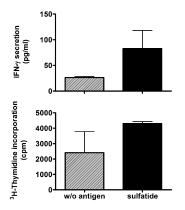
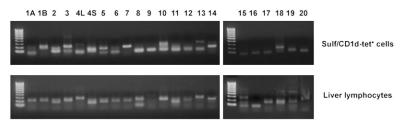
## **Supporting Information**

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**Fig. S1.** Sorted sulfatide/CD1d-tetramer<sup>+</sup> cells are reactive to sulfatide. Bar graphs depict IFN- $\gamma$  secretion (*Upper*) and [<sup>3</sup>H]thymidine incorporation (*Lower*) of sorted sulfatide/CD1d-tetramer<sup>+</sup> 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. 52.** Predominant expression of V $\alpha$ 3, V $\alpha$ 1, and V $\alpha$ 7 gene segments by sulfatide/CD1d-tetramer<sup>+</sup> cells. Gel images show RT-PCR products of indicated V $\alpha$  chains expressed by sorted sulfatide/CD1d-tetramer<sup>+</sup> cells from 10 J $\alpha$ 18<sup>-/-</sup> mice or by unsorted liver lymphocytes. A 100-bp DNA ladder was used. Data are representative of two individual experiments. A minimal expression of V $\alpha$ 2, V $\alpha$ 5, V $\alpha$ 10, V $\alpha$ 14, and V $\alpha$ 18 also was detected. Only the weaker upper band of V $\alpha$ 2 and V $\alpha$ 10 represents the specific PCR product. Sequencing of V $\alpha$ 7 and V $\alpha$ 14 PCR products did not yield any functional TCR sequences, and for V $\alpha$ 2 only 3/50 sequences were functional.

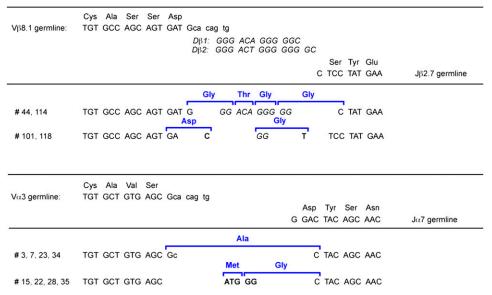
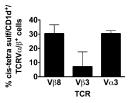
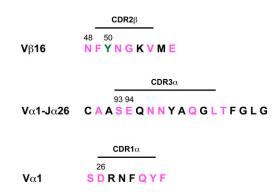


Fig. S3. CDR3 regions of both TCR  $\alpha$ - and  $\beta$ -chains among type II NKT cells are encoded by germline or N-additions. A typical example for V $\beta$ 8.1-J $\beta$ 2.7 and V $\alpha$ 3-J $\alpha$ 7 sequences is shown. Sequences at top are encoded by germline, whereas sequences below that have N-additions (depicted in bold letters). D $\beta$ -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 V $\beta$ 8, V $\beta$ 3, and V $\alpha$ 3 chains. Liver MNCs from J $\alpha$ 18<sup>-/-</sup> mice were analyzed by flow cytometry following staining with cis-tetracosenoyl sulfatide/CD1d-tetramer and respective antibodies against V $\beta$ 8, V $\beta$ 3, and V $\alpha$ 3 chains. Percentage was calculated in relation to total cis-tetracosenoyl sulfatide/CD1d-tetramer\*TCR $\alpha$ / $\beta$ <sup>+</sup> cells after subtraction of background (unloaded CD1d-tetramer). Data are representative of two individual experiments.



**Fig. S5.** CDR2 $\beta$ , CDR3 $\alpha$ , and CDR1 $\alpha$  regions of the lyso-sulfatide-reactive hybridoma Hy19.3 are similar to those of sulfatide/CD1d-tetramer<sup>+</sup> cells. The amino acid sequence of the V $\alpha$ 1-J $\alpha$ 26/V $\beta$ 16-J $\beta$ 2.1 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<sup>+</sup> cells (Fig. 3*B*) are depicted in purple.