**Supporting Information**

**Arrenberg et al. 10.1073/pnas.1000576107**

**Fig. S1.** Sorted sulfatide/CD1d-tetramer+ cells are reactive to sulfatide. Bar graphs depict IFN-γ secretion (Upper) and [3H]thymidine incorporation (Lower) of sorted sulfatide/CD1d-tetramer+ cells, following coculture with dendritic cells in the absence (without antigen) or presence of sulfatide (20 μg/mL). Data are representative of two individual experiments.

**Fig. S2.** Predominant expression of Vα3, Vα1, and Vα7 gene segments by sulfatide/CD1d-tetramer+ cells. Gel images show RT-PCR products of indicated Vα chains expressed by sorted sulfatide/CD1d-tetramer+ cells from 10 Jα18−/− mice or by unsorted liver lymphocytes. A 100-bp DNA ladder was used. Data are representative of two individual experiments. A minimal expression of Vα2, Vα5, Vα10, and Vα18 also was detected. Only the weaker upper band of Vα2 and Vα10 represents the specific PCR product. Sequencing of Vα7 and Vα14 PCR products did not yield any functional TCR sequences, and for Vα2 only 3/50 sequences were functional.

**Fig. S3.** CDR3 regions of both TCR α- and β-chains among type II NKT cells are encoded by germline or N-additions. A typical example for Vβ8.1-Jβ2.7 and Vα3-Jα7 sequences is shown. Sequences at top are encoded by germline, whereas sequences below that have N-additions (depicted in bold letters). DJi-nucleotides are italic. The junctional amino acid residues are depicted in blue.
Fig. S4. Hepatic MNCs stained with CD1d-tetramers loaded with a single immunodominant cis-tetracosenoyl sulfatide also predominantly use TCR Vβ8, Vβ3, and Vα3 chains. Liver MNCs from Jα18−/− mice were analyzed by flow cytometry following staining with cis-tetracosenoyl sulfatide/CD1d-tetramer and respective antibodies against Vβ8, Vβ3, and Vα3 chains. Percentage was calculated in relation to total cis-tetracosenoyl sulfatide/CD1d-tetramer+ TCRα/β+ cells after subtraction of background (unloaded CD1d-tetramer). Data are representative of two individual experiments.

Fig. S5. CDR2β, CDR3α, and CDR1α regions of the lyso-sulfatide-reactive hybridoma Hy19.3 are similar to those of sulfatide/CD1d-tetramer+ cells. The amino acid sequence of the Vα1-Jα26/Vβ16-Jβ2.1 TCR used by Hy19.3 is depicted. Conserved tyrosine residue between type I and type II NKT cells is shown in green. Residues identical or similar to the sulfatide/CD1d-tetramer+ cells (Fig. 3B) are depicted in purple.