

24 h







Supplementary Figure S1.

Liver injury and hepatocyte proliferation in sham-operated mice treated with α -GalCer. ALT levels, representative photomicrographs of H&E-stained liver sections, and PCNA⁺-hepatocytes (%) after administration of α -GalCer to sham-operated mice. Data are expressed as mean \pm SD (four–six biological replicates per group). Scale bars, 200 μ m.



Supplementary Figure S2. Infiltration of immune cells after hepatic I/R injury. (A) Flow cytometry gating strategy used to identify neutrophils and Kupffer cells (KCs) in livers from sham-operated mice. (B,C) Changes in the percentage of neutrophils (B) and KCs (C) in livers from mice treated with α -GalCer or vehicle after hepatic I/R injury. Neutrophils were defined as CD45+/Ly6G+/CD11b+ cells; KCs, CD45+Ly6G^{low}/Ly6C-/CD11b^{low}/F4/80^{high} -cells. Data are expressed as the mean \pm SD (four biological replicates per group). * *P* <0.05, ** *P* <0.01, **** *P* <0.0001.



Supplementary Figure S3.

Effect of IL-4 or IFN- γ blockade on the percentage of neutrophils and Kupffer cells after hepatic I/R injury. Anti-IL-4 or IFN- γ neutralizing antibodies were administered to mice 1 h prior to the induction of ischemia, and α -GalCer was given at the induction of ischemia. The percentage of neutrophils and Kupffer cells in livers from mice treated with anti-IL-4 antibodies and IgG. Neutrophils and Kupffer cells are defined as CD45+/Ly6G+/CD11b+ cells and CD45+Ly6G^{low}/Ly6C-/CD11b^{low}/F4/80^{high} -cells, respectively. Data are expressed as the mean \pm SD (four biological replicates per group). * P < 0.05.

CD1dF4/80DAPI



В





CD1d expression in the livers after hepatic I/R injury treated with α -GalCer.

(A) Representative photo showing double staining of CD1d (red) and F4/80 (green) in livers treated with α -GalCer at 6 h post-reperfusion. Yellow indicates double-positive cells. Nuclei are stained by DAPI (blue). Scale bar, 20 µm. (B) The expression of CD1d in Ly6C^{high} macrophages (Ly6G⁻/Ly6C^{high}/CD11b^{high}/F4/80^{high} cells) and Ly6C^{low}macrophages (Ly6G⁻/Ly6C^{low}/CD11b^{high}/F4/80^{high} cells) at 6, 24, and 48 h post-reperfusion (light gray, isotype control; light green, Ly6C^{low}macrophages and Ly6C^{high} macrophages, light blue).

Α



Supplementary Figure S5.

Characterization of adherent and non-adherent cells in co-culture of macrophages and activated iNKT cells.

(A,B) Expression of mRNA for *Itgam* (A) and *Cd3e* (B) in adherent and non-adherent cells in co-culture of macrophages and iNKT cells. Cultured bone marrow-derived macrophages and isolated iNKT cells were stimulated with α -GalCer for 72 h. Then, mRNA levels of *Itgam* and *Cd3e* were determined from adherent and non-adherent cells to the wells. Data are expressed as the mean \pm SD (four mice per group, representative data from two independent experiments).



Supplementary Figure S6.

Production of IL-4 and IFN- γ from iNKT cells in vitro.

(A) The levels of mRNA for *Il4* and *Ifng* from cultured activated iNKT cells and macrophages. Mf, macrophages. Data are expressed as the mean \pm SD (four mice per group, representative data from two independent experiments). * P < 0.05, *** P < 0.001. (B) The levels of IL-4 and IFN- γ in supernatants from activated iNKT cells and macrophages. Mf, macrophages. Data are expressed as the mean \pm SD (four mice per group, representative data from two independent experiments). * P < 0.05.



В



Supplementary Figure S7.

Liver repair after hepatic I/R injury in $Cd1d^{-/-}$ mice treated with α -GalCer or vehicle. (A) ALT levels, hepatic necrosis, and PCNA⁺-hepatocytes (%), and (B) expression of genes related to pro-inflammatory (*Tnf, Il1b, Il6, Nos2, and Ifng*) and reparative macrophages phenotypes (*Mrc1, Retnla, Il4, and Il13*) at 72 h post-reperfusion. Data are expressed as the mean \pm SD (four biological replicates per group). Veh, vehicle.