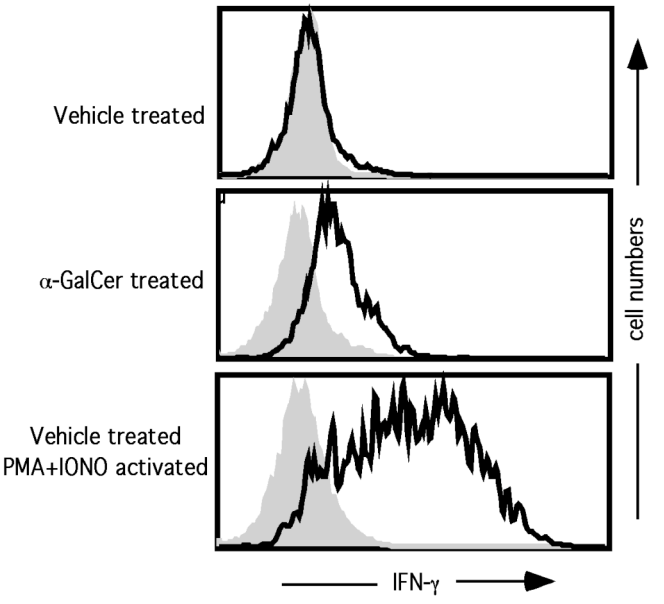
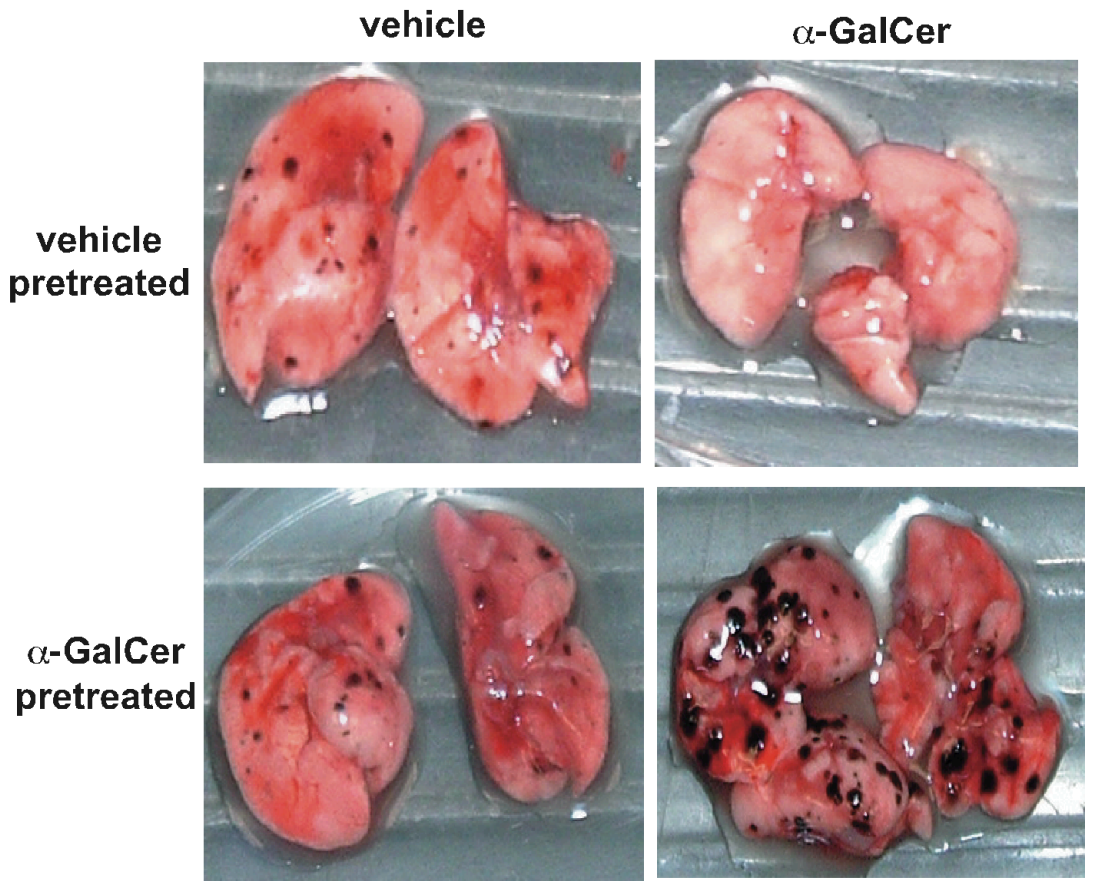
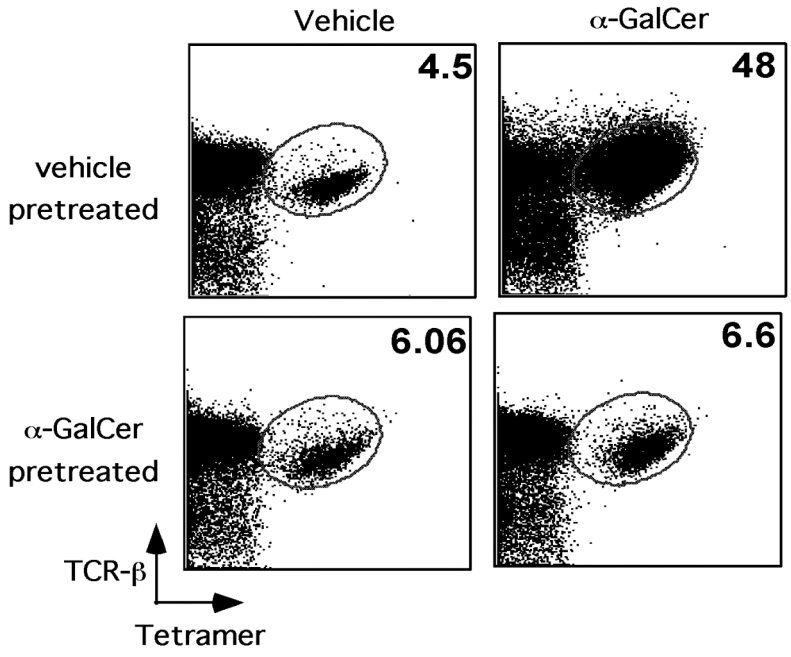
Supplementary Materials:



Supplementary Figure 1. IFN-γ synthesis by NK cells. Mice were injected with vehicle or α-GalCer. Twenty four hrs later, splenocytes were prepared, cultured with Golgi Plug™ for 6 h in the presence or absence of PMA plus ionomycin (IONO) and stained with anti-CD3-PerCP and anti-NK1.1-PE followed by anti-IFN-γ-FITC (line) or FITC-labeled isotype control (shaded) mAb. Intracellular IFN-γ expression by $NK1.1^{high}CD3^{-}$ cells was evaluated. No IFN-γ staining was obtained over the isotype control from NK cells in vehicle treated mice, whereas specific staining was obtained for α-GalCer-injected mice, as well as for spleen cells from vehicle-injected mice treated with PMA+IONO.



Supplementary Figure 2. Determination of B16 tumor lung metastases. B6 mice were injected with α-GalCer or vehicle and one month later these animals were challenged i.v. with $3x10^5$ B16 melanoma cells. The mice were also treated with α-GalCer (5 μg/injection) or vehicle at 0, 4 and 8 days after the tumor challenge. A representative lung from each group is shown. Data are representative of 3 experiments with a total of at least 18 mice per group. Data are presented in a graphical format in Figure 4A.



Supplementary Figure 3. Anergy induction is independent of IFN-γ. IFN-γ-deficient mice were injected with vehicle or α-GalCer (5 μg, i.p.), re-injected with vehicle or α-GalCer 1 month later, sacrificed 3 days later, and the prevalence of NKT cells in the spleen was evaluated by flow cytometry. Representative data from 3 animals are shown. Data indicate that NKT cells from IFN-γ-deficient mice that were injected 1 month earlier with α-GalCer are refractory to α-GalCer-induced expansion.

Supplementary Table 1.

α-GalCer can prevent EAE in mice injected one month earlier with a single dose of α-GalCer.

Pretreatment	Treatment	Number of mice	Disease Frequency	Mean onset of disease (days)	Mean maximum score	Mean Cumulative score
Vehicle	Vehicle	16	100%	12±0	4.18±0.18	103±3.7
Vehicle	α-GalCer	10	30%	16.4±0.24	0.5±0.16	7.4±3.8*
α-GalCer	Vehicle	15	100%	12.4±0.28	4.6±0.27	113.6±6.3
α-GalCer	α-GalCer	15	60%	15.5±0.5	1.13±0.19	23.3±5.4*

B6 mice were injected with vehicle or α-GalCer. One month later, EAE was induced as described in the Methods and animals were treated with α-GalCer or vehicle. Data shown represents mean values± SE. A graphical presentation of the data is shown in Figure 4B.

*, $p < 0.0001$ as compared with vehicle-treated group.