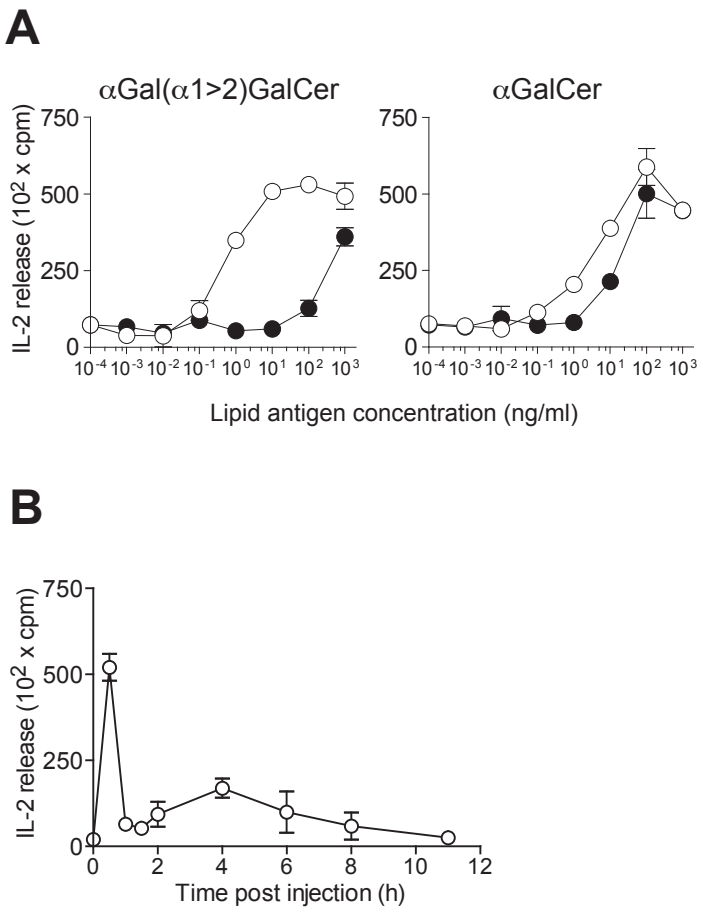


Supplemental Figure 1 (**A**) CD1d presentation of αGalCer(α1>2)GalCer and αGalCer by bone marrow-derived DCs derived from *Ldlr* KO (●) and WT (○) mice as assessed by stimulation of DN32.D3 hybridoma cells *in vitro*. Mean ± SD of triplicates from one of three similar experiments are shown. (**B**) Presence of stimulatory bioactivity in serum of C57BL/6 mice (n=4) after i.v. injection of 1 μg αGalCer. Serum samples were collected at indicated times after injection, pulsed on splenic APCs, and tested for presence of bioactive compound using DN32.D3 NKT cells as a read-out.

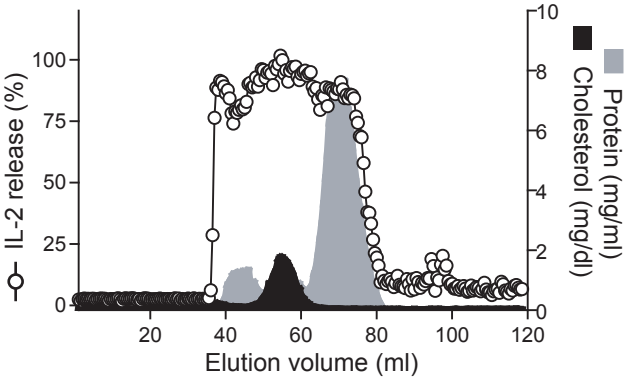
A

RQGLLYGVPVSLKE
RFPSAFCGICGLKPTGNRL
KGTNCVTSYLTDCETQLSQAPRQ
KAMNLDVVLTPMLGPALDLNTPGRA
KKGIGLPVAVQCVALPWQEELCLRF
KSPGGSSGGEGALIGSGGSPLGLGTDIGGSIRF

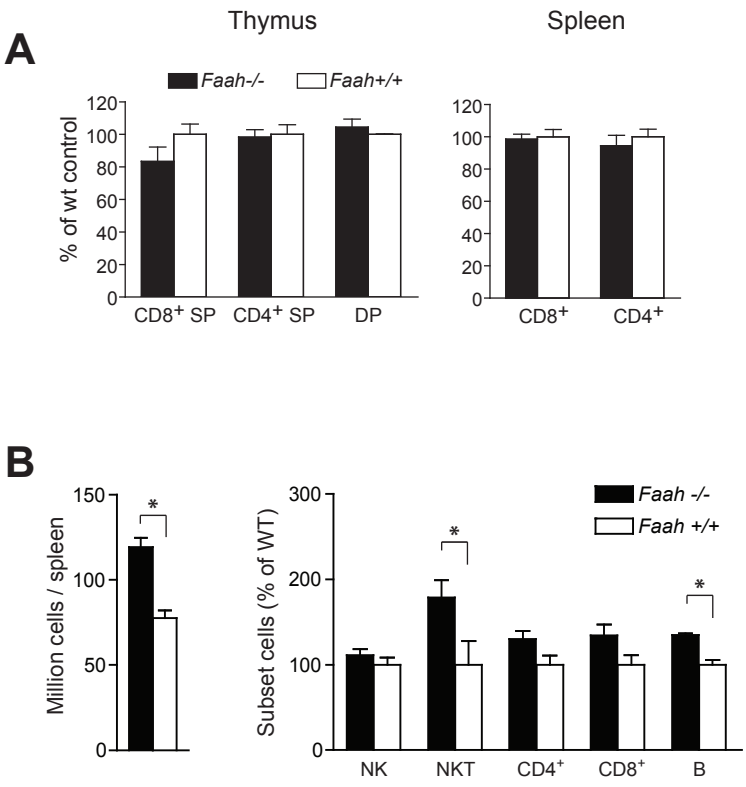
B

KDLELLASGVDRY
KQIFSAEFEVKE
REVVADSVWVDVKD
KSSVAVPYVIVPLKI
KTIYTPGSTVLYRI
RLVAYYTLIGASGQRE
KNYAGVFMDAGLAFKT
KTVVILIETPDGIPVKR
KYVTVVANFGETVVEKA
KVYSYYNLEESCTRF
RDICEGQVNSLPGSINKA
RSELEEDIIPEEDIISRS
KVDVPAADLSDQVPDTDSETRI
RIILQGSPVVQMAEDAVDGERL
REPGQDLVVLSLPITPEFIPSFRL

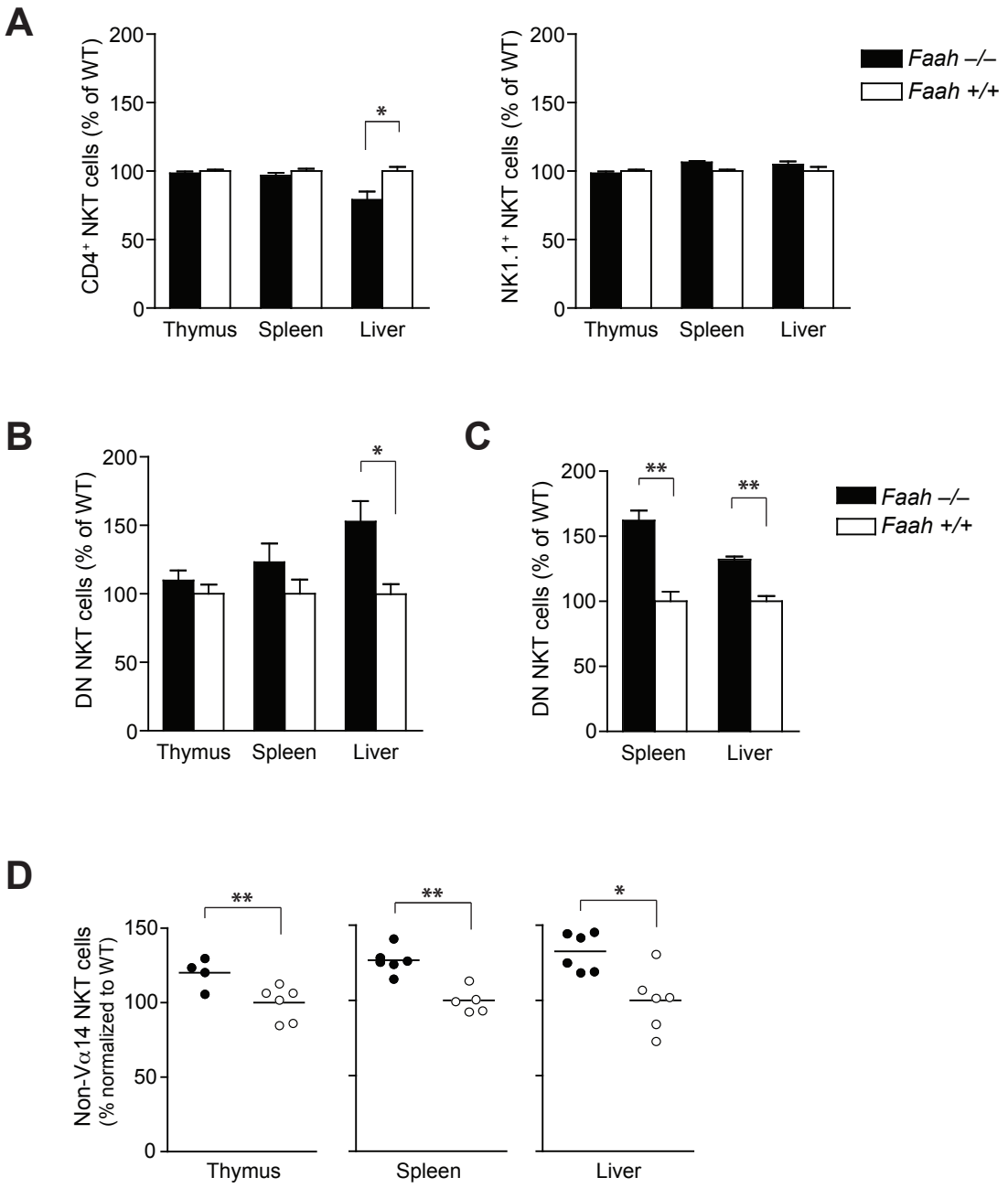
C



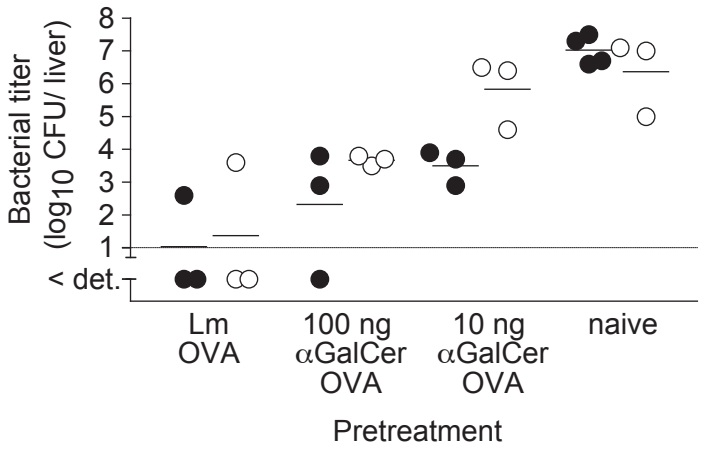
Supplemental Figure 2 Peptide sequences identified by mass spectrometry sequencing in αGalCer-containing serum protein fractions and distribution of αGalCer in C3-deficient serum. (**A**) Peptides isolated from the 70 kDa sized band that identified murine FAAH. (**B**) Peptide sequences identified from C3 in the 150 kDa sized band. (**C**) Distribution of αGalCer bioactivity in C3-deficient serum fractionated by gel filtration chromatography as detected by DN32.D3 hybridoma cells in an antigen presentation assay.



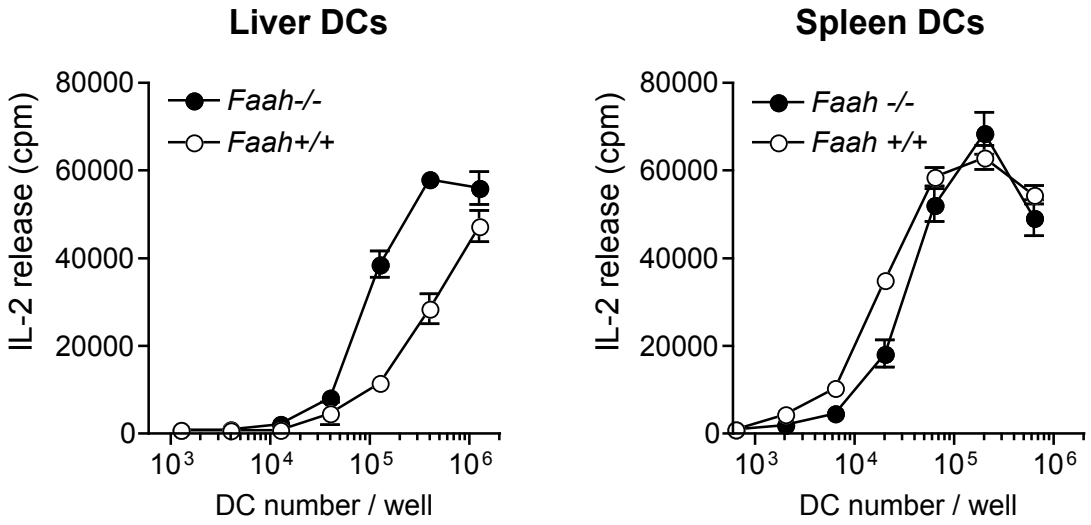
Supplemental Figure 3 (**A**) T cell populations in thymus and spleen of naive *Faah* KO (■) and WT (□) mice as assessed by flow cytometry. Mean ± SD of groups of 3 mice from one of two similar experiments are shown. (**B**) Expansion of individual lymphocyte subsets in spleens of αGalCer immunized WT and *Faah*-deficient mice. Subsets were identified by their expression of cell surface markers as NK cells (NK1.1+/CD3ε-), NKT cells (NK1.1+/CD3ε+), CD4+ T cells (CD4+/CD3ε+), CD8+ T cells (CD8+/CD3ε+), or B cells (B220+).



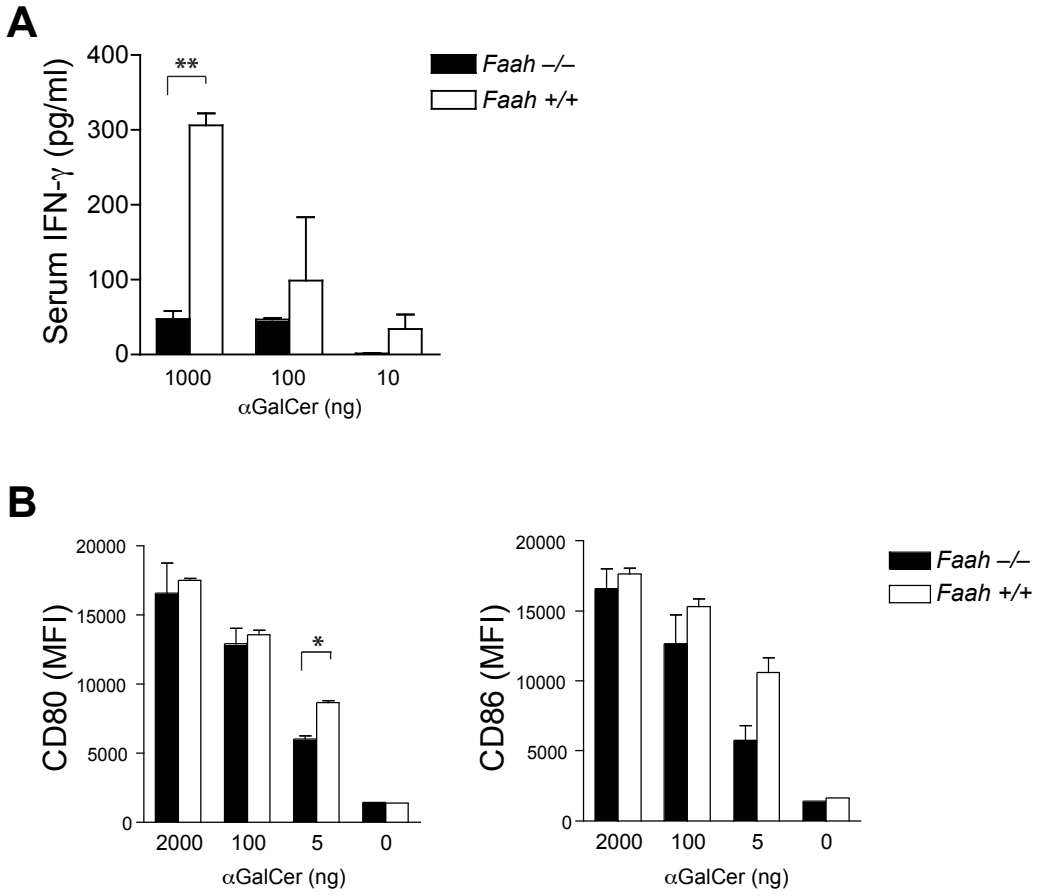
Supplemental Figure 4 NKT cell subpopulations in thymus, spleen and liver of *Faah* KO (closed symbols) and WT (open symbols) mice as assessed by flow cytometry. (**A**) Vα14 NKT cell subsets in naive WT and *Faah*-deficient mice as characterized by CD4 and NK1.1 expression. (**B,C**) Double negative (DN) Vα14 NKT cells in naive mice (**B**) and 3 days after immunization with αGalCer (**C**). Mean ± SD of groups of 4-6 mice from one of two experiments are shown. (**D**) Type II (non-Vα14) NKT cells in different organs were assessed as the CD1d/αGalCer tetramer-negative population of NK1.1+/CD3ε+ NKT cells. Symbols represent individual mice.



Supplemental Figure 5 *Listeria* titers in livers of LmOVA-immunized, αGalCer/OVA-immunized and naive *Faah*-deficient (●) and WT (○) mice 3 days after i.v. challenge with $1x10^5$ CFU LmOVA. Data of individual mice from one of two similar experiments is shown.



Supplemental Figure 6 Altered CD1d presentation of αGalCer in *Faah*-deficient mice. CD1d presentation of αGalCer by hepatic and splenic DCs was determined ex vivo on MACS-purified CD11c+ cells from livers and spleens of αGalCer-injected WT (○) and *Faah* KO (●) mice using the DN32.D3 NKT cell hybridoma in an antigen presentation assay.



Supplemental Figure 7 The cytokine response of NKT cells is more sensitive to the antigenic potency of stimulation than the NKT cell-mediated induction of DC maturation. (**A**) Peak IFN-γ concentrations in serum of WT and *Faah*-deficient mice in response i.v. injection of titrated doses of αGalCer. (**B**) DC maturation in WT and *Faah*-deficient mice at 18 hours post injection of indicated amounts of αGalCer as assessed by surface expression of the maturation markers CD80 and CD86.