Supplementary Figure Legends

Supplementary Figure 1. The memory response of conventional T cells following *L. monocytogenes* infection. Mice were infected with 5×10^4 CFU of *L. monocytogenes*, sacrificed 3 weeks later, and splenocytes (2×10^5 per well) were cultured with graded doses of heat-killed *L. monocytogenes*. After 3 days, proliferation was assessed by [^{3}H]thymidine incorporation and culture supernatants were evaluated for IL-2 by ELISA. Proliferation and cytokine results represent the mean ± SEM of 4 mice. *, $p<0.05$ as compared with naïve splenocytes cultured with the same dose of heat-killed *L. monocytogenes*.

Supplementary Figure 2. In vivo dynamics of NK1.1 expression by iNKT cells in response to heat-killed *E. coli* or live *L. monocytogenes*. Mice were injected with α-GalCer (5 μg/mouse, i.p.), heat-killed *E. coli* (**A**), or live *L. monocytogenes* (**B**), sacrificed at the indicated time points, and spleen and liver mononuclear cells were prepared and stained for the identification of iNKT cells with anti-TCRβ-FITC, anti-NK1.1-PE, anti-B220-PerCP, and tetramer-APC. The percentage of NK1.1$^-$ cells among iNKT cells is shown. The shaded area represents the NK1.1 staining of naïve iNKT cells, and the solid line represents the staining of iNKT cells from mice treated with α-GalCer or bacteria. Representative plots from 4-10 mice per group are shown.

Supplementary Figure 3. (A) Surface expression of Ly49, NKG2D and CD94 on iNKT cells. Mice were treated with α-GalCer, heat-killed *E. coli*, or live *L. monocytogenes* and spleen mononuclear cells were stained with different combinations of anti-Ly49-FITC cocktail, anti-NK1.1, anti-NKG2D-biotin, anti-CD94-biotin, streptavidin-FITC, anti-TCRβ-PE, anti-B220-PerCP, and tetramer-APC. Histograms were gated on B220$^-$ TCRβ$^+$tetramer$^+$. Shaded areas indicate isotype controls for NKG2D, CD94 and Ly49

histograms, and NK1.1 expression on naïve iNKT cells for NK1.1 histograms. **(B)** Surface expression of Ly49 on NK cells. Naïve mice were sacrificed and spleen mononuclear cells were stained with anti-Ly49-FITC cocktail, anti-TCRβ-PE, anti-B220-PerCP and anti-NK1.1-APC. Histograms were gated on $B220^{-}TCR\beta^{-}NK1.1^{+}$ cells. Shaded area indicates the isotype control.

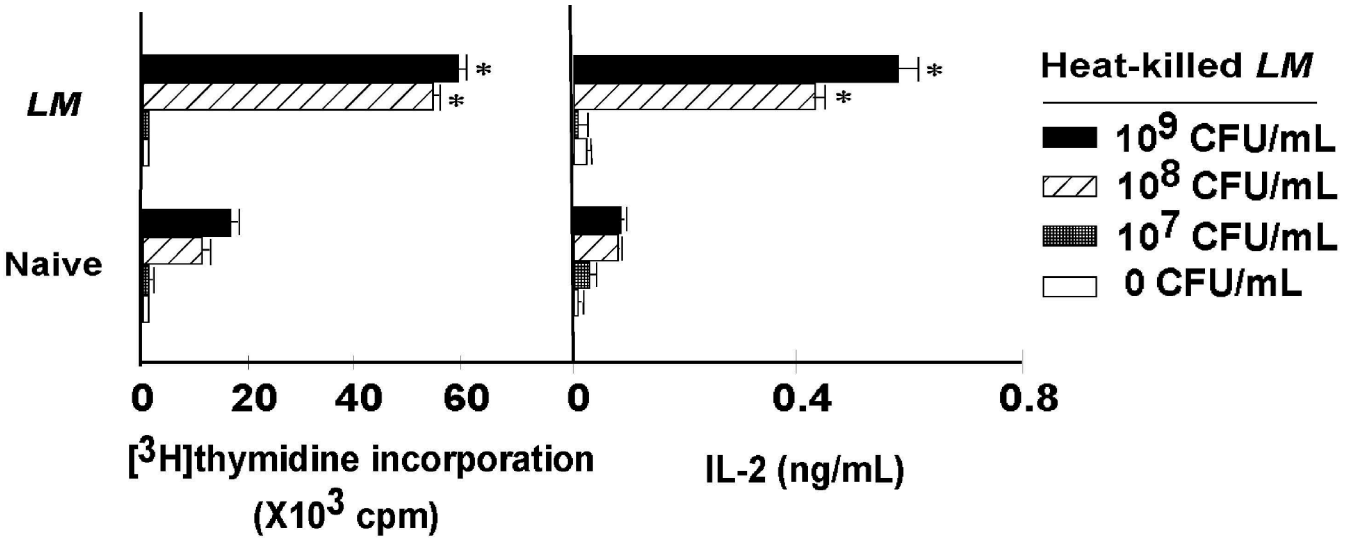
Supplementary Figure 4. Both heat-killed and live bacteria induce iNKT cell hyporesponsiveness. (**A)** Mice were injected with heat-killed or live *E. coli* or *L. monocytogenes* and, 3 weeks later, rechallenged in vivo with vehicle or α-GalCer (1 μg/mouse, i.p.). Mice were sacrificed 3 days later and spleen cells were stained with anti-TCRβ-FITC, anti-B220-PerCP, and tetramer-APC, and analyzed by flow cytometry. Numbers indicate the percentage of $TCR\beta^{+}tetramer^{+}$ cells among $B220^{-}$ cells from representative plots of 5-6 mice per group from 2 experiments. (**B**) Graphical representation of the total spleen iNKT cells calculated from the experiments shown in (A). *, $p<0.05$ as compared with naive mice rechallenged with α-GalCer.

Supplementary Figure 5. The induction of iNKT cell hyporesponsiveness by LPS and flagellin requires IL-12. Wild type mice and IL-12p40-deficient mice were treated with α-GalCer or *Salmonella* LPS + Flagellin. Three weeks later, mice were sacrificed and splenocytes (2×10^5 per well) were cultured with graded doses of α-GalCer, proliferation was assessed 3 days later by [^{3}H]thymidine incorporation and culture supernatants were evaluated for IL-4 and IFN-γ levels by ELISA. Proliferation and cytokine results represent the mean ± SEM.

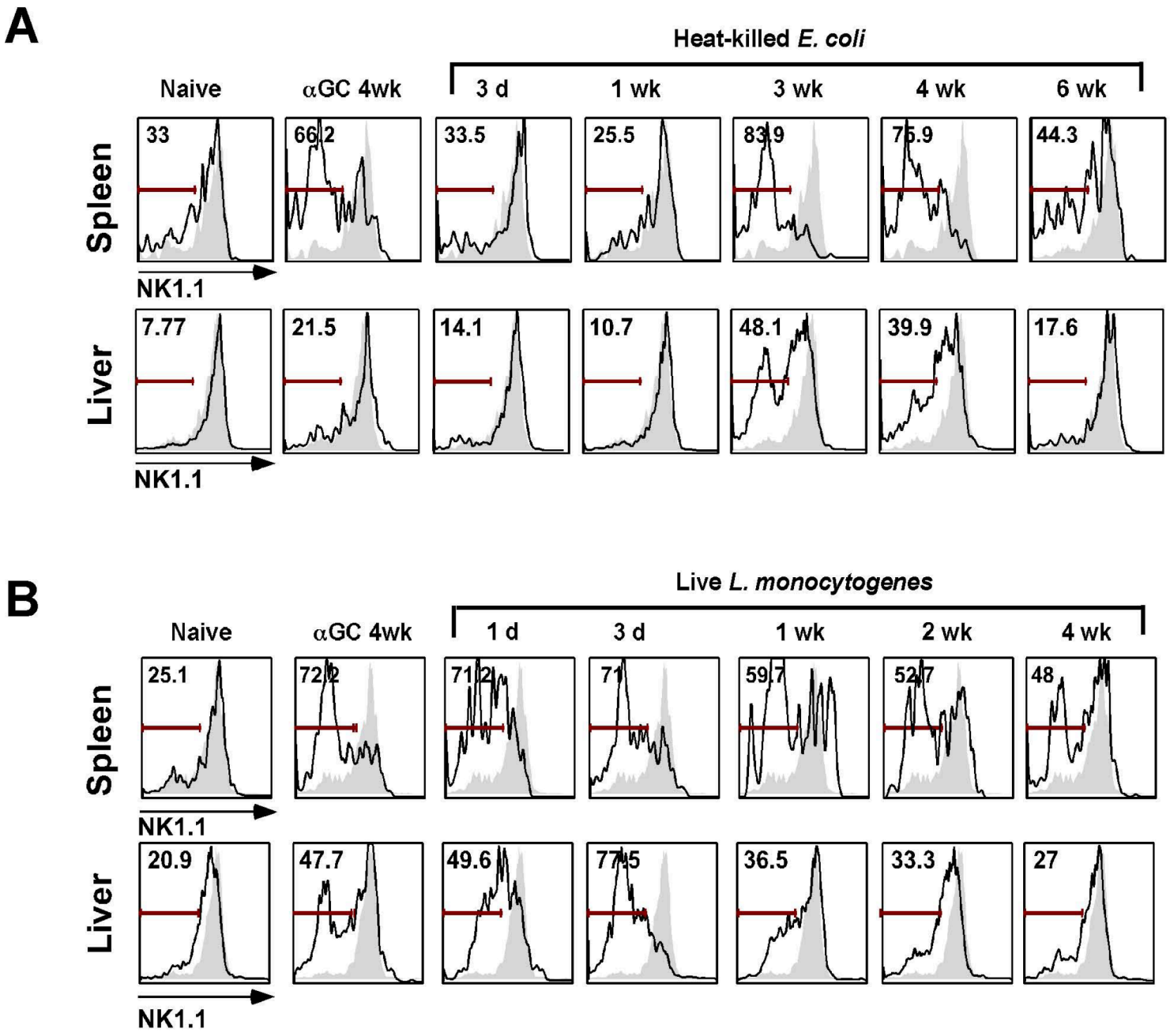
Supplementary Figure 6. Expression of NK1.1 in euthymic and athymic mice treated with bacteria. Euthymic and athymic B6 mice were treated with α-GalCer (5 μg/mouse, i.p.) or the indicated bacteria. Three weeks later, mice were sacrificed and spleen

mononuclear cells were stained with anti-TCRβ-FITC, anti-NK1.1-PE, anti-B220-PerCP, and tetramer-APC and analyzed by flow cytometry. Data shown are gated on $B220^{-}$ $TCR\beta^{+}tetramer^{+}$ cells. Data shown are representative of 2 mice per group.

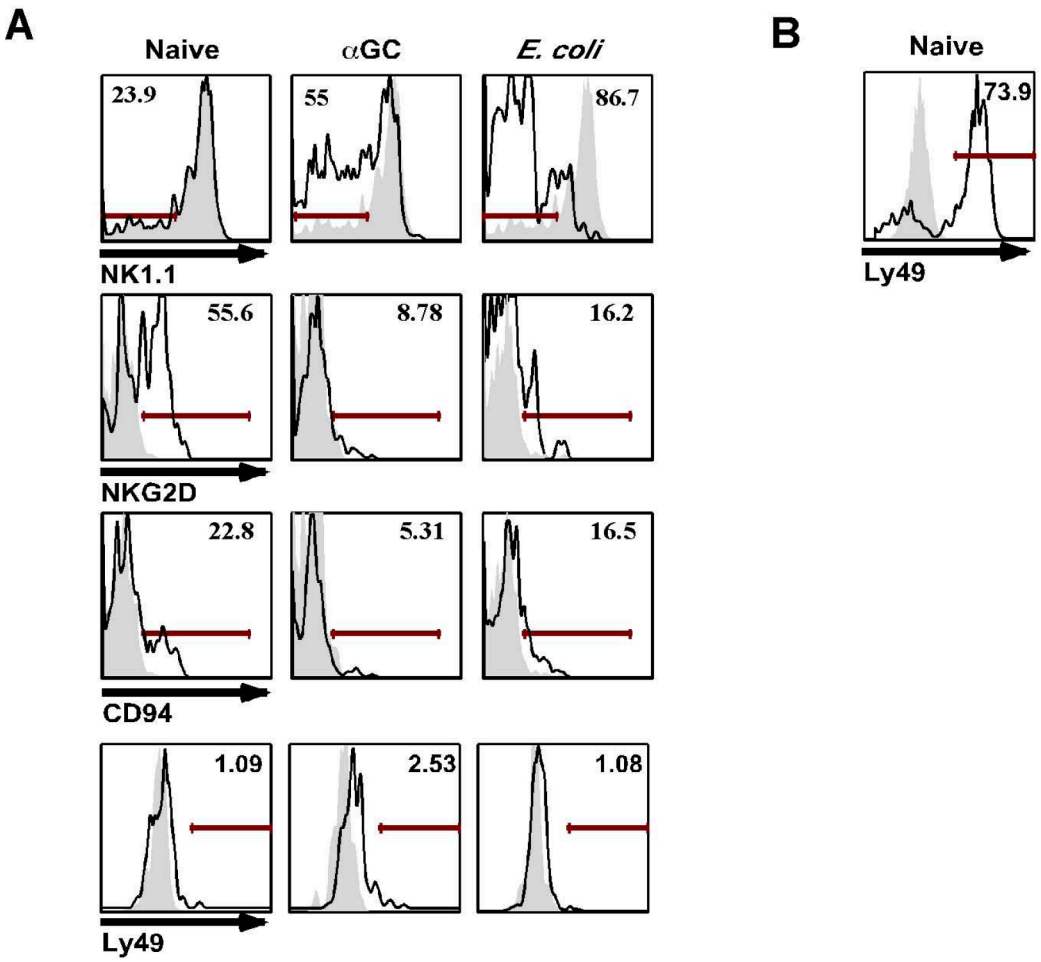
Supplementary Figure 7. Development of tolerogenic DCs following α-GalCer or heat-killed *E. coli* treatment. Mice were injected with α-GalCer (5 μg/mouse, i.p.) or heat-killed *E. coli* (indicated as 1°) and, 3 weeks later, rechallenged with vehicle or α-GalCer (5 μg/mouse, i.p.) (indicated as 2°). Mice were sacrificed 24 hrs following α-GalCer rechallenge and DCs were MACS-purified from spleens. Purified DCs were then cultured for 48 hours in the presence of vehicle, 10 μg/ml *Salmonella* LPS or 1 μM CpG ODN. Supernatants were analyzed for IL-12 and IL-10 by sandwich ELISA. Data shown represents mean ± SD from 3 wells per group.



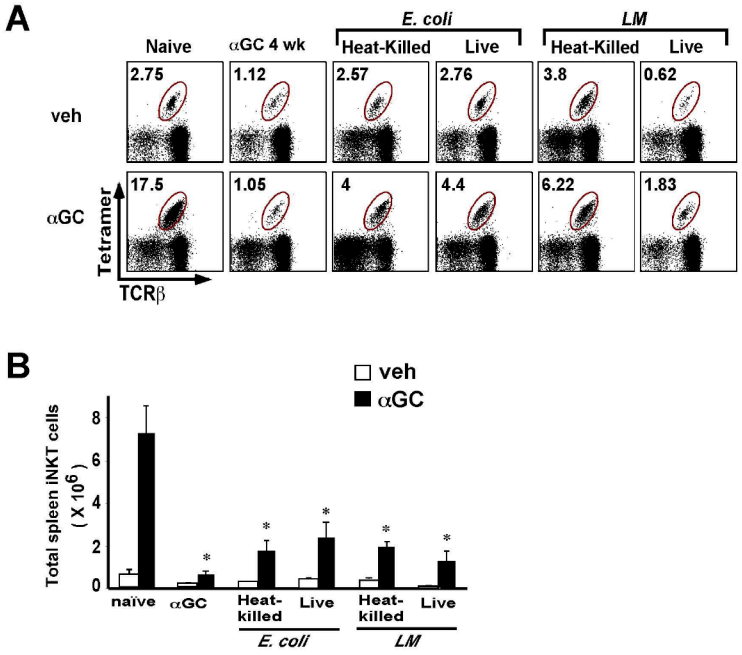
Supplementary Figure 1



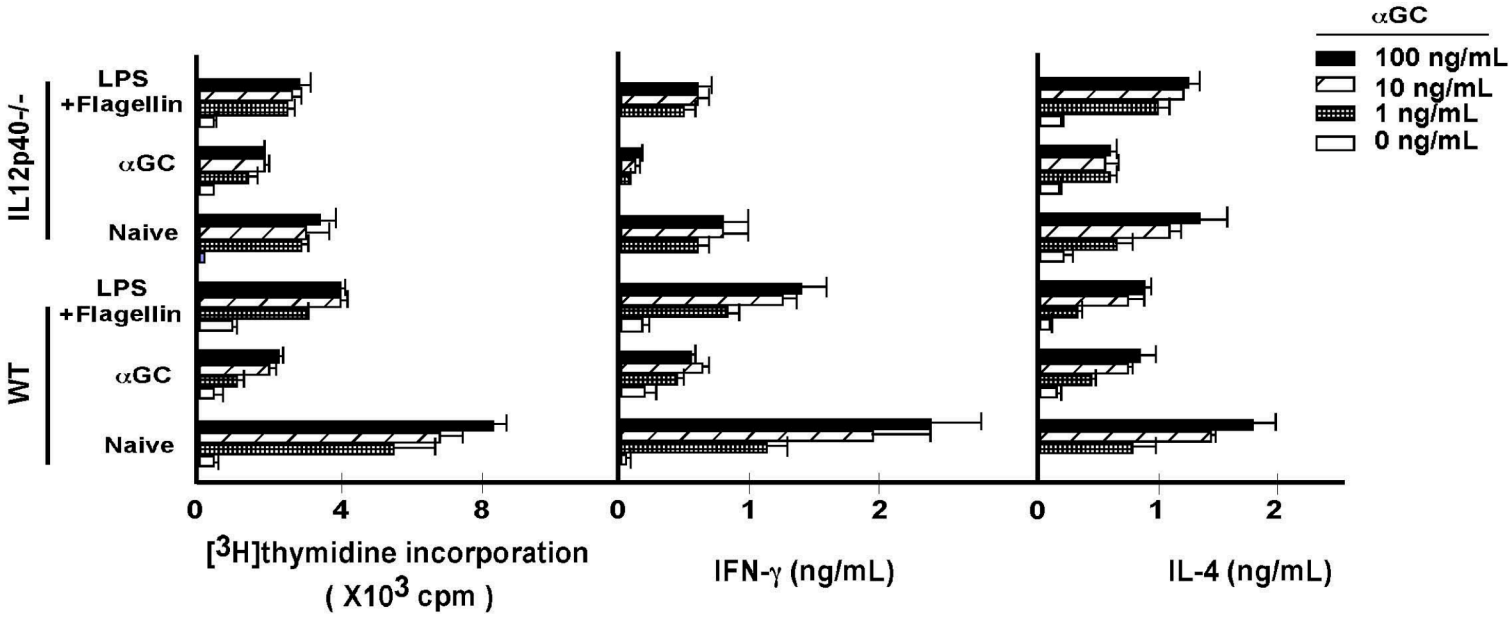
Supplementary Figure 2



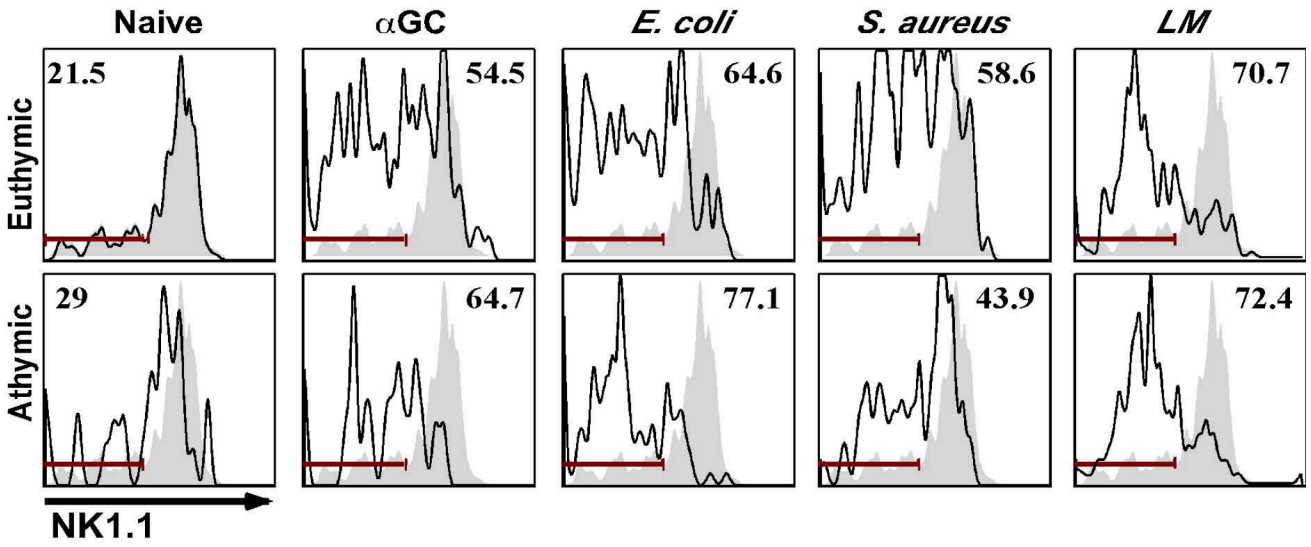
Supplementary Figure 3



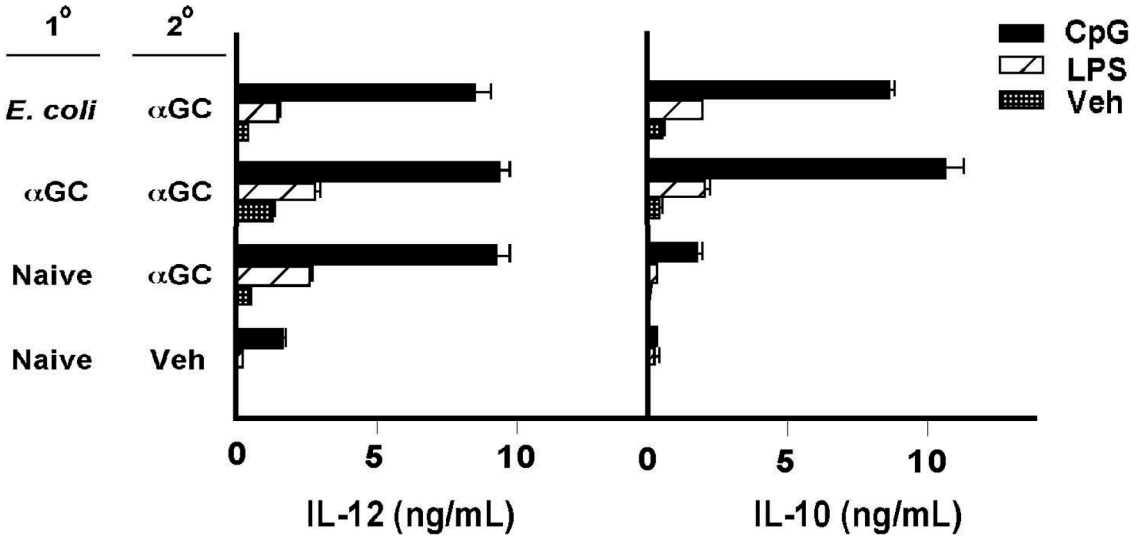
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7