Supporting Information

Muindi et al. 10.1073/pnas.0915056107

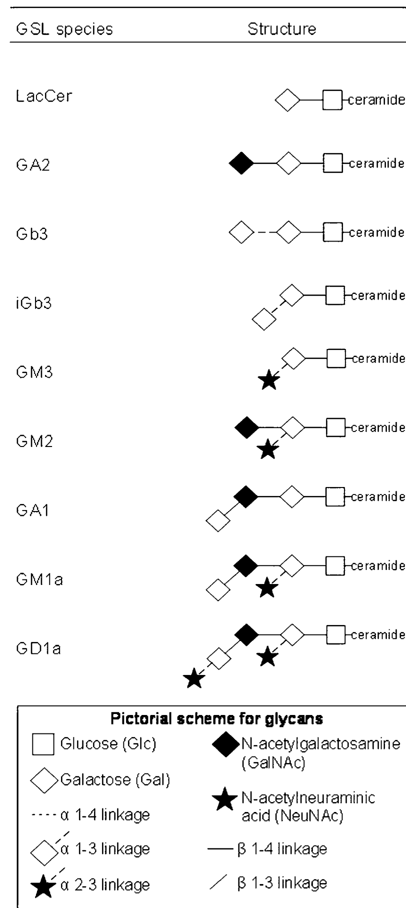

Fig. S1. Symbolic representation of glycosphingolipid (GSL) glycan headgroup structures. GSL structures were drawn using the glycan drawing system proposed by ref. 1.

1. Harvey DJ, et al. (2009) Proposal for a standard system of drawing structural diagrams of N- and O-linked carbohydrates and related compounds. *Proteomics* 9:3796–3801.

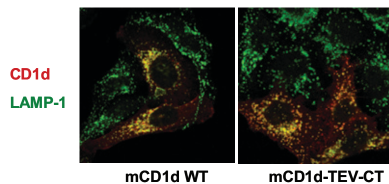

Fig. S2. Wild-type cluster of differentiation 1 (mCD1d) and mCD1d Tobacco Etch Virus (TEV) cytoplasmic tail (CT) molecules colocalize with LAMP-1. HeLa cells transiently transfected with wild-type, full-length mCD1d (mCD1d WT) or mCD1d-TEV-CT cDNA were allowed to adhere to glass coverslips. The cells were fixed, permeabilized with saponin, stained with Alexa 488-conjugated mouse anti-lysosomal-associated membrane protein-1 (LAMP-1) mAb (H4A3; BD Biosciences) and rat anti-mCD1d mAb (1B1) or isotype control detected with Cy3-conjugated donkey anti-rat IgG mAb (Jackson ImmunoResearch). The slides were analyzed with an inverted microscope (TE2000; Nikon) with a 60× oil (NA 1.4) objective lens using EZ-C1 software (Nikon). Only representative images were acquired. CD1d molecules were labeled in red (Cy3), and LAMP-1 was labeled in green (Alexa 488). The mCD1d construct transfected cells allow for the detection of the intracellular compartments expressing both CD1d and LAMP-1 (yellow). These findings confirm that mCD1d-TEV-CT molecules (*Right*) have an identical trafficking pattern to wild-type mCD1d (*Left*) that also strongly colocalize with the lysosomal marker, LAMP-1.

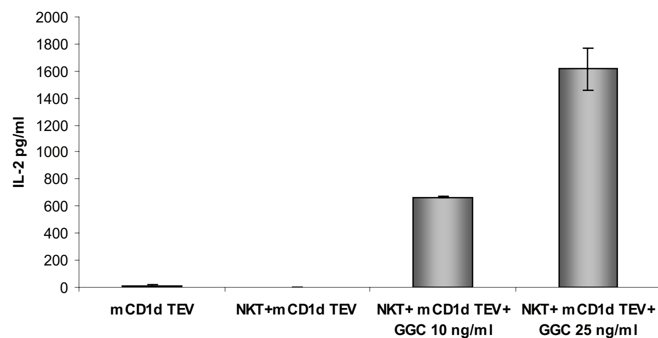

Fig. S3. mCD1d-TEV-CT molecules present α-GalGalCer to natural killer T (NKT) cells. Stable mCD1d-TEV-CT (mCD1d-TEV) RAW cell transfectants were plated in flat-bottomed, 96-well microtiter plates at 5×10^3 cells per well and incubated with 2.5×10^4 invariant DN32 NKT hybridoma cells (provided by A. Bendelac, University of Chicago, Chicago, IL, USA) in the presence of media, 10 or 25 ng/mL α-GalGalCer (GGC) in RPMI complete media overnight. NKT cell activation was determined by measuring the concentration of IL-2 in the culture supernatants by ELISA. Murine IL-2 in culture supernatants was detected by standard sandwich ELISA and normalized to a recombinant IL-2 standard curve. Error bars indicate the SD for each sample.

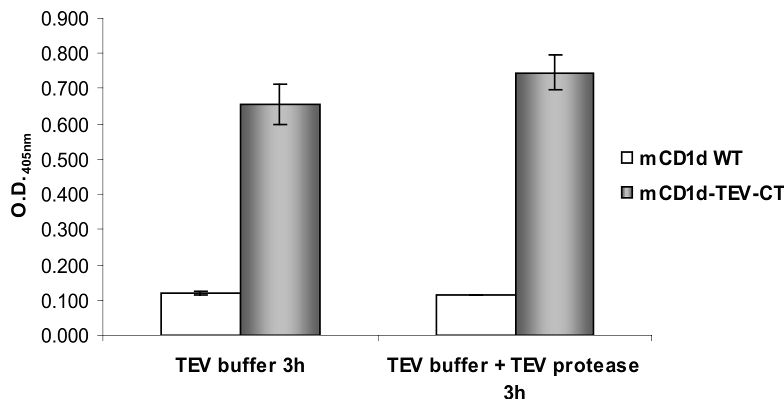

Fig. S4. mCD1d-TEV is cleaved from the surface of stable mCD1d-TEV-CT RAW cell transfectants. Detached mCD1d WT and cell surface-cleavable mCD1d-TEV-CT RAW cell transfectants were washed and incubated in TEV buffer for 3 h at 4 °C. mCD1d molecules were not shed from mCD1d WT RAW cell transfectants (white bars) after incubation with TEV buffer. However, intact mCD1d-TEV was cleaved from the mCD1d-TEV-CT transfectants (gray bars) as determined by ELISA performed on the digest supernatants. AcTEV protease (Invitrogen), an enhanced form of TEV protease, was added as per manufacturer's instructions in several of the assays. However, because the increase in protein cleavage (TEV buffer + TEV protease) over the TEV buffer alone was modest, TEV protease was not used in the GSL-elution experiments. These findings suggest that the TEV buffer, likely in addition to endogenous proteases, is sufficient to mediate cleavage of mCD1d-TEV from the transfectants. mCD1d-Fc and cleaved mCD1d-TEV were assayed by a standard sandwich ELISA using anti-mouse CD1d mAb (1B1) and in the case of MHC class I-Fc, using anti-β2m mAb (BBM.1; ATCC) as the capture antibodies. They were detected with biotinylated rabbit anti-β2m-microglobulin mAb (Rockland Immunochemicals), streptavidin-conjugated alkaline phosphatase (BD Pharmingen), and *p*-nitrophenyl phosphate (Sigma-Aldrich) in standard fashion. Error bars indicate the SD for each sample.