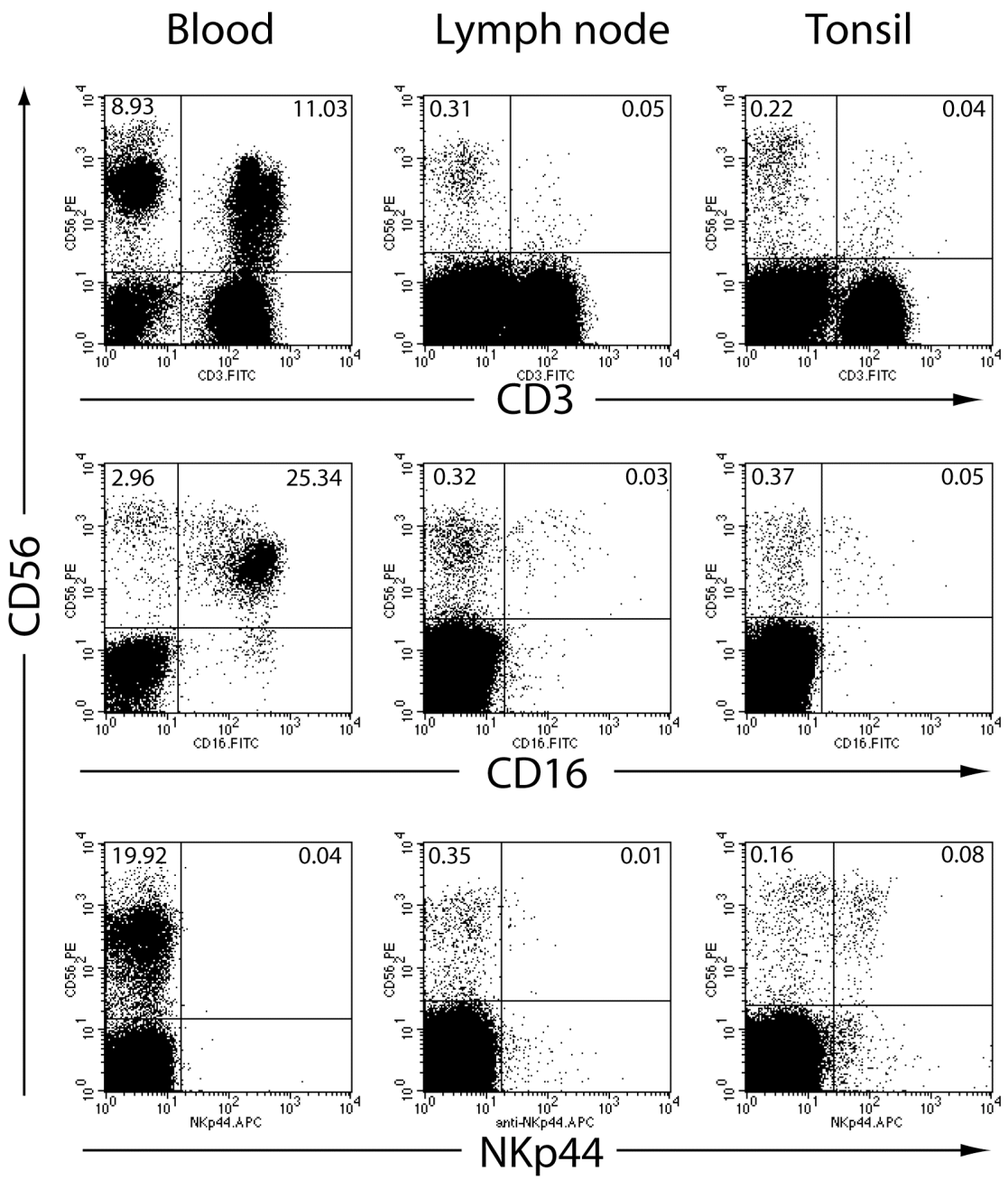
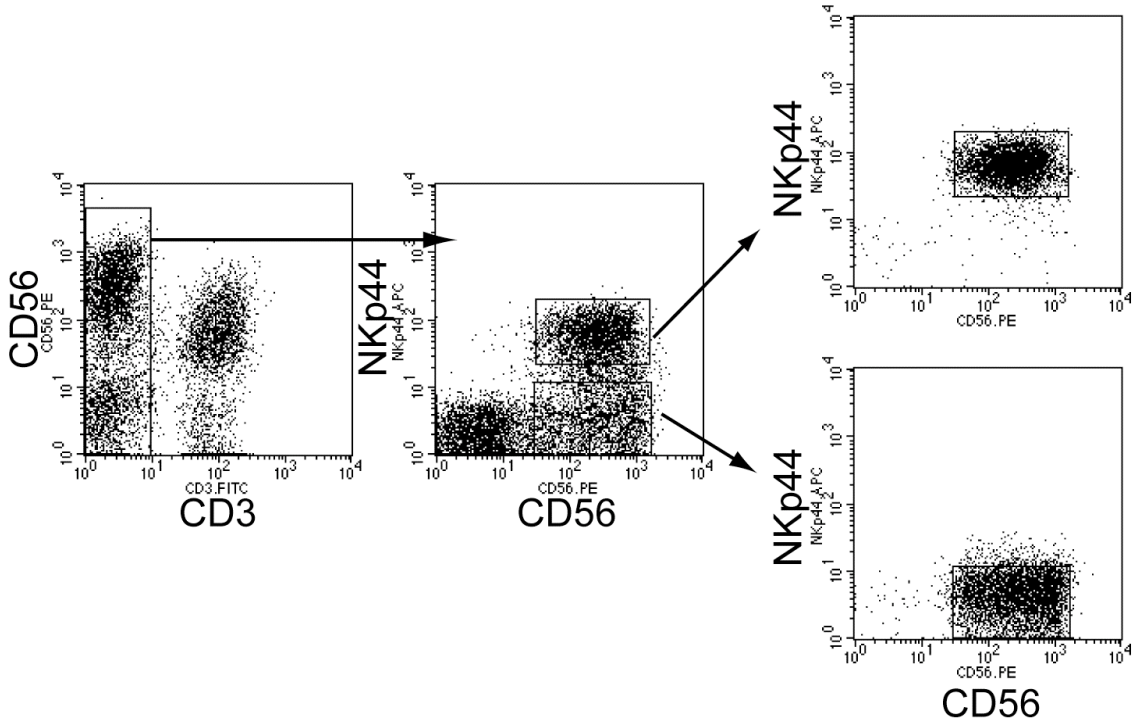
Supplementary Figure 1.



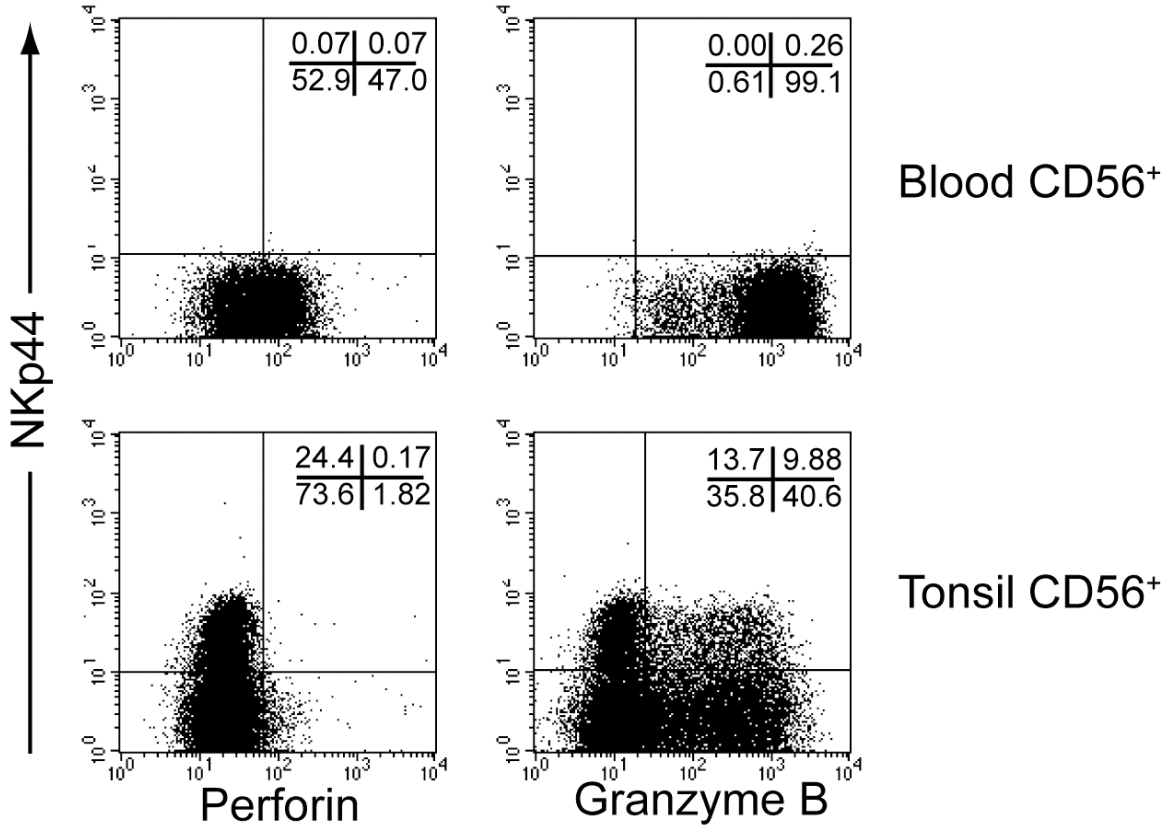
Supplementary Figure 1. A subset of tonsil NK cells expresses NKp44. Blood, lymph node and tonsil mononuclear cells were stained with anti-CD3, anti-CD56, anti-CD16 and/or anti-NKp44 to identify subsets of NK cells. $CD3^-$ $CD56^+NKp44^+$ cells were identified in tonsil but not in peripheral blood or lymph nodes.

Supplementary Figure 2.



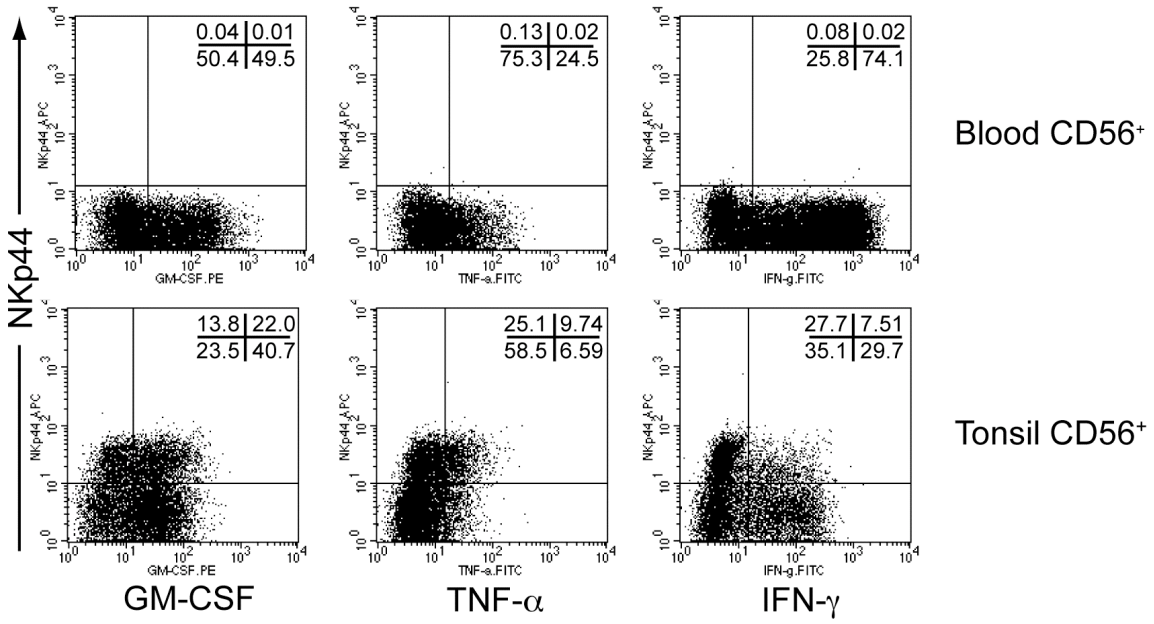
Supplementary Figure 2. **Sorting of NKp44^{+} and NKp44^{-} tonsilar NK cells**. NK cells were pre-enriched with CD56 microbeads and then stained with anti-CD3, -CD56 and -NKp44 antibodies. CD3^{-}CD56^{+}NKp44^{-} and CD3^{-}CD56^{+}NKp44^{+} cells were FACS sorted. In some sortings, anti-CD19 was added to remove contaminating B cells.

Supplementary Figure 3.



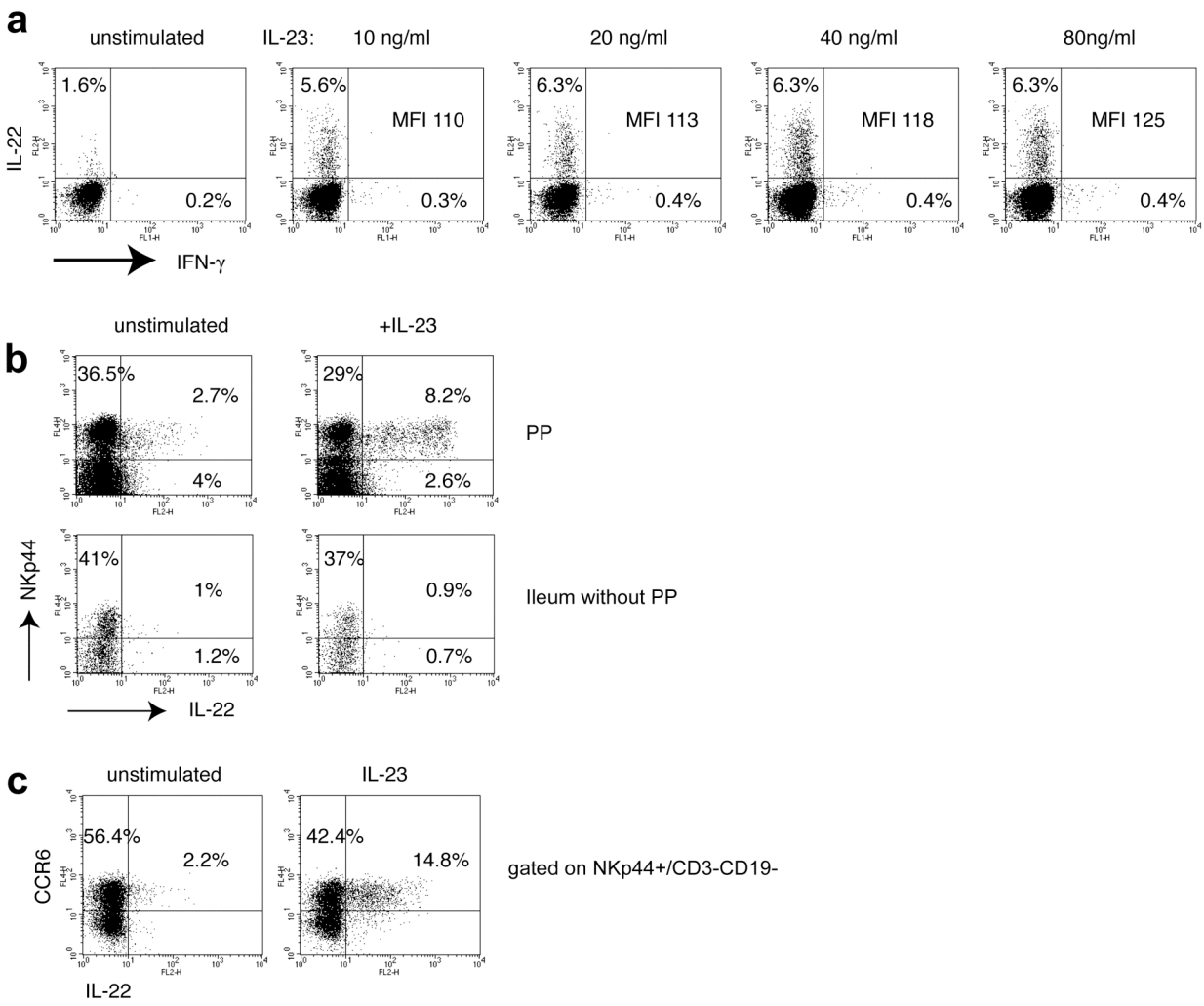
Supplementary Figure 3. Tonsil NK cells have low intracellular content of lytic mediators. Tonsil and peripheral blood mononuclear cells were stained for cell surface expression of CD3, CD56 and NKp44 and counter-stained for intracellular perforin and granzyme B. A gate is applied on $CD3^{-}CD56^{+}$ NK cells.

Supplementary Figure 4.



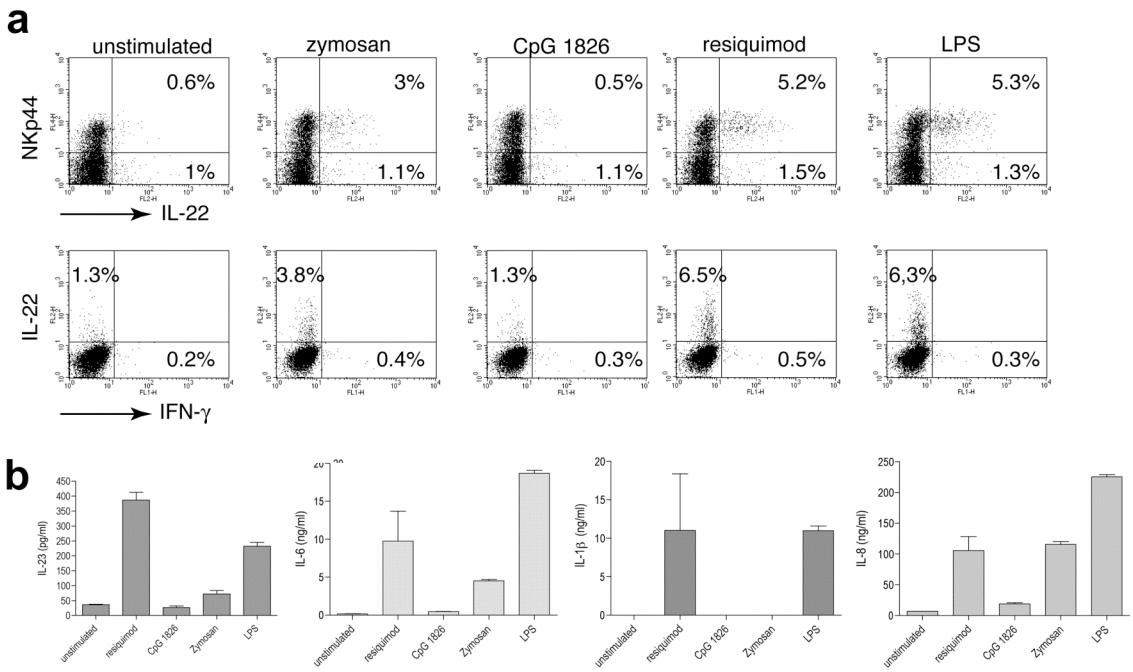
Supplementary Figure 4. NKp44^{+} tonsil NK cells produce low levels of IFN-γ. After stimulation with PMA/ionomycin, peripheral blood or tonsil NK cells were analyzed for intracellular content of IFN-γ, GM-CSF and TNF-α.

Supplementary Figure 5.



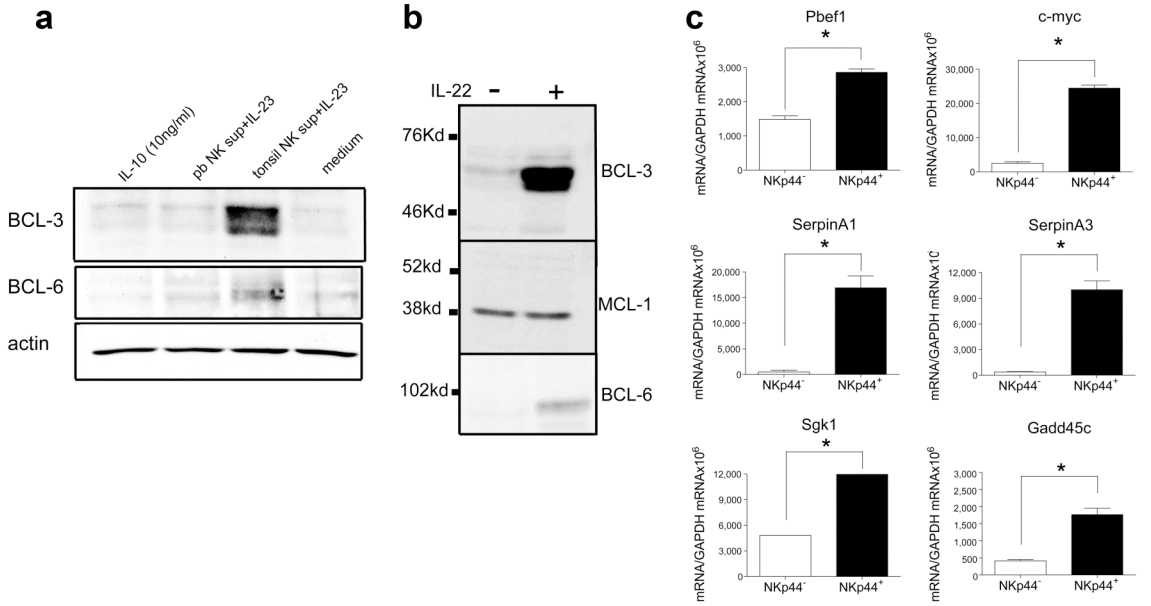
Supplementary Figure 5. NK-22 cells are MALT NKp44^{+} NK cells that secrete IL-22 in response to IL-23. a, Activation of tonsil NK cells with graded doses of IL-23 shows that maximal IL-22 production is already achieved at 10 ng/ml of IL-23. **b,** NK-22 are present in human Peyer's patches (PP). Human NKp44^{+} NK cells from PP produce IL-22 in response to IL-23. In contrast, NK-22 cells are not detected in a specimen of human ileum that lacks PP. A gate was drawn on CD3^{-}CD19^{-} cells. Presence or absence of PP was macroscopically determined by a pathologist. **c,** IL-22 production is restricted to NKp44^{+}CCR6^{+} NK cells. A gate was drawn on CD3^{-}NKp44^{+} cells were gated.

Supplementary Figure 6.



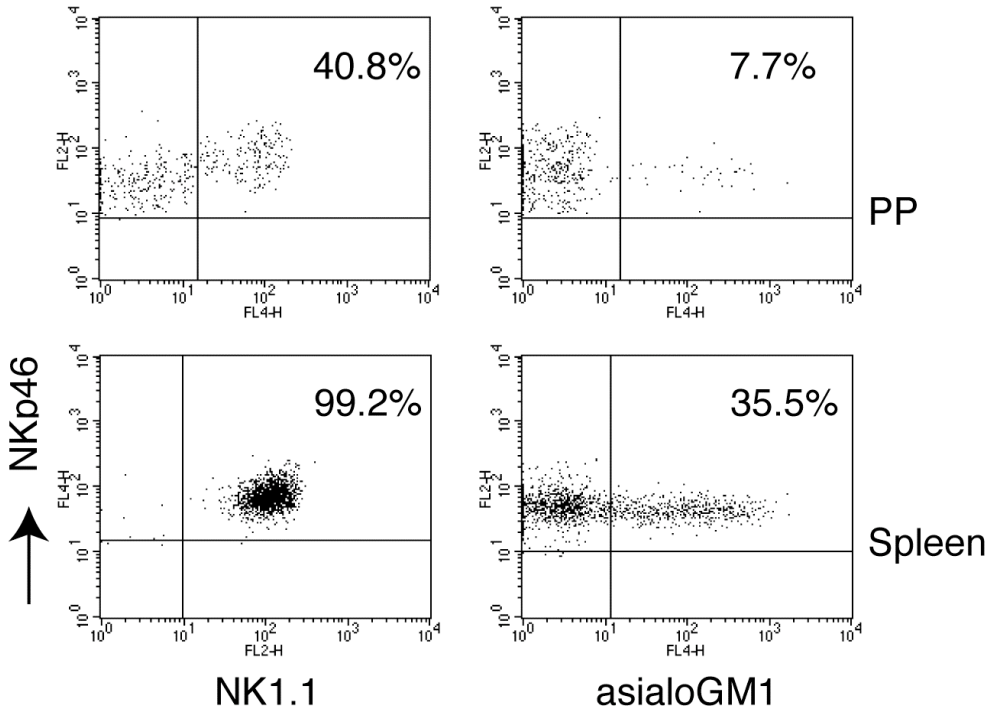
Supplementary Figure 6. Supernatants of monocytes stimulated with TLR agonists contain IL-23 and activate NK-22 cells. a, Tonsil NK cells were pulsed with cell culture supernatants derived from monocytes stimulated with different TLR agonists for 36 hours. Production of IL-22 versus IFN-γ was analyzed by intracellular staining. Supernatants of polyI:C stimulated monocytes induced IFN-γ production in NKp44^{-} cells (data not shown). **b,** Quantification of IL-23, IL-6, IL-1β, and IL-8 secreted in cell culture supernatants of monocytes stimulated with different TLR agonists. The amount of cytokines/10^6 cells is shown.

Supplementary Figure 7.



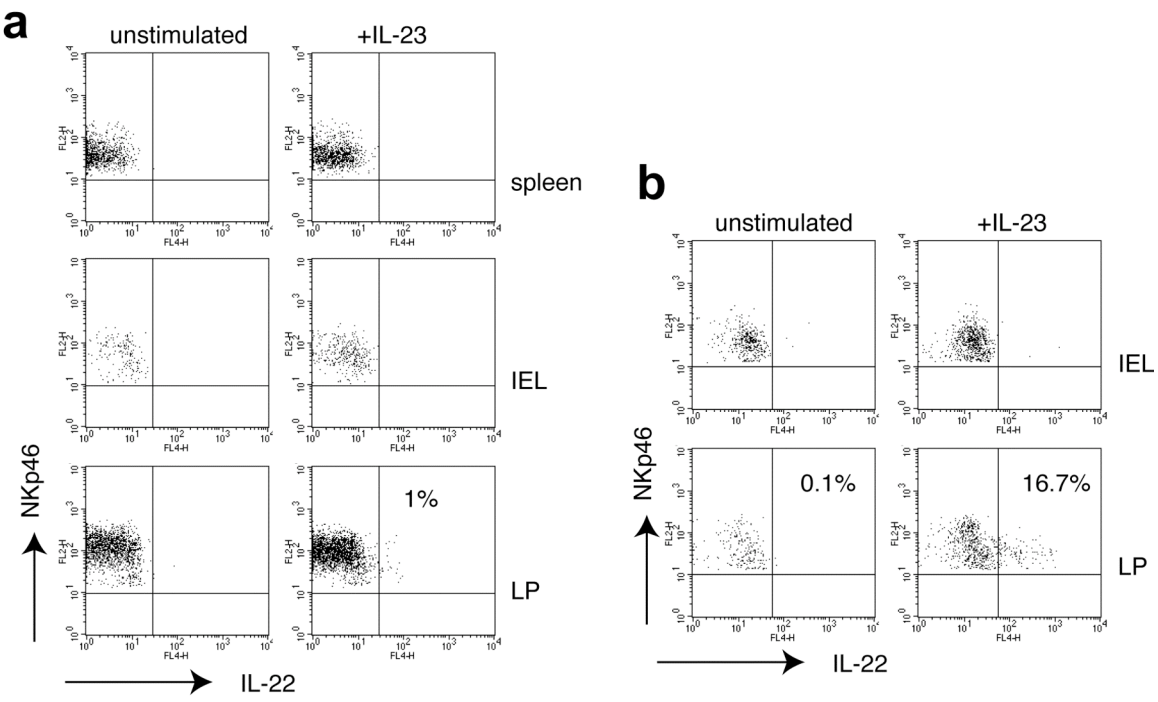
Supplementary Figure 7. NK-22 cell-secreted cytokines stimulate epithelial cells to activate mitogenic and anti-apoptotic intracellular pathways. a, b, Immunoblot analysis of BCL-3 and BCL-6 induction in Colo205 stimulated with supernatants of IL-23-activated tonsil NK cells. Supernatants from IL-23-activated peripheral blood NK cells (**a**), recombinant IL-10 (**a**) and IL-22 (**b**) are included as control stimuli. Note that IL-22 but not IL-10 upregulates BCL-3 expression in Colo205 cells. Actin (**a**) and MCL-1 (**b**) are shown as loading controls. **c**, Real-time PCR analysis of PBEF1, c-Myc, SerpinA1, SerpinA3, SGK1 and GADD45γ transcripts in Colo205 cells stimulated with supernatants of IL-23-activated NKp44^{+} or NKp44^{-} NK cells. GAPDH cDNA was amplified as control.

Supplementary Figure 8.



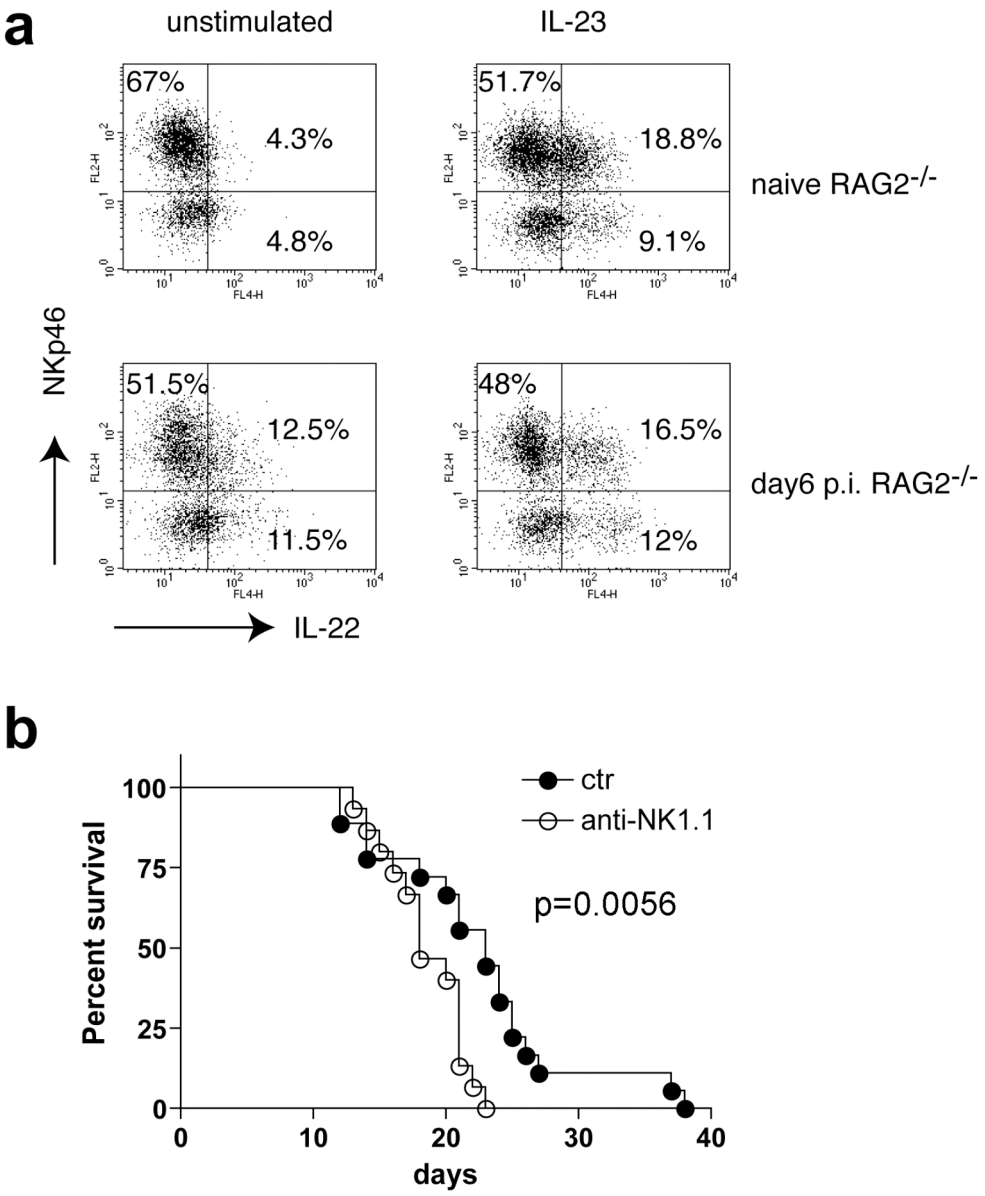
Supplementary Figure 8. NK cells subsets in mouse Peyer's patches.
Staining of mouse Peyer's patches cells with the NK cell specific markers NKp46 and NK1.1 reveals the presence of two NK cell subsets, $NKp46^{+}NK1.1^{+}$ and $NKp46^{+}NK1.1^{-}$. This latter is not present in the spleen. A gate was drawn to exclude $CD3^{+}CD19^{+}$ cells. Only a minute fraction of $NKp46^{+}$ cells expresses asialo-GM1 in Peyer's patches. In spleen a larger fraction of NK cells (30-50%) express asialo GM-1, which are mostly $CD11b^{high}$ $CD27^{-}$ NK cells (not shown).

Supplementary Figure 9.



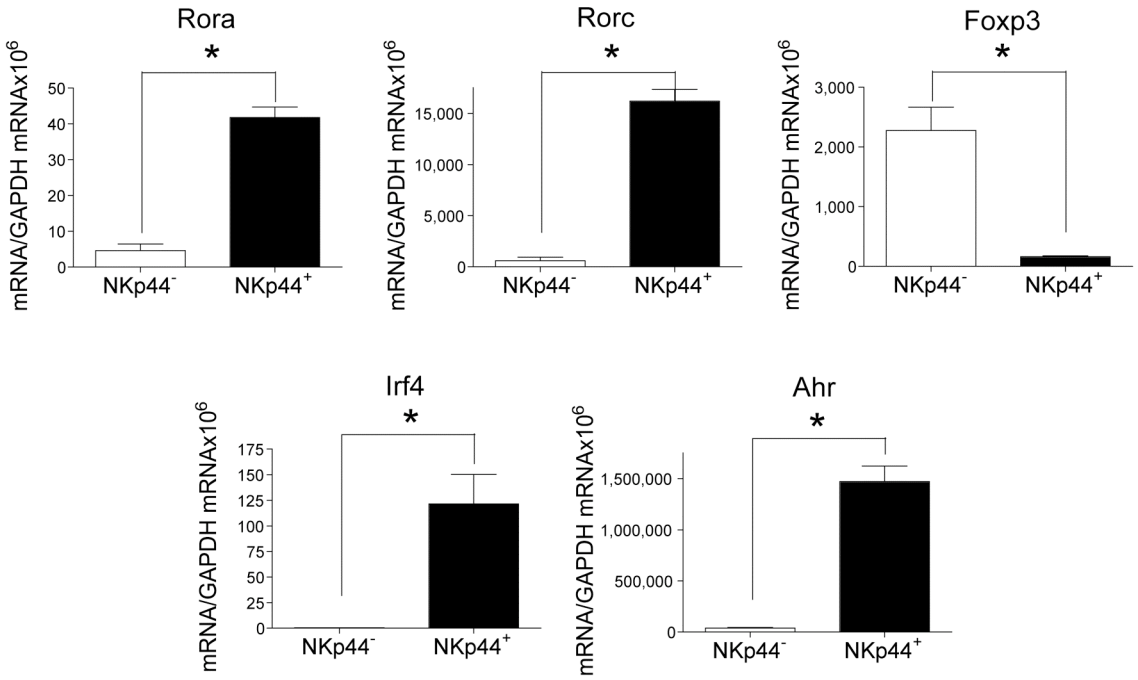
Supplementary Figure 9. NK-22 cells are present in the lamina propria of *C. Rodentium*-infected mice. a, NKp46^{+} IL-22-producing cells are absent in intestinal epithelium (IEL) and lamina propria (LP) of naïve mice. Lymphocytes from spleens, IEL or LP of naïve mice were stimulated in vitro with IL-23 and stained for IL-22 intracellular content. A gate was applied on NKp46^{+}CD3^{-}CD19^{-} cells. **b,** NK-22 cells become detectable in the lamina propria upon *C. rodentium* infection. Cells from intestinal epithelium or lamina propria of mice challenged with *C. rodentium* were isolated at day 6 post-infection, pulsed in vitro with IL-23 and stained for intracellular IL-22 content.

Supplementary Figure 10.



Supplementary Figure 10. NK-22 cells are detectable in the intestinal epithelium of naïve and *C. Rodentium*-infected RAG2$^{-/-}$ mice and depletion of NK1.1 cells during early phases *C. rodentium* infection accelerates death. a, IEL cells were prepared from naïve or *C. rodentium*-infected RAG2$^{-/-}$ mice and IL-22 secretion was determined by intracellular staining. In infected mice a higher spontaneous secretion of IL-22 was observed. **b,** Depletion of NK cells with an anti-NK1.1 antibody during the early phases of *C. Rodentium* infection accelerates death. Anti-NK1.1 was administered 24 hours before and 48 hours after bacterial challenge. Noteworthy, anti-NK1.1 depletes only a subset of IL-22 producing NK cells.

Supplementary Figure 11.



Supplementary Figure 11. NK-22 cells express transcription factors that are essential for T_H17 T cell differentiation and lymphoid tissue inducer function. Real-time-PCR shows that tonsil NKp44$^+$ NK cells exhibit higher levels of RORA, RORC, IRF4 and AHR transcripts than NKp44$^-$ NK cells. FoxP3 transcript shows the opposite pattern.

Online-only Methods

Immunohistochemistry and immunofluorescence. Acetone-fixed 4μ sections from tonsils, skin, Peyer's patches, appendix and LC microgranulomas skin biopsies were stained with anti-NKp44, -CD56 (Thermo Scientific), -Cytokeratin 5 (Covance), followed by SuperSensitive IHC Detection System (BioGenex) and 3-Amino-9-Ethylcarbazole (AEC) or DAB as chromogens. Digital images were taken with an Olympus BX60 microscope equipped with a DP-70 Olympus digital camera and processed using Analysis Image Processing software.

Cell preparation, isolation and FACS sorting. Tonsils were obtained from children undergoing elective tonsillectomy (Children's Hospital, WUSM, St. Louis). Lymph nodes and ileum specimens were obtained from the Division of Surgical Pathology, WUSM. Approvals were obtained from the WUSM Human Studies Committee Board. Tissues were mechanically disrupted and digested with collagenase for 1h. NK cells were enriched from tonsils with CD56 microbeads (Miltenyi). $CD3^-CD19^-CD56^+NKp44^-$ and $CD3^-CD19^-CD56^+NKp44^+$ NK cells were sorted from enriched NK cells on a MoFlo cytometer (Cytomation) or a FACSVantage sorter (BD). Cells were stimulated in vitro with 10^{-7} M phorbol 12-myristate-13-acetate (PMA) and 0.5 μg/ml ionomycin (Sigma-Aldrich), 40 ng/ml IL-23, 10ng/ml IL-12p70 or 10 ng/ml IL-15. Preparation of murine Peyer's patches (PP), small intestinal intraepithelial lymphocytes (IEL) and lamina propria (LP) cells was performed as described [Lefrancois and Lycke, Current Protocols in Immunology (1996) 3.19.1-3.19.16 (Wiley & Sons, Inc)]. In some experiments, IEL and LP cells were further enriched with CD45 microbeads (Miltenyi).

Cytokine/chemokine detection by ELISA and intracellular staining. IL-22 and CCL20 concentrations in cell culture supernatants were determined using ELISA Kits (Antigenix and R&D Systems, respectively).
For intracellular cytokine detection, 10^6/ml cells were stimulated with 10^{-7} M PMA and 0.5 μg/ml ionomycin, IL-23 (40ng/ml), IL-15 (10ng/ml), IL-12p70 (10ng/ml) and IL-6 (10ng/ml) for two hours at 37°C. Cells were cultured for 4 additional hours in the presence of 2 μM Monensin (Sigma-Aldrich) and then stained for cell surface markers, fixed in 2% paraformaldehyde, permeabilized in PBS/5% BCS/0.5% saponin (Sigma-Aldrich), counter-stained with fluorochrome-labelled antibodies anti-cytokines and analysed on a FACSCalibur using the Cell Quest program (BD). Intracellular detection of human and murine IL-22 was performed using a biotinylated antibody (Antigenix).

NK cell adhesion assays. Enriched tonsil NK cells (5-10x10^5) were added to 24 well plates containing confluent layers of the Colo3 colon carcinoma (ATCC). Cells were spun down briefly to allow cell-cell contact, and cultured for one hour at 37°C. Non-adherent or loosely adherent cells were then removed by gentle pipetting. Wells were washed twice with 0.5 ml RPMI 1640/10% BCS to remove residual non-adherent cells. Firmly adherent cells were then detached with PBS/1mM EDTA. The ratio of $CD3^-CD56^+NKp44^+$: $CD3^-CD56^+NKp44^-$ cells in non-adherent versus adherent fractions was assessed by FACS.

Gene chip analyses. Tonsil NK cell subsets were FACS-sorted into $CD3^-CD56^+NKp44^-$ and $CD3^-CD56^+NKp44^+$ cell populations. RNA was isolated using the Pico Pure RNA isolation kit (Arcturus). Equal quantities of RNA from $NKp44^+$ and $NKp44^-$ samples from three donors were pooled for RNA amplification and subjected to two rounds of amplification using the RiboAmp OA RNA amplification kit (Arcturus). During the last step of amplification, RNA was biotin-labelled using the BioArray High Yield RNA Transcript Labeling Kit (T7) (Enzo Life Sciences). Hybridization of U133 Plus Affymetrix gene chips was performed by the Siteman Cancer Center Genechip Facility (WUSM). For gene chip analysis of IL-22-induced genes, 10^7 Colo205 were kept unstimulated or stimulated with IL-22 (80ng/ml) for 7.5 hours at 37°C. RNA was extracted with Trizol (Invitrogen) and used for Hybridization of U133 Plus Affymetrix gene chips following standard procedures. Gene chip data was analyzed using the dChip gene analysis program (Department of Biostatistics, Harvard School of Public Health, Boston, MA).

Quantification of mRNA levels by Real-time PCR

cDNA was synthesized using Superscript III reverse transcriptase (Invitrogen). Real-time quantitative PCR reactions were performed on a Bio-rad I-Cycler using a iQ™ SYBR Green supermix (Bio-rad) and specific primers. Relative quantification of target mRNA expression was calculated and normalized to the expression of GAPDH. The following primers sequences were used (forward, reverse): c-Myc: 5'-ACCCTGAGATGCCCAAAC-3', 5'-CAGTGACGGCGATGAAGT-3'; SerpinA1: 5'-TGAGCATCGCTACAGCCTTTGC-3', 5'-ATCATAGGCACCTTCACGGTGG-3'; SerpinA3: 5'-GTTCACGGAGGATGCCAAGA-3', 5'-TCTTCACGTAGTCGTTGATGAGCTT-3'; Sgk1: 5'-GATCTCCCAACCTCAGGAGCC-3', 5'-CTGGAAAGAGAAGTGAAGGCCC-3'; Pbef1: 5'-CAAGGACCCAGTTGCTGATC-3', 5'-CTCCTTTTCCTTCCTCCAGTG-3'; Gadd45g: 5'-CTGCATGAGTTGCTGCTGTC-3', 5'-TTCGAAATGAGGATGCAGTG-3'; IL-26: 5'-TTTGAGGTGTGGGTTGCTGTTA-3', 5'-TCAACAGCTTGGGACAATGTTC-3'; Rorc: 5'-TGAGAAGGACAGGGAGCCAA-3', 5'-CCACAGATTTTGCAAGGGATCA-3'; Rora: 5'-GCACCTGACCGAAGACGAAA-3', 5'-GAGCGATCCGCTGACATCA-3'; Foxp3: 5'-GCACCTTCCCAAATCCCAGT-3', 5'-GGCCACTTGCAGACACCAT-3'; Irf4: 5'-GAAGCCTTGGCGTTCTCAAC-3', 5'-CGGGTCCTTCACGAGGATTTCCCG; Ahr: 5'-CAGAAAACAGTAAAGCCAATCC-3', 5'-AATACAAAGCCATTCAGAGCC-3'; Lta: 5'-TGTTGGCCTCACACCTTCAG-3', 5'-

TGCTGTGGGCAAGATGCAT-3'; Ltb: 5'-ACTTCTCTGGTGACCTTGTTGCT-3', 5'-AGCTTCTGAAACCCCAGTCCTT-3'; GAPDH: 5'-CAAAGTTGTCATGGATGACC-3', 5'-CCATGGAGAAGGCTGGGG-3'

Monocytes isolation, stimulation and coculture with NK cells. Peripheral blood monocytes were purified using CD14 microbeads (Miltenyi Biotec). $2x10^5$ cells were cultured in 96 well flat bottom plates and either left unstimulated or stimulated with zymosan (Sigma-Aldrich) (100ng/ml), LPS (Ultrapure LPS from *E.Coli*, Biolist Laboratories) (100ng/ml), resiquimod (InVivogen) (6μM) or the CpG B oligonucleotide 1826 (Quiagen) (6μg/ml). After 36 h tonsil NK cells ($2X10^5$/well) were added to the culture for additional 6 h. In the last 4 h of incubation monensin (2μM) was added to the culture. To measure IL-23 production, 10^6 monocytes were stimulated in 200μl of complete medium. Supernatants were collected and IL-23 tested by ELISA (R&D).

In vitro migration assay. Chemotaxis of tonsil NK cells was measured in a 2 h transwell migration assay using 24-well Costar Transwell chambers (3-μm pore size; Corning). Recombinant human CCL20 (Peprotech) was added at different concentrations to the lower wells, and $3X10^5$ cells were added to the upper chamber of the Transwell insert. The original population used for the assay and migrated cells recovered from the bottom chamber of the transwell were stained with anti-CD3, -CD56 and -NKp44. The ratio of $NKp44^+$ to $NKp44^-$ NK cells was calculated.

Proliferation of epithelial cells. Colo205 cells ($5X10^3$-10^4cells/well) were incubated for 7-9 h with IL-22 or NK-derived supernatants and pulsed overnight with 0.5μCi of ^{3}H-Thymidine. Samples were harvested on a Micro96 harvester (Molecular Devices) and counted on a Microbeta Trilux luminescence counter (Perkin Elmer).

Detection of phospho-STAT1 and -STAT3. Colo205 cells were stimulated with IL-22 (80ng/ml) or NK cell derived supernatants (1:2 dilution) for 15 min at 37°C, washed, fixed in 2% paraformaldehyde, permeabilized in 90% ice-cold methanol and then stained for 1 h at RT with AlexaFluor-488 anti-pSTAT1 (pY701) and AlexaFluor-647 anti-pSTAT3 (pY705).

Immunoblot analyses. Colo205 cells ($5X10^6$) were incubated for 9.5 h with IL-22 or NK cell-derived supernatant and lysed on ice in 0.1% TritonX100 in 100 mM Tris, 150mM NaCl pH 7.4, in the presence of protease inhibitors. Proteins from cell lysates were separated by standard SDS-PAGE and analyzed by immunoblotting with antibodies specific for BCL-3 (sc-185, rabbit polyclonal) and BCL-6 (sc-7388, mouse monoclonal IgG3) (Santa Cruz).

***C. rodentium* infection and NK cell depletion.**
$RAG2^{-/-}$ mice were fasted for 8 hrs before intraoral inoculation of $2x10^9$ *C. rodentium* strain DBS100 (ATCC) as described [Zheng, Y. et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens.

Nat Med 14, 282-9 (2008)] One group of mice was injected intraperitoneally with 200μL of NK1.1 ascites 24 hrs before and 24hrs after bacterial challenge. A control group received 200 μL of a control IgG2a ascites. Survival was monitored for 40 days. The hybridomas producing anti-asialoGM1 (mouse IgM) and NK1.1 (mouse IgG2a) were purchased from ATCC. The anti-asialoGM1 antibody was purified on protein L (Sigma) and conjugated to biotin using a Fluororeporter mini-biotin-XX protein labelling kit (Molecular Probes). The anti-NK1.1 antibody was used as ascites.

Supplementary Table 1

Gene name	Gene symbol	Gene ID	Fold change
NKp44	NCR2	9436	15.29
Carbonic anhydrase XII	CA12	771	9.73
CCL20	CCL20	6364	9.49
Sclerosteosis, sclerostin	SOST	50964	8.23
IL-22	IL22	50616	7.94
Maba1, estrogen induced gene 121	KIAA1324	57535	7.47
Layilin	LAYN	143903	6.95
IL-26	IL26	55801	6.89
Leucine rich repeat neuronal 3	LRRN3	54674	5.94
Rhotekin 2	RTKN2	219790	5.69
Protocadherin 9	PCDH9	5101	5.08
BAFF	TNFSF13B	10673	4.65
IREM	CD300F	146722	4.56
Breast carcinoma amplified sequence 1	BCAS1	8537	4.46
BCSC1	LOH11CR2A	4013	4.41
GPI deacylase	PGAP1	80055	4.21
ICOS, CD278	ICOS	29851	4.13
Actin filament associated protein 1-like 1	AFAP1L1	134265	4.07
Lysosomal associated protein transmembrane 4 beta	LAPTM4B	55353	4.03
AMICA, JAM-like	AMICA1	120425	3.84
Mannosidase, alpha, class 1C, member 1	NCOA7	57134	3.83
RANKL	TNFSF11	8600	3.82
MST150, NID67	MST150	85027	3.77
Protein kinase C, alpha	PRKCA	5578	3.66
Keratin 86	KRT86	3892	3.62
Interferon gamma receptor 1, CD119	IFNGR1	3459	3.40
Ankyrin 3	ANK3	288	3.28
Purinergic receptor P2Y, G-protein coupled 5	P2RY5	10161	3.20
LIF	LIF	3976	3.15
CD39	ENTPD1	953	3.13
Low density lipoprotein receptor-related protein 11	LRP11	84918	2.96
OX40L	TNFSF4	7292	2.92
PCI domain containing 2	PCID2	55795	2.92
Aryl hydrocarbon receptor	AHR	196	2.90
G protein-coupled receptor 55	GPR55	9290	2.87
Glycoprotein A33	GPA33	10223	2.85
Phosphatase and actin regulator 2	PHACTR2	9749	2.81
CCR6	CCR6	1235	2.71
Rap guanine nucleotide exchange factor 6	RAPGEF6	51735	2.71
Phosphatidic acid phosphatase type 2A	PPAP2A	8611	2.63
NEL-like 2 (chicken)	NELL2	4753	2.53
Transducin-like enhancer of split 1	TLE1	7088	2.52
Dysferlin, limb girdle muscular dystrophy 2B	DYSF	8291	2.49
Jumonji, AT rich interactive domain 1B	JARID1B	10765	2.45
Chimerin 1	CHN1	1123	2.45
FYVE and coiled-coil domain containing 1	FYCO1	79443	2.44
RAR-related orphan receptor A	RORA	6095	2.42
Dual specificity phosphatase 16	DUSP16	80824	2.38
CXCR6	CXCR6	10663	2.25
Coiled-coil domain containing 41	CCDC41	51134	2.23
Early growth response 3	EGR3	1960	2.18
ATP-binding cassette, sub-family C (CFTR/MRP), member 1	ABCC1	4363	2.15
WD repeat domain 67	WDR67	93594	2.13
Nuclear receptor coactivator 7	NCOA7	135112	2.11
Sprouty homolog 1	SPRY1	10252	2.10
Activin A receptor, type IB	ACVR1B	91	2.05
CD96	CD96	10225	2.04
Interleukin 1 receptor, type 1; CD121A	IL1R1	3554	2.02
RCAN family member 3	RCAN3	11123	2.02
Alpha E integrin, CD103	ITGAE	3682	2.01
Ataxin 1	ATXN1	6310	2.00

Supplementary Table 1. Genes upregulated 2-fold or higher in $NKp44^+$ as compared to $NKp44^-$ tonsil NK cells. Results are mean values from the hybridisation of two U133 Plus gene chips for each $NKp44^+$ and $NKp44^-$ NK cells. Each gene chip was hybridised with a pool of RNA from three donors. Shown are the 61 most significant hits from a total of 112 genes.

Supplementary Table 2

Gene name	Gene symbol	Gene ID	Fold change
SOCS3 (suppressor of cytokine signaling 3)	SOCS3	9021	79.25
Serpin A3, AACT, ACT	SERPINA3	12	24.86
Gadd45 gamma	GADD45G	10912	19.6
Gadd45 beta	GADD45B	4616	19.31
IRF-1	IRF1	3659	15.48
Protein tyrosine phosphatase type IVA, PRL-1	PTP4A1	7803	14.97
BATF2, basic leucine zipper transcription factor, ATF-like 2	BATF2	116071	9.04
Serpin A1	SERPINA1	5265	9.15
Bcl-6	BCL6	604	8.79
CCAAT/enhancer binding protein (C/EBP), delta	CEBPD	1052	8.71
STAT3	STAT3	6774	7.15
Cytokine inducible SH2-containing protein, CIS, SOCS	CISH	1154	5.67
Pre-B-cell colony enhancing factor,	NAMPT	10135	5.61
Phospholipid scramblase 1	PLSCR1	5359	4.53
Inositol 1,4,5-triphosphate 3-kinase C	ITPKC	80271	4.27
Interferon-induced protein with tetratricopeptide repeats 3, ISG60, RIG-G, IFIT4	IFIT3	3437	4.07
Serum/glucocorticoid regulated kinase, SGK	SGK1	6446	4.07
Endothelial PAS domain protein 1, EPAS1, HIF2A	EPAS1	2034	4.06
Nuclear receptor coactivator 7	NCOA7	135112	3.96
Inhibitor of DNA binding 1	ID1	3397	3.87
Coagulation factor II (thrombin) receptor-like 1	F2RL1	2150	3.82
TRIM15	TRIM15	89870	3.73
IL-10	IL10	3586	3.66
CXCL1, GRO1	CXCL1	2919	3.53
Phosphodiesterase 4D, camp-specific	PDE4D	5144	3.37
Interferon gamma receptor 1, CD119	IFNGR1	3459	3.36
Kruppel-like factor 9, BTEB	KLF9	687	3.32
6-phosphofructo-2kinase/fructose-2,6-biphosphatase 3, PFK2	PFKFB3	5209	3.18
Glutathione S-transferase A4	GSTA4	2941	3.11
Discs, large (Drosophila) homolog-associated protein 4, DAP4	DLGAP4	22839	3.04

Supplementary Table 2. Genes upregulated in Colo205 cells after treatment with 80 ng/ml IL-22 (30 most significant hits).