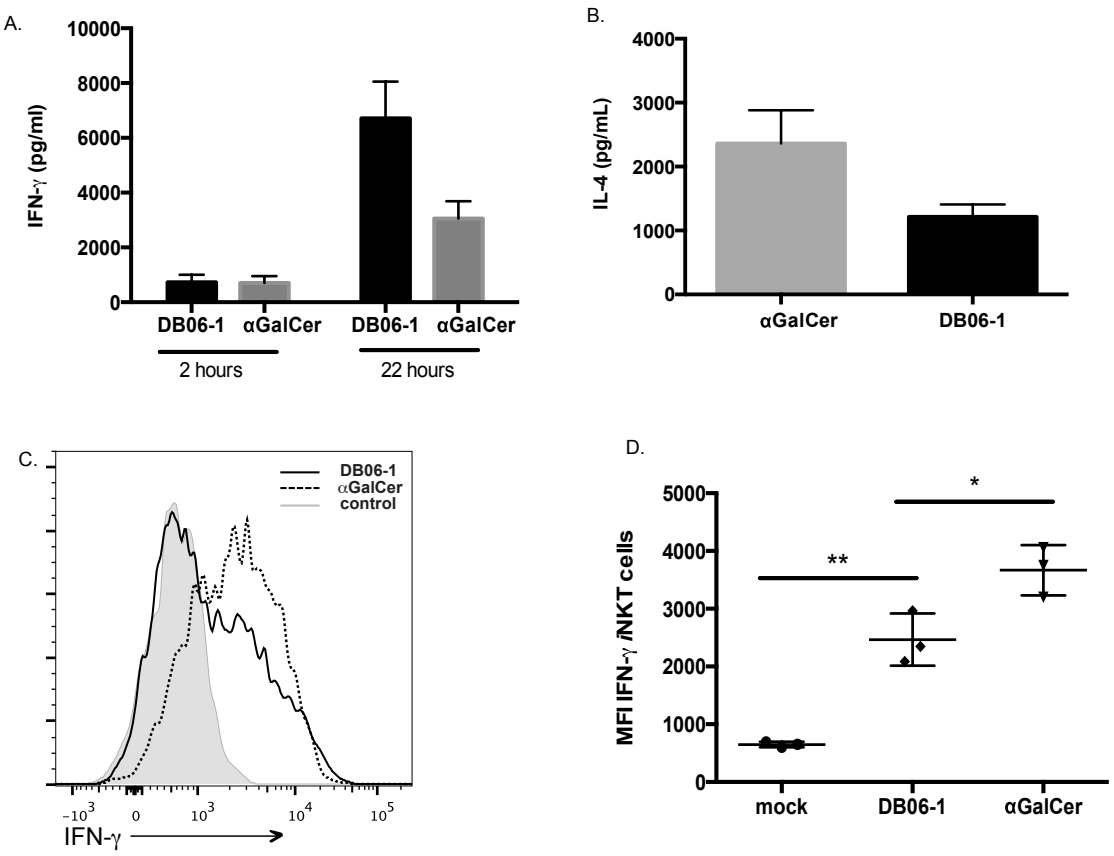
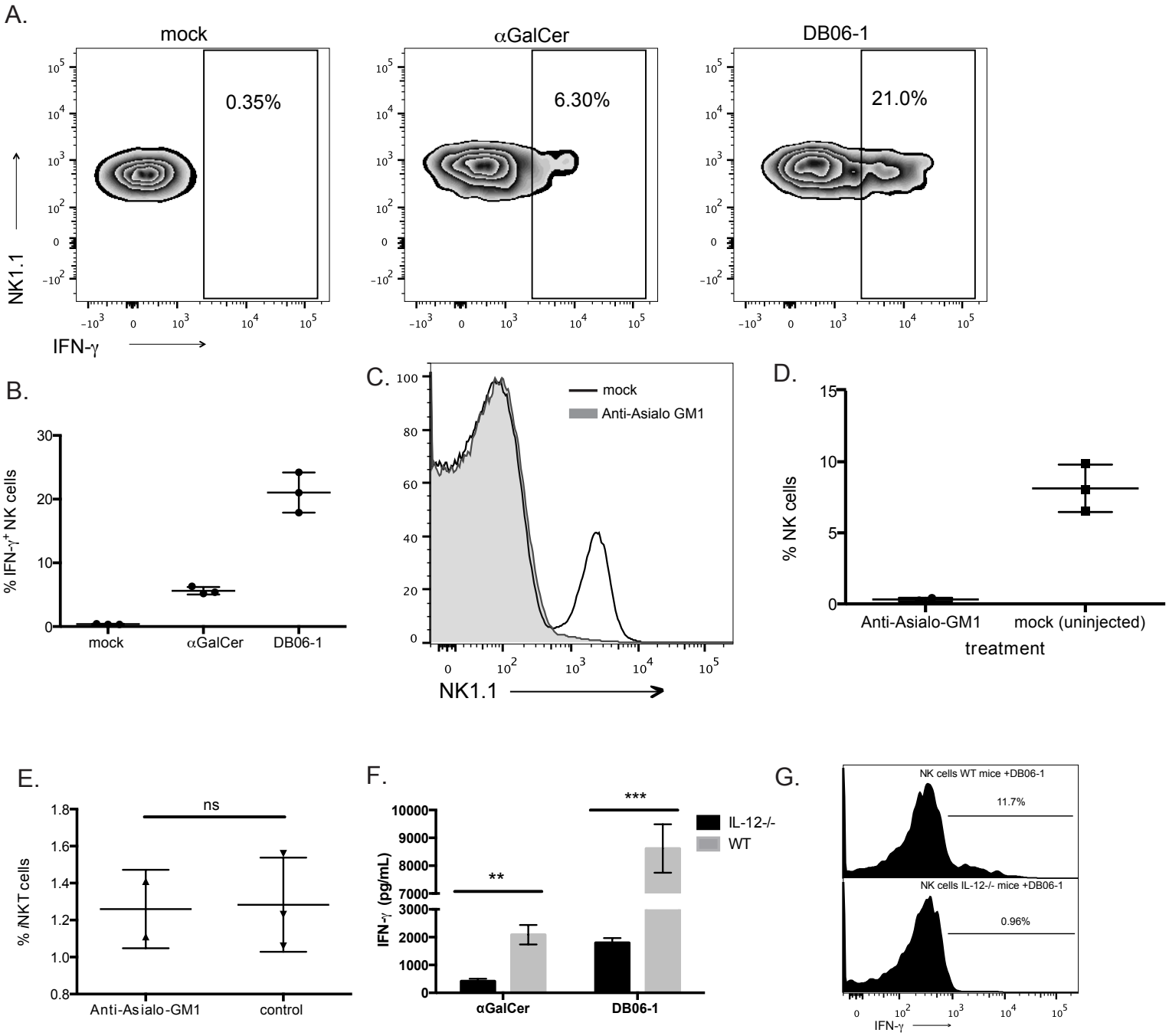
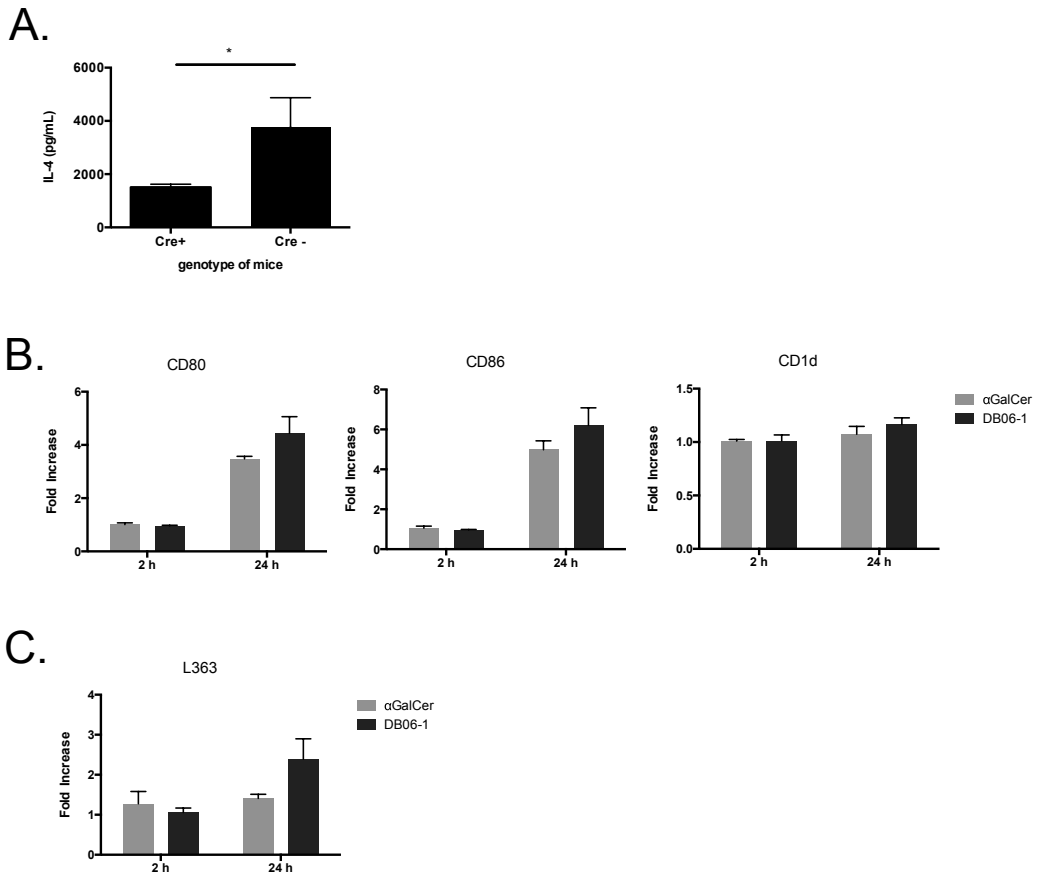


Supplemental Figure 1. *In vivo* cytokine responses of C57Bl/6 mice to GSL Ags. (A) IFN-γ in the sera of mice injected with αGalCer or DB06-1 2 or 22 h earlier. Cytokine was measured by sandwich ELISA. 3 mice per group, error bars represent ± SEM. (B) IL-4 in sera at 2 h post injection. Cytokine was measured by sandwich ELISA. 3 mice per group, error bars represent ± SEM. (C) ICCS to measure IFN-γ production by splenic *i*NKT cells, gated as live cells, $B220^-$, $CD8\alpha^-$, $CD3\varepsilon^+$ and CD1d-tet^+. (D) Summary graph of MFI IFN-γ of *i*NKT cells 2 h post injection. 3 mice per group. Error bars represent ± SEM.


A.
mock
αGalCer
DB06-1
0.35%
6.30%
21.0%
NK1.1
IFN-γ
B.
% IFN-γ+ NK cells
mock
αGalCer
DB06-1
C.
mock
Anti-Asialo GM1
NK1.1
D.
% NK cells
Anti-Asialo-GM1
mock (uninjected)
treatment
E.
% iNKT cells
ns
Anti-Asialo-GM1
control
F.
IFN-γ (pg/mL)
IL-12-/-
WT
**
***
αGalCer
DB06-1
G.
NK cells WT mice +DB06-1
11.7%
NK cells IL-12-/- mice +DB06-1
0.96%
IFN-γ

Supplemental Figure 2. In vivo NK cell responses to glycolipid Ags. (A) Representative flow cytometry plots for intracellular IFN-γ showing splenic NK cells, gated as live cells that are $B220^-$, $CD3\varepsilon^-$, and $NK1.1^+$. Data were obtained from mice injected with no GSL (mock), αGalCer or DB06-1 and analyzed at 24 h post injection. (B) Representative data of one of three experiments showing % IFN-γ^+ NK cells at 24 h post injection with 3 mice per group. (C) Staining of splenic NK cells in control mice and mice treated with anti-Asialo-GM1 antibody showing the effectiveness of NK cell depletion. NK cells were gated as live cells that were $B220^-$, $CD3\varepsilon^-$ and $NK1.1^+$. Un-injected mice (mock) were compared to anti-asialo-GM1 treated mice. (D) Representative data from one of two experiments depicting the percent NK cell depletion with anti-asialo-GM1 treatment. (E) Percent *i*NKT cells after anti-asialo-GM1 Ab treatment, showing that *i*NKT cells were not affected. (F) IFN-γ in sera comparing WT and IL-$12^{-/-}$ mice 24 h post injection. Combined data from 2 different experiments, 5 mice per group. (G) ICCS IFN-γ from splenic NK cells 24 h post injection comparing WT and IL-$12^{-/-}$ mice. Representative data from one of two experiments.



Supplemental Figure 3. DC responses in GSL injected mice. (A) IL-4 in sera at 2 h after injection of DB06-1 measured by sandwich ELISA comparing *Cd1d*$^{f/f}$ *mice* x *CD11c-Cre* mice (Cre^{+}) to *Cd1d*$^{f/f}$ controls lacking the Cre transgene (Cre-). (B,C) Splenic CD8α^{+} DCs (CD11c^{+} CD8α^{+}) were analyzed by flow cytometry for the indicated cell surface molecules at 2 and 24 h following i.v. injection of 1 μg of either DB06-1 or αGalCer (gray, αGalCer; black, DB06-1). Changes in cell surface expression molecules are shown as the fold increase compared to uninjected control mice. CD1d and activation markers CD80 and CD86 are shown in (B) and GSL-CD1d complex formation as measured by L363 is shown in (C). Data shown are representative of one of two experiments showing means ± SD for triplicate mice in each group.

Supplemental Table I. Data collection and refinement statistics from CD1d-DB06-1-TCR mouse ternary crystal structure (PDB ID 4ZAK).

Data collection statistics	**CD1d-DB06-1-TCR**
Space group	$C222_1$
Cell dimension	
a, b, c, (Å)	78.9, 190.5, 150.8
a, b, g (°)	90.0, 90.0, 90.0
Resolution range (Å) [outer shell]	50-2.83 (2.93-2.83)
No. reflections	27210 (2706)
R_{merge} (%)	12.2 (57.3)
R_{pim} (%)	6.1 (28.2)
Multiplicity	3.9 (3.8)
Average I/sI	10.4 (1.9)
Completeness (%)	98.1 (98.6)
Refinement statistics	
No. atoms	6,341
Protein	6,225
Ligand	60
Carbohydrate	56
R/R_{free} (%)	21.6/25.6
Ramachandran plot (%)	
Favored	97.7
Allowed	100
R.m.s. deviations	
Bonds (Å)	0.006
Angles (°)	0.915
B-factors ($Å^2$)	
Protein	50.2
Ligand	40.4
Carbohydrate	58.9