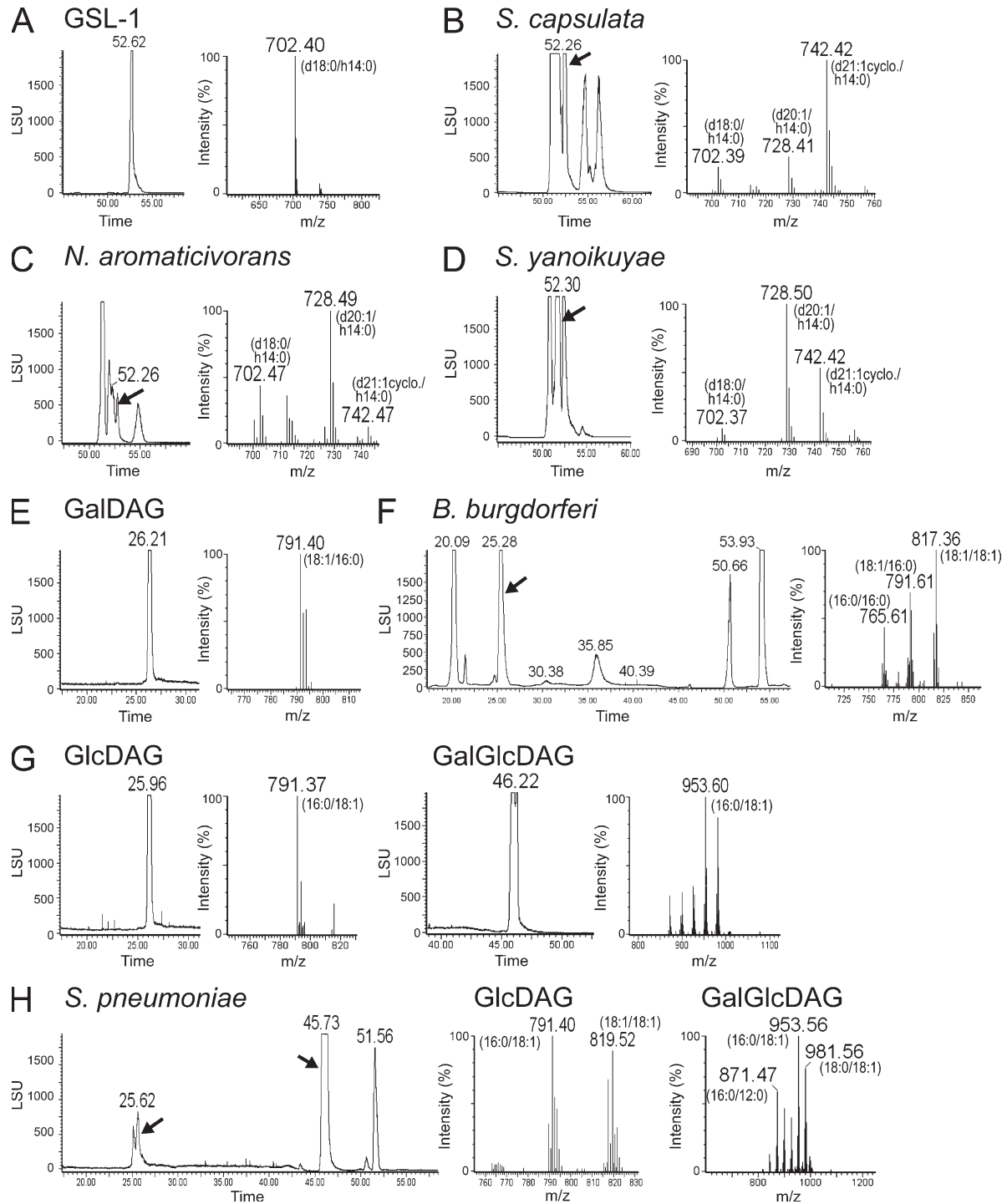
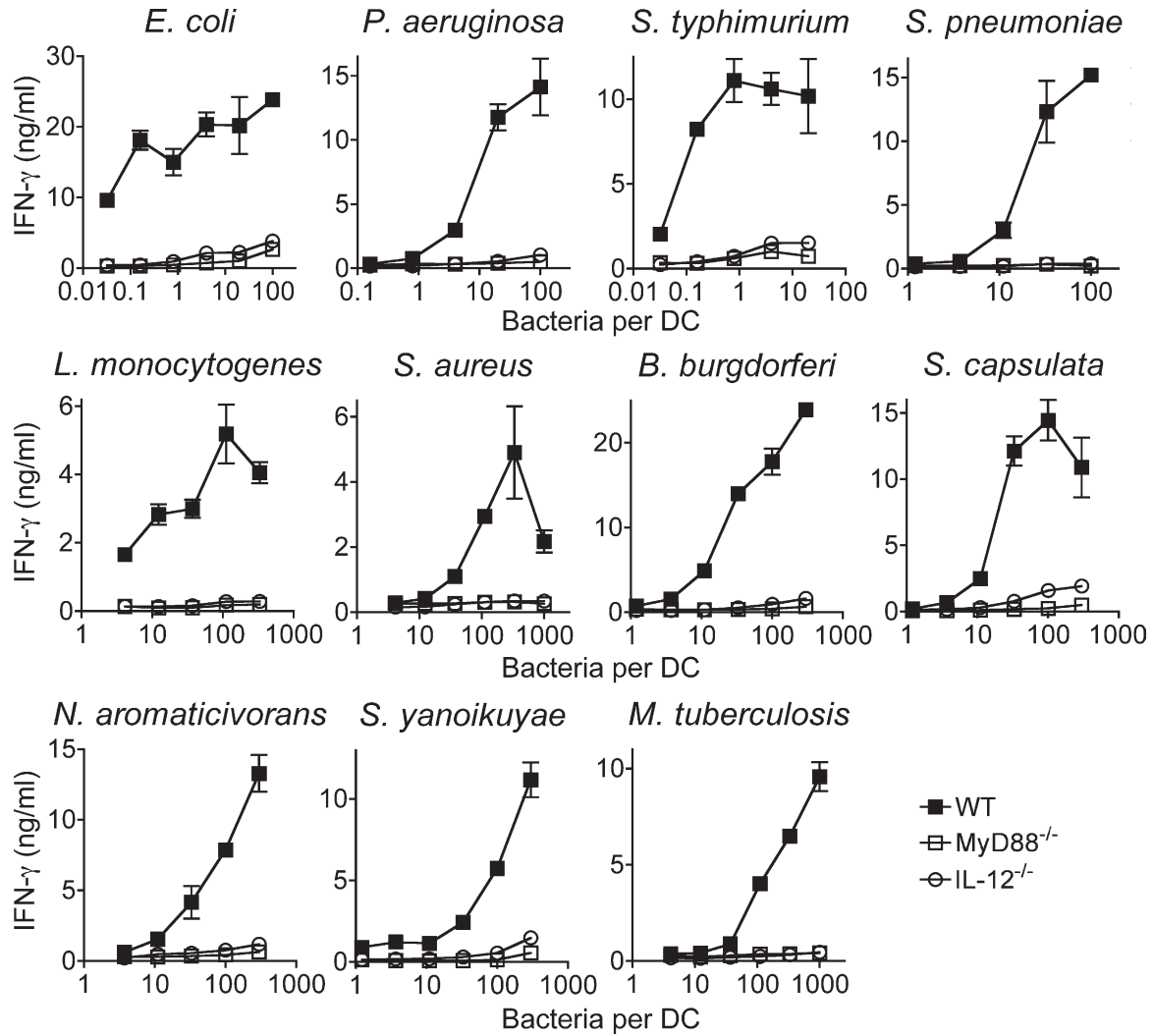


SUPPLEMENTAL MATERIAL

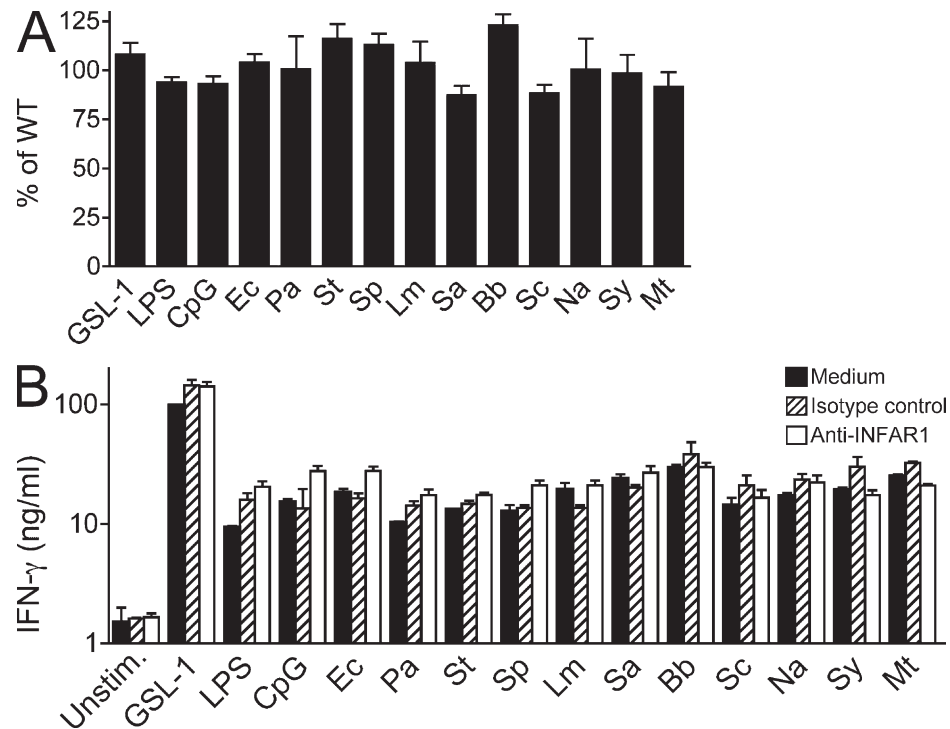
Brigl et al., <http://www.jem.org/cgi/content/full/jem.20102555/DC1>



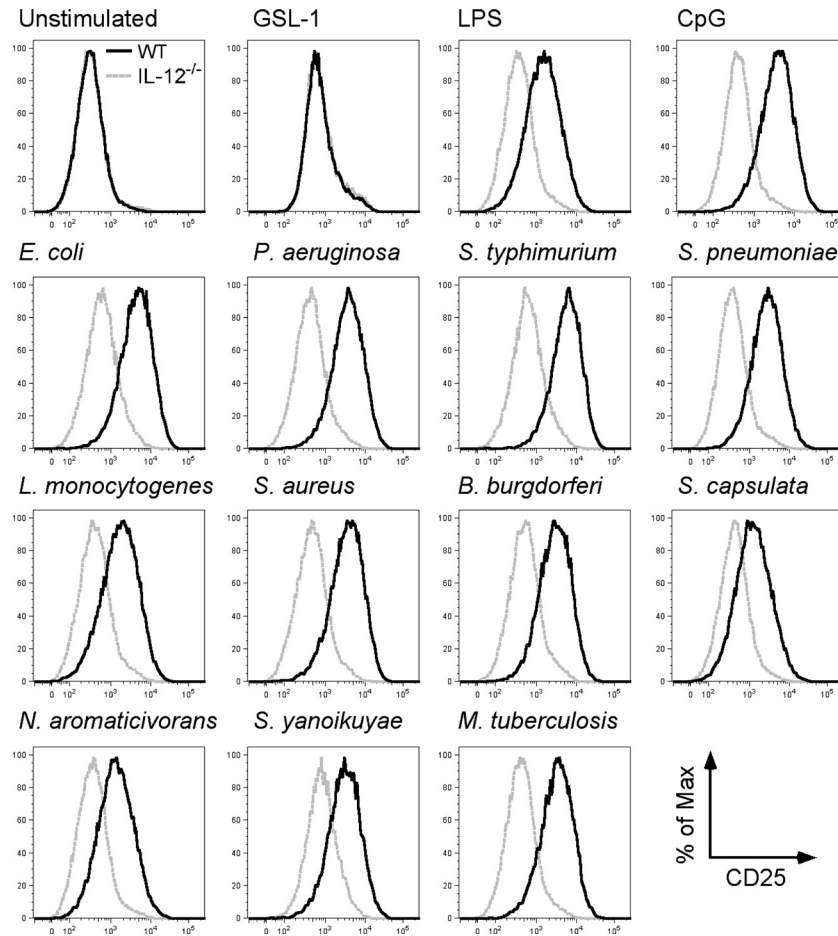
**Figure S1. Detection of microbial iNKT cells antigens expressed in bacteria.** Lipids were extracted from bacteria, separated by liquid chromatography (LC), and analyzed by mass spectrometry (MS). (A) LC separation and detection by light scattering of synthetic GSL-1 (GlcAGSL) antigen (eluting at 52 min; left) and corresponding MS data (right). (B–D) Analysis of lipids extracted from *S. capsulata*, *N. aromaticivorans*, and *S. yanoikuyae*. Light scattering profiles with peaks corresponding to GSL-1 (left; arrows) are shown. Corresponding MS data of GSL-1 lipid and annotation of lipid species is shown on the right. (E) LC separation of synthetic GalDAG (BbGL-II) eluting at 26 min (left) and corresponding MS data (right). (F) LC separation of lipids extracted from *B. burgdorferi* showing GalDAG peak (arrow) and corresponding MS data. (G) LC separation and corresponding MS data of synthetic GlcDAG (eluting at 26 min; left) and purified GalGlcDAG (eluting at 46 min; right). (H) LC separation of lipids extracted from *S. pneumoniae* and light scattering peaks corresponding to GlcDAG and GalGlcDAG (arrows). MS data for bacterial GlcDAG and GalGlcDAG are shown on right. LSU, light scattering units.



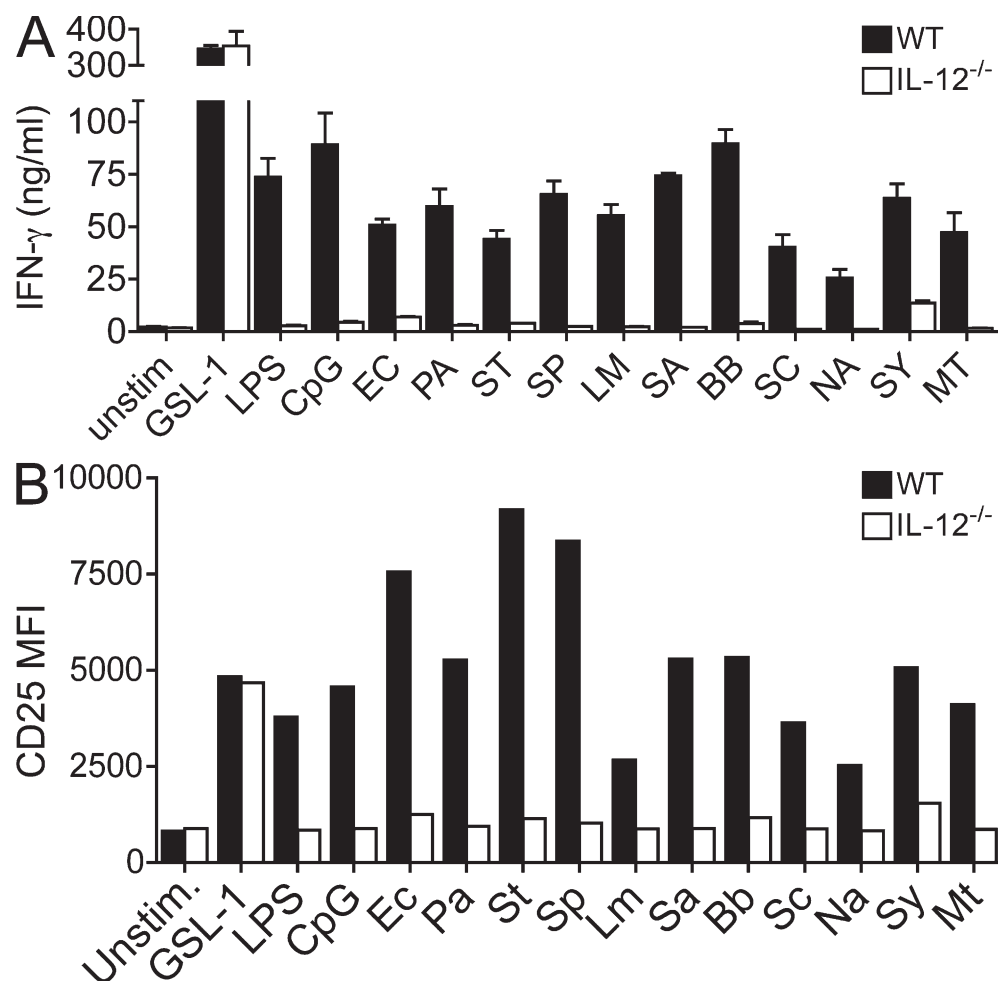
**Figure S2. Cytokine responses of iNKT cell activation in response to diverse bacteria.** iNKT cell lines were cultured with WT (filled squares), MyD88-deficient (open squares), or IL-12-deficient (open circles) DCs in the presence of heat-inactivated bacteria. IFN- $\gamma$  concentrations were measured in culture supernatants by ELISA after 16–24 h. Data represent means of duplicate values  $\pm$  SD and are representative of at least three independent experiments.



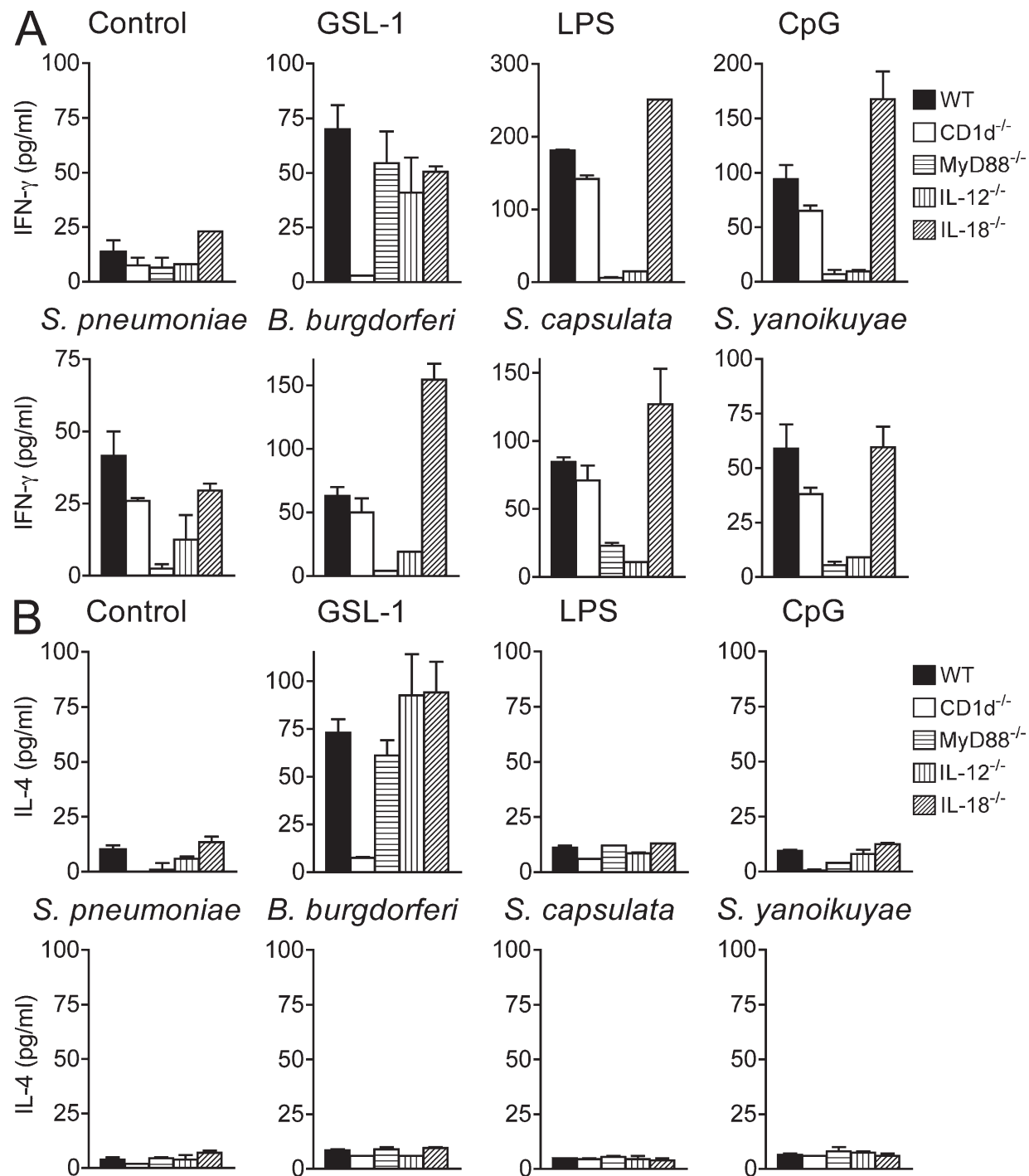
**Figure S3. IL-18- and type I IFN-independent activation of iNKT cells in response to microbes.** (A) iNKT cell lines were cultured with WT or IL-18-deficient BM-derived DCs. Data are shown as percent inhibition of IFN- $\gamma$  secretion in culture supernatants comparing IL-18-deficient to WT DCs after stimulation with 2  $\mu$ g/ml GSL-1, 10 ng/ml LPS, 2  $\mu$ g/ml CpG, or 10 bacteria per DC for *E. coli* (Ec), *P. aeruginosa* (Pa), and *S. typhimurium* (St) and with 100 bacteria per DC for *S. pneumoniae* (Sp), *L. monocytogenes* (Lm), *S. aureus* (Sa), *B. burgdorferi* (Bb), *S. capsulata* (Sc), *N. aromaticivorans* (Na), *S. yanoikuyae* (Sy), and *M. tuberculosis* (Mt). Data are summarized from two independent experiments (mean  $\pm$  SD). (B) iNKT cell lines were cultured with WT BM-derived DCs in the presence of medium alone (filled bars), 10  $\mu$ g/ml of control isotype antibody (hatched bars), or 10  $\mu$ g/ml anti-IFNAR1 antibody (open bars). Stimulation with antigen, TLR agonists, or bacteria was as described in A. Cytokine concentrations in culture supernatants were measured by ELISA. Data are presented as means of triplicate values  $\pm$  SD and are representative of two independent experiments.



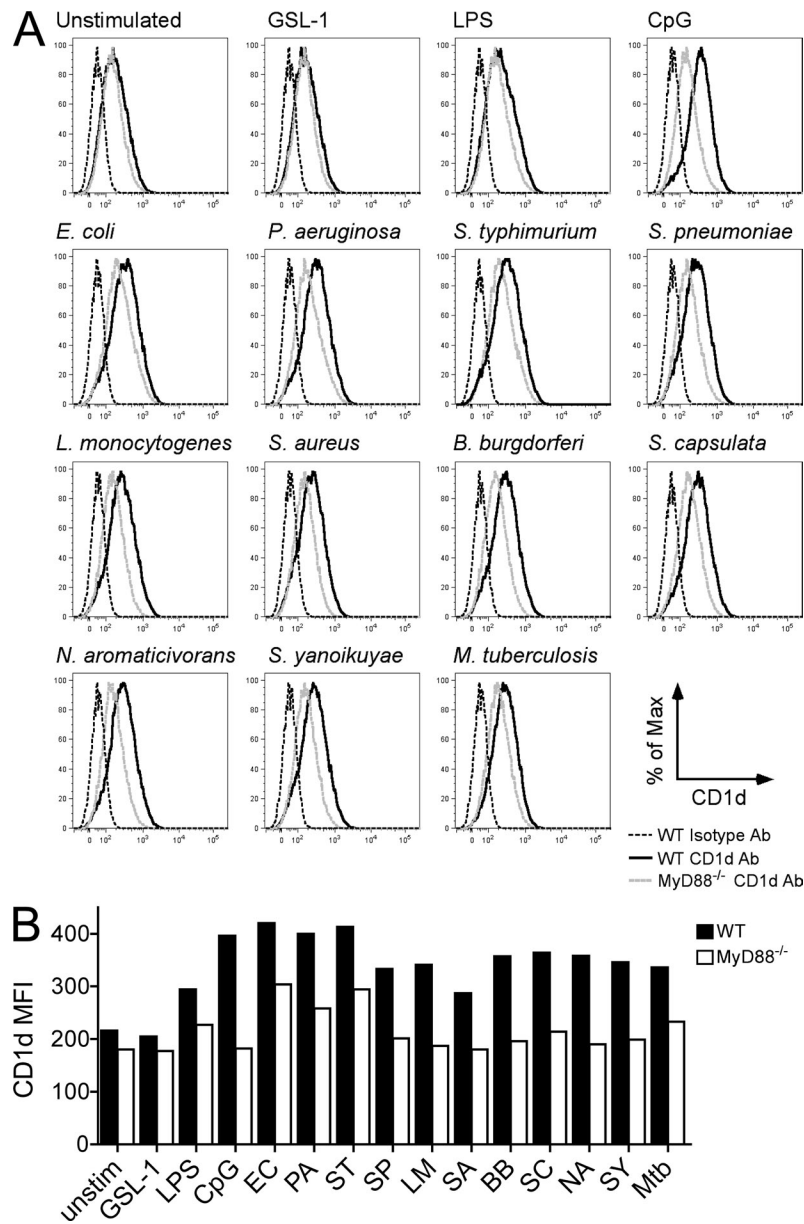
**Figure S4. Expression of the early activation marker CD25 on iNKT cells after stimulation with microbial products.** Expression of CD25 by iNKT cells after 16–24 h of co-culture with WT (black line) or IL-12-deficient (gray dotted line) DCs.  $10^5$  iNKT cells/well were cultured with  $2 \times 10^4$  DCs/well in triplicate in 96-well plates in the presence of stimuli, as in Fig. 4 D, and cells were pooled for analysis. CD25 expression was determined on CD11c-negative cells by FACS analysis. Results are representative of at least two independent experiments.



**Figure S5. IL-12 dependence of iNKT cell activation.** iNKT cells were incubated with WT (filled bars) or IL-12p35-deficient (open bars) DCs in the presence of stimuli as described in Fig. 4 D for 36–48 h. IFN- $\gamma$  production in culture supernatants (A; mean  $\pm$  SD) and surface expression of CD25 (B) were analyzed. Results are representative of at least two independent experiments.

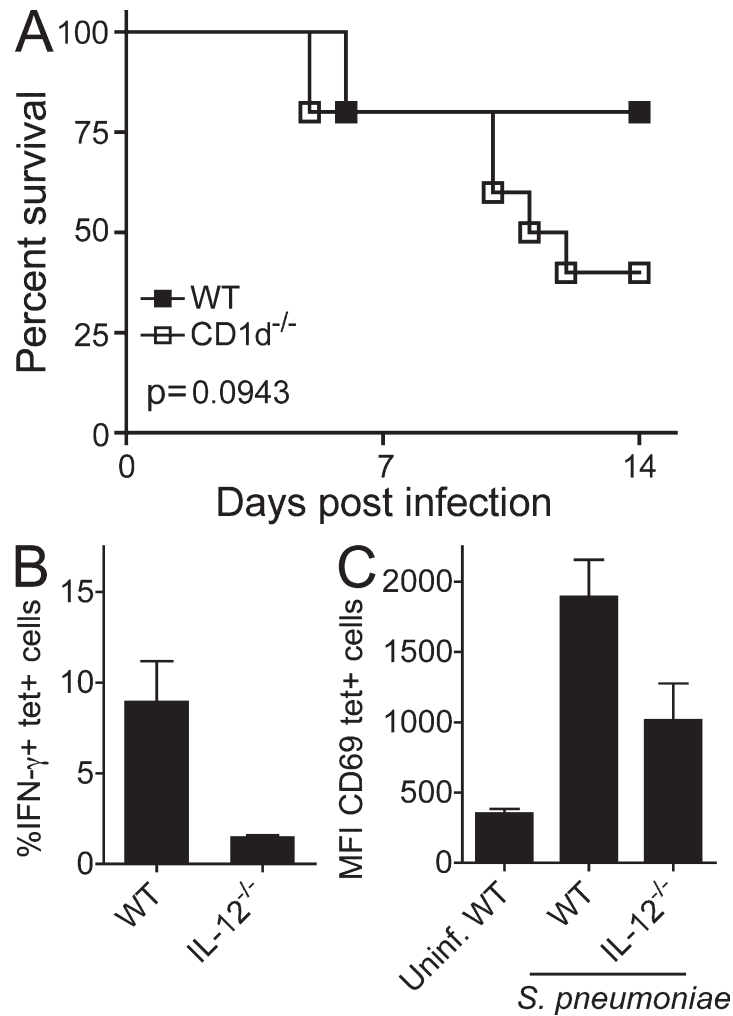


**Figure S6. Cytokine responses of freshly isolated iNKT cells in response to diverse bacteria.** iNKT cells were isolated from WT mice using CD1d-tetramer staining followed by cell sorting.  $1-3 \times 10^4$  iNKT cells/well were cultured with  $5 \times 10^4$ /well of WT, CD1d<sup>-/-</sup>, MyD88<sup>-/-</sup>, IL-12<sup>-/-</sup>, or IL-18<sup>-/-</sup> deficient DCs in the presence of stimuli as described in Fig. 4 D. IFN- $\gamma$  (A) and IL-4 (B) concentrations were determined in culture supernatants after 36-48 h by ELISA (mean  $\pm$  SD). Results are representative of at least two independent experiments.



**Figure S7. CD1d expression by DCs after stimulation with microbial products.**  $5 \times 10^5$  WT or MyD88-deficient DCs/well were stimulated with microbial products, as described in Fig. 4 D, for 16–24 h and analyzed by FACS for expression of CD1d. (A) Individual FACS plots show staining of WT DCs with isotype antibodies (dotted thin black line) or CD1d antibodies (black line), or MyD88-deficient DCs stained with CD1d antibodies (dotted gray line). (B) CD1d MFI of WT (filled bars) or MyD88-deficient (open bars) DCs are shown. Results are representative of two independent experiments.





**Figure S8. In vivo activation of iNKT cells during *S. pneumoniae* infection.** WT or CD1d-deficient mice were infected intranasally with *S. pneumoniae*. (A) Survival was recorded daily for 2 wk for WT (filled squares;  $n = 9$ ) and CD1d<sup>-/-</sup> (open squares;  $n = 9$ ) mice. Results are representative of two independent experiments. (B and C) WT or IL-12p35-deficient mice were infected intratracheally with *S. pneumoniae* and secretion of IFN- $\gamma$  (B) and expression of CD69 by iNKT cells (C) was determined on day 4 after infection (as described in Fig. 7, E and F). Data represent means  $\pm$  SD for three to four mice per group and one experiment of two similar experiments is shown.

**Table S1.** iNKT cell antigens expressed by bacteria

Lipid	Adducts	Ions ( <i>m/z</i> ) Acyl chains
<i>Sphingomonas</i> spp. GSL-1 (GlcAGSL)	[M – H] <sup>–</sup>	702.6 d18:0/h14:0 <b>714.5</b> d19:1/h14:0 728.5 d20:1/h14:0 <b>742.6</b> d21:1cyclo./h14:0
<i>B. burgdorferi</i> BbGL-II (GalDAG)	[M + CH <sub>3</sub> COO] <sup>–</sup>	789.6 16:0/16:0 813.6 18:1/16:1 <b>815.6</b> 18:1/16:0 839.6 18:2/18:1 <b>841.6</b> 18:1/18:1
<i>S. pneumoniae</i> GlcDAG	[M + Na] <sup>+</sup>	777.6 18:1/16:1 <b>779.6</b> 16:0/18:1 805.6 18:1/18:1 <b>807.6</b> 18:0/18:1
GalGlcDAG	[M + Na] <sup>+</sup>	913.6 16:0/16:1 939.6 18:1/16:1 <b>941.6</b> 16:0/18:1 967.6 18:1/18:1 <b>969.6</b> 18:0/18:1

Microbial lipids were analyzed by electrospray MS and multiple-stage ion-trap tandem mass spectrometry, and detected adduct ions are listed with annotation of the corresponding iNKT cell antigen species. Dominating ions are in bold. See Table I for references. d, sphingosine base; h, hydroxylated fatty acid; cyclo., cyclopropyl ring-containing sphingosine.