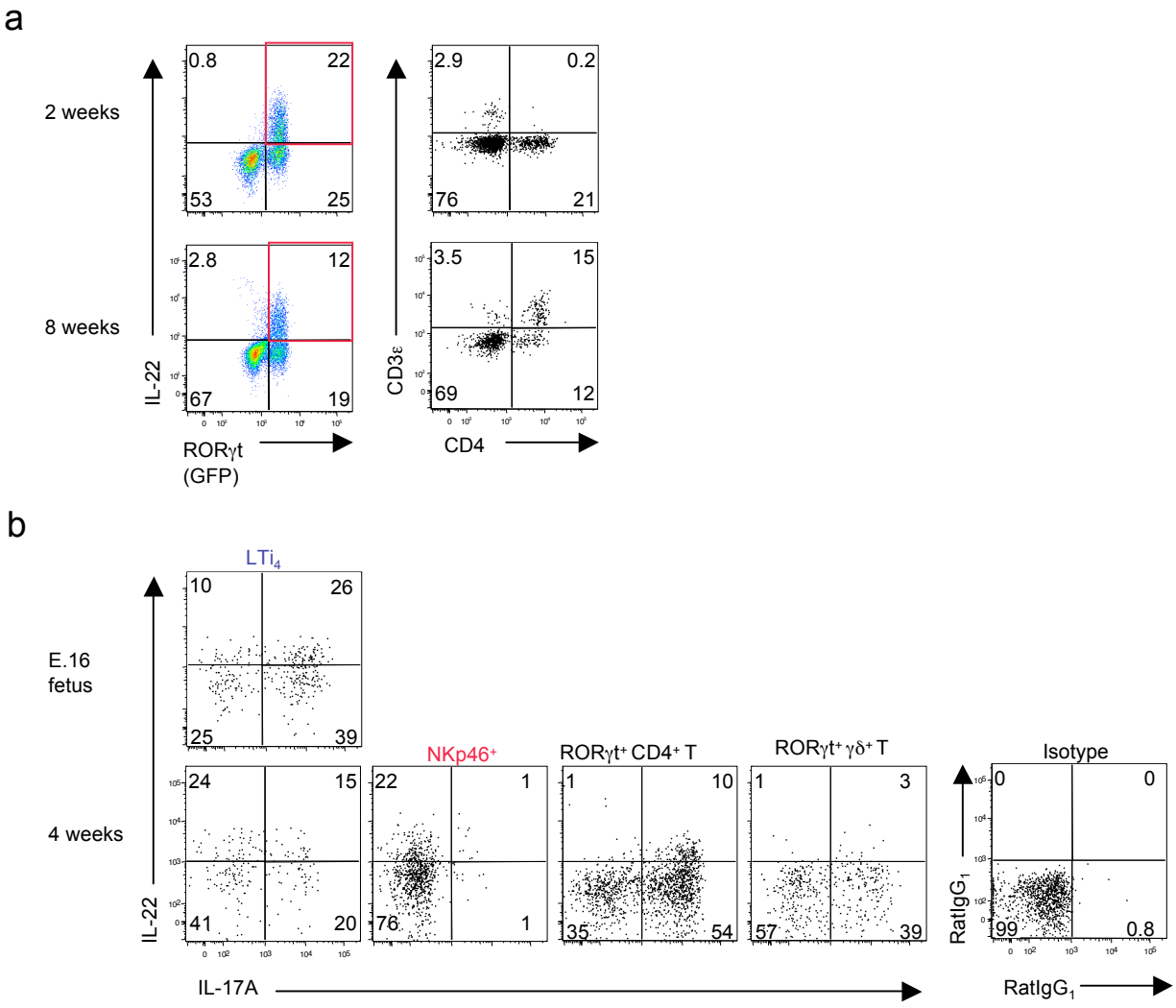



b




**Supplementary Figure 1**

RORγt$^{+}$ ILCs in IELs and secondary lymphoid tissues. (**a**) RORγt$^{+}$ ILCs in the SI-LPL, intraepithelial lymphocytes (IEL), mesenteric lymph nodes (mLN) and spleen of 4 weeks old *Rorc(γt)-Gfp*$^{TG}$ mice. Data are representative of $n = 4$ mice. **(b)** RORγt (GFP) expressions in SI-LPL B220$^{+}$, CD11c$^{+}$ or CD11b$^{+}$ cells. Numbers indicate percent positive cells. Data are representative of $n = 3$ mice.

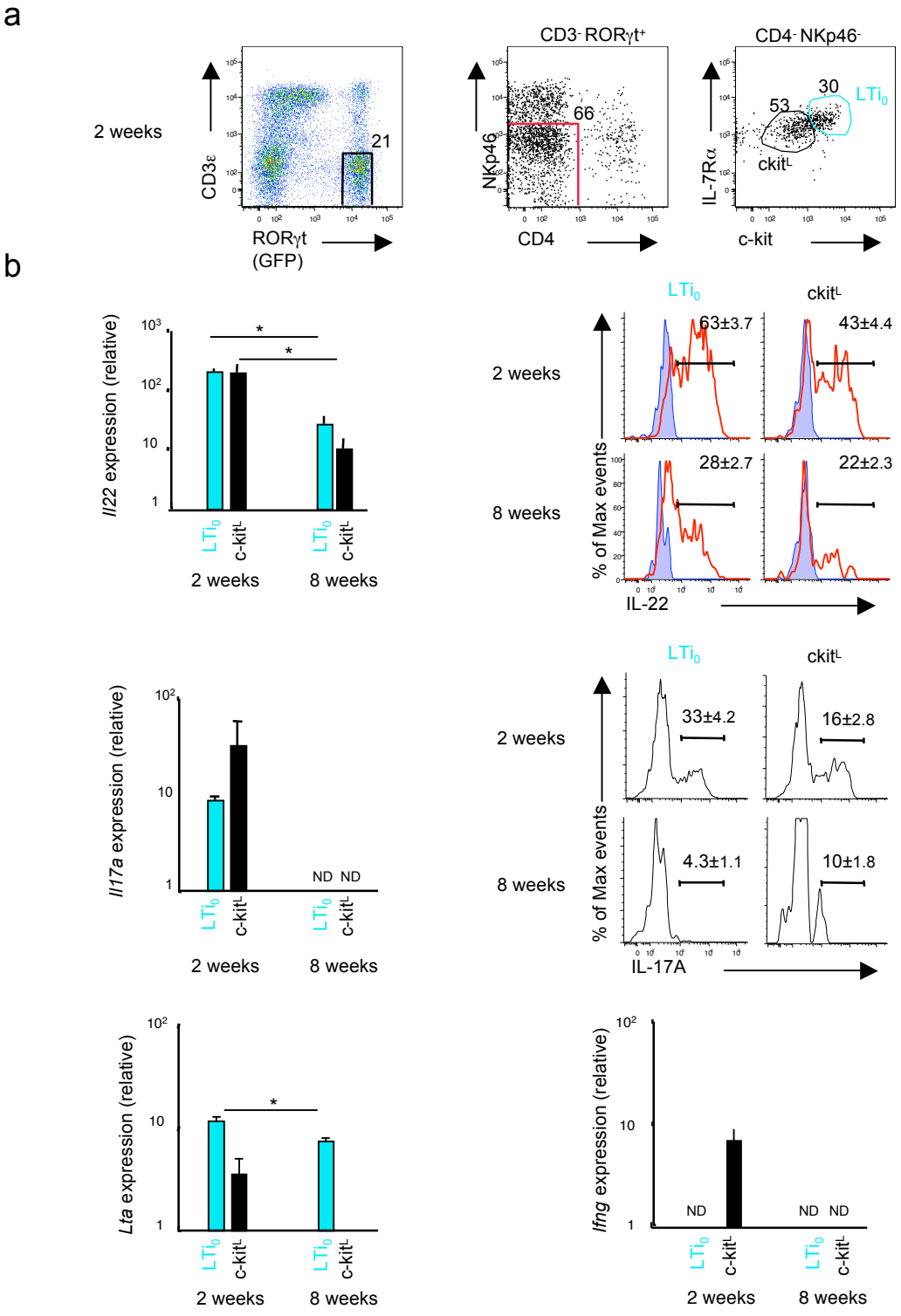



**Supplementary Figure 2**

The production of pro-inflammatory cytokines by RORγt$^+$ ILCs. (**a**) Small intestinal lamina propria leukocytes (SI-LPL) from 2 weeks and 8 weeks old *Rorc(γt)-Gfp*$^{TG}$ mice were analyzed by flow cytometry. The production of IL-22 was assessed by intracellular cytokine staining after 3 hours of stimulation with PMA/ionomycine *ex vivo*, and IL-22$^+$ RORγt$^+$ cells were further analyzed for the expression of CD3ε and CD4. Numbers indicate percent cells per quadrant. The data are representative of three independent experiments. (**b**) RORγt$^+$ ILCs from E16 mice, and RORγt$^+$ ILCs, RORγt$^+$ CD4$^+$ T cells and RORγt$^+$ γδ T cells from 4 weeks old mice were analyzed for intracellular expression of IL-17 and IL-22 after 3 hours stimulation *ex vivo* with PMA/ionomycin. Data are representative of 3 independent experiments. (**c**) SI-LPL from E15, 2 weeks and 8 weeks old *Rorc(γt)-Gfp*$^{TG}$ mice were analyzed by real-time qPCR for the expression of transcripts for TRANCE. Data are mean of three independent experiments. $^*P < 0.05$, unpaired *t*-test.



Nature Immunology doi:10.1038/ni.2002

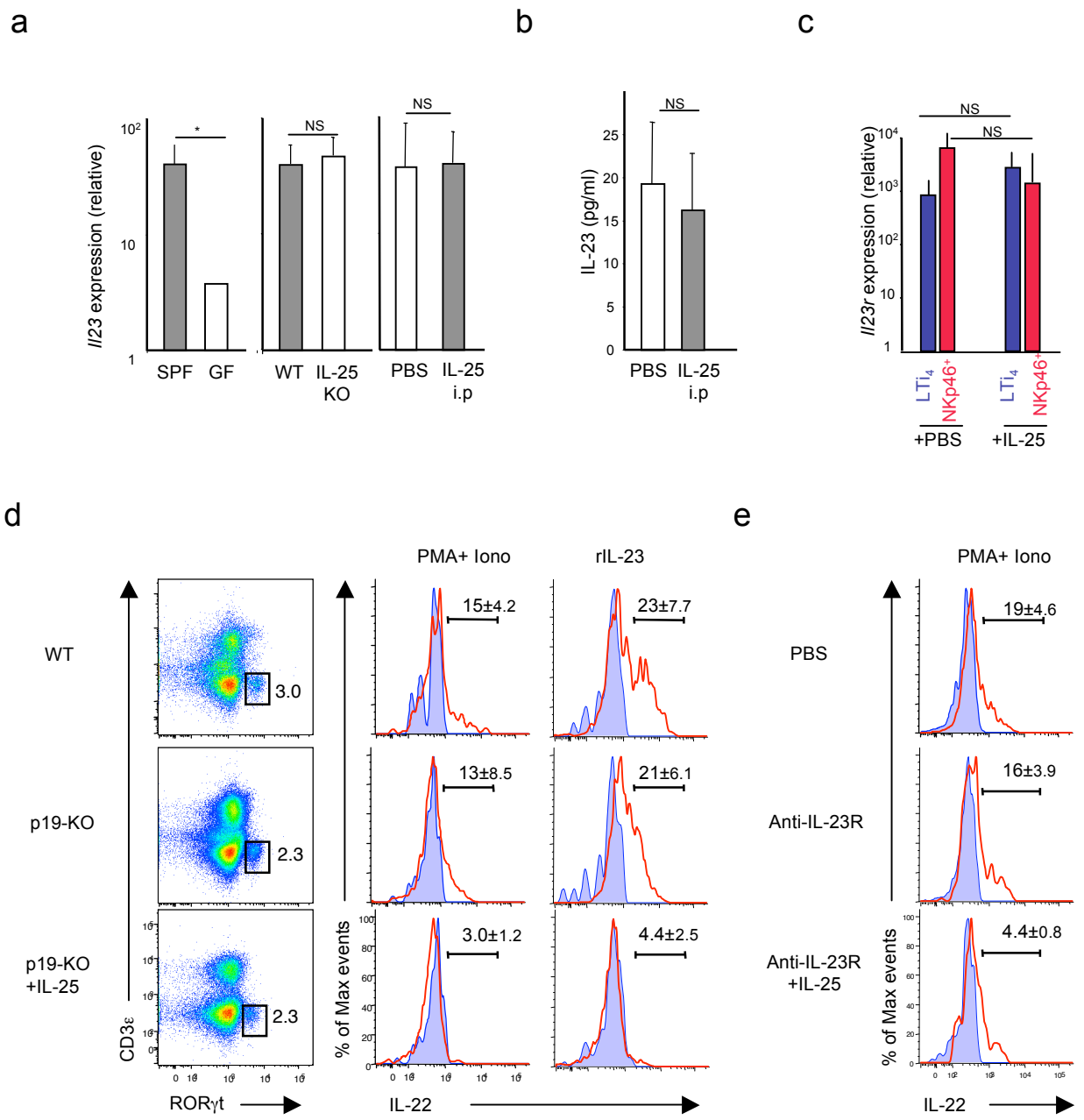



**Supplementary Figure 3**

The production of pro-inflammatory cytokines by CD4$^-$ NKp46$^-$ RORγt$^+$ ILCs. (**a**) SI-LPL from 2 weeks old *Rorc(γt)-Gfp*$^{TG}$ mice were analyzed by flow cytometry. CD4$^-$NKp46$^-$ RORγt$^+$ILCs were subdivided into c-kit$^{hi}$ (LTi$_0$) and c-kit$^{lo}$ (c-kit$^L$) cells. Data are representative of at least $n$ = 8 mice. (**b**) Expression of transcripts and proteins for IL-22, IL-17, LTα and IFNγ by LTi$_0$ and c-kit$^L$ cells from 2 weeks and 8 weeks old *Rorc(γt)-Gfp*$^{TG}$ mice. Intracellular IL-22 and IL-17 protein expression was measured after 3 hours stimulation *ex vivo* with rIL-23 or PMA/ionomycin, respectively. The data are representative of five independent experiments. In histograms, numbers are mean of $n$ = 10 mice ± S.E.M percentages of IL-22$^+$ or IL-17$^+$ cells. $^*P < 0.05$, unpaired $t$-test, ND, non-detected.



Nature Immunology doi:10.1038/ni.2002

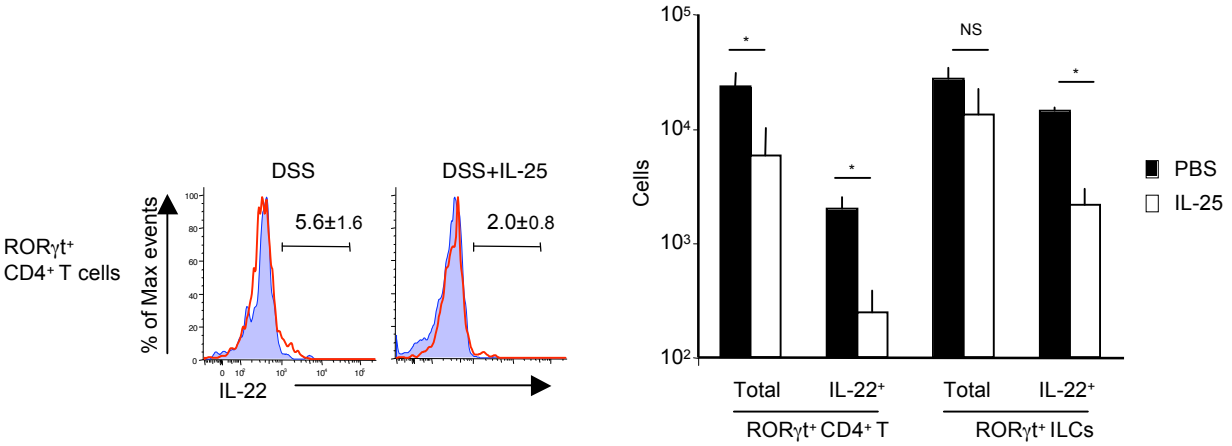



**Supplementary Figure 4**

No role for IL -23 in the IL-25-mediated regulation of IL-22 production by R ORγt$^+$ ILCs. (**a**) Expression of transcripts for IL-23p19 (*Il23a*) in the terminal ileum of 8 weeks-old SPF, GF, wild type and IL-25-deficient mice, and 6 weeks-old wild-type mice injected with rIL-25 or PBS. (**b**) IL-23a protein expression in the terminal ileum of wild-type mice injected with rIL-25 or PBS. (**c**) IL-23 receptor (*Il23r*) expression by RORγt$^+$ ILCs from 6 weeks-old RAG2-deficient mice treated with rIL-25 or PBS. $^*P < 0.05$, NS, statistically not significant, unpaired *t*-test. (d) Expression of IL-22 by RORγt$^+$ ILCs in the small intestine of adult p19-deficient mice treated with rIL-25 or PBS. Cells were stimulated for 3 hours *ex vivo* with PMA/ionomycin (middle columns) or rIL-23 (right columns). Data are mean ± S.E.M of $n = 3$ mice per group. (d) Expression of IL-22 by RORγt$^+$ ILCs in 8 weeks old *Rorc(γt)-Gfp*$^{TG}$ mice treated with neutralizing anti-IL23R antibody and rIL-25 or PBS. Cells were stimulated for 3 hours *ex vivo* with PMA/ionomycin. Data are mean ± S.E.M of $n = 3$ mice per group.



Nature Immunology doi:10.1038/ni.2002




**Supplementary Figure 5**

IL-25 represses IL-22 production by RORγt$^+$ T cells during colitis. (Left) Expression of IL-22 by small intestinal lamina propria CD4$^+$ RORγt$^+$ T cells in 9 weeks old C57/BL6 wild type mice treated with DSS. Data are mean ± S.E.M of $n$ = 3 mice per group. Cells were stimulated for 3 hours *ex vivo* with PMA/ionomycin. (Right) Absolute numbers of small intestinal lamina propria cells in DSS-treated 9 weeks old C57/BL6 wild type mice. Data are mean ± S.E.M of $n$ = 3 mice per group. $^*P < 0.05$, unpaired $t$-test.



Nature Immunology doi:10.1038/ni.2002