## Supplemental Figure 1. Wingender et al.



**Supplemental Figure 1**. Gating strategy to detect cytokine production in *i*NKT cells as shown in figure 1. V $\alpha$ 14*i* NKT cells were defined throughout as live CD8 $\alpha$ <sup>-</sup> CD19<sup>-</sup> (or CD45R<sup>-</sup>) CD44<sup>+</sup> TCR/CD3<sup>+</sup> CD1d/ $\alpha$ GalCer-tetramer<sup>+</sup> cells. Representative data from at least three independent experiments are

Supplemental Figure 2. Wingender et al.



**Supplemental Figure 2: Plasma cytokines**. C57BL/6 (B6) mice were either left untreated or injected i.v. with 4µg of OCH, C-Gly or  $\alpha$ GalCer as indicated. One month later mice were injected i.v. with 1µg  $\alpha$  GalCer, and 90 min later IFN $\gamma$  and IL-4 levels in the plasma were analyzed. Statistically significant differences of treated groups versus the control group are indicated. Representative data from two independent experiments are shown.

## Supplemental Figure 3. Wingender et al.



Supplemental Figure 3: Infection with MCMV or Sphingobium yanoikuyae does not induce *i*NKT cell hypo-responsiveness. C57BL/6 (B6) mice were injected i.v. with 4µg of  $\alpha$ GalCer or infected with 5 x 10<sup>4</sup> PFU MCMV i.p. (A, B) or 1 x 10<sup>8</sup> Sphingobium yanoikuyae (S.yanoi.) bacteria i.p. (C, D) as indicated. One month later mice were injected i.v. with 1µg  $\alpha$ GalCer, and 90 min later expression of CD69 (A, C) and of indicated cytokines (B, D) by splenic *i*NKT cells was analyzed. Statistically significant differences of treated groups versus the control group are indicated. Representative data from at least two independent experiments are shown.

Supplemental Figure 4. Wingender et al.



## Supplemental Figure 4. Depletion of CD11c<sup>high</sup> DCs in CD11c-DOG mice:

(A, B) CD11c-DOG mice either left untreated (control) or were i.p. injected with 8ng per gram body weight of diphtheria toxin (DTx) and 24 h later the frequency of CD11c<sup>+</sup> cells was determined in the spleen. Representative data (A, gated for CD19<sup>-</sup> CD122<sup>-</sup> Ly6C/G<sup>-</sup> TCR $\beta$ <sup>-</sup> cells), and a summary graph of the percentage of all DCs within splenocytes (B, left) and of CD8<sup>+</sup> DCs within all DCs (B, right) are shown. Representative data from one of three independent experiments are shown.