SUPPLEMENTARY MATERIAL

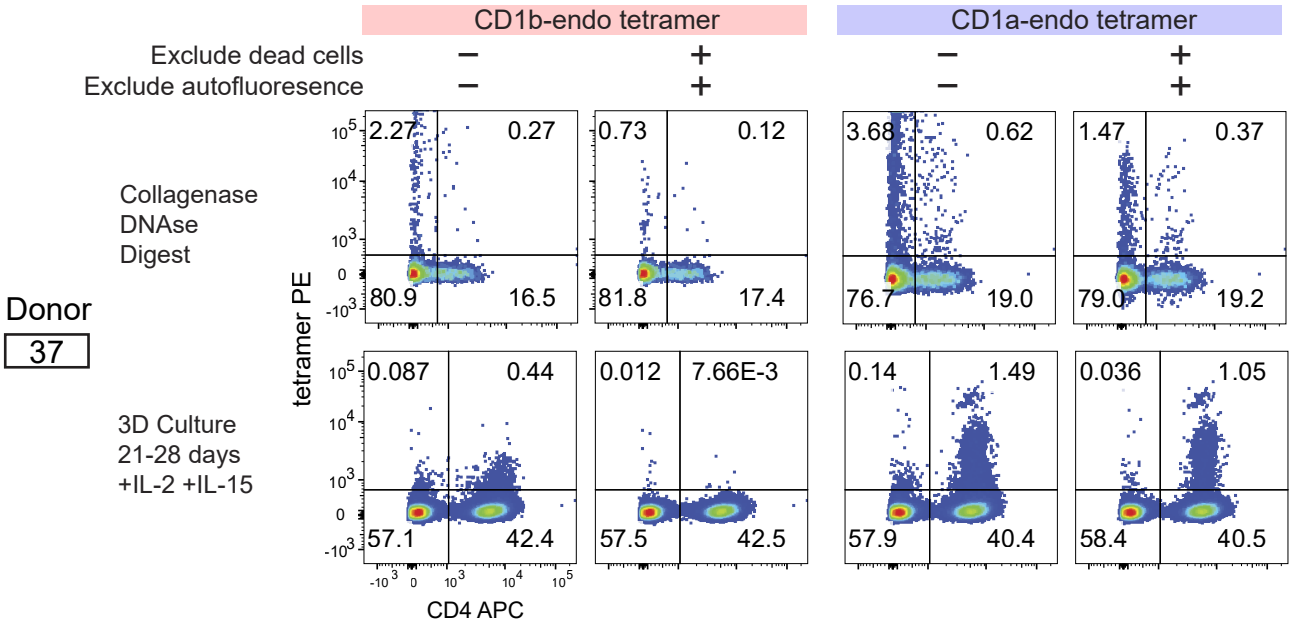


Figure S1. Exclusion of dead cells and autofluorescence improves signal to noise ratios of tetramer detection. Skin from donor 37 was allocated for enzymatic digestion or for 3D culture as in main figure 1. Cells were stained for flow cytometry with CD1b-endo or CD1a-endo tetramers, Blue (AF350) LIVE/DEAD amine-reactive dye. As shown, pre-gated CD3+, lymphocyte size and granularity and singlets by FSC and SSC. (+) indicates additional gating to exclude cells based on autofluoresence in the FITC channel and uptake of amide-reactive dye.

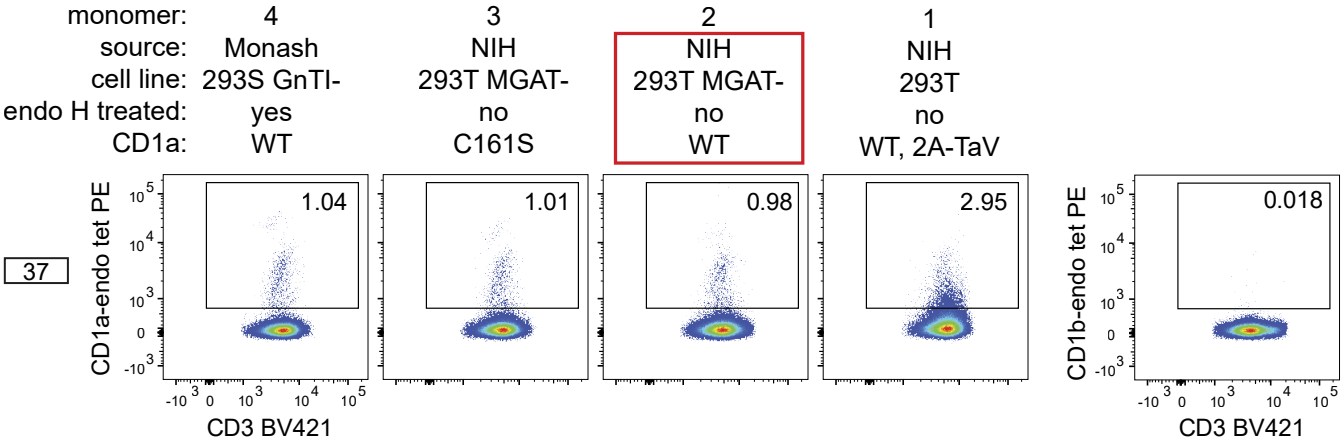
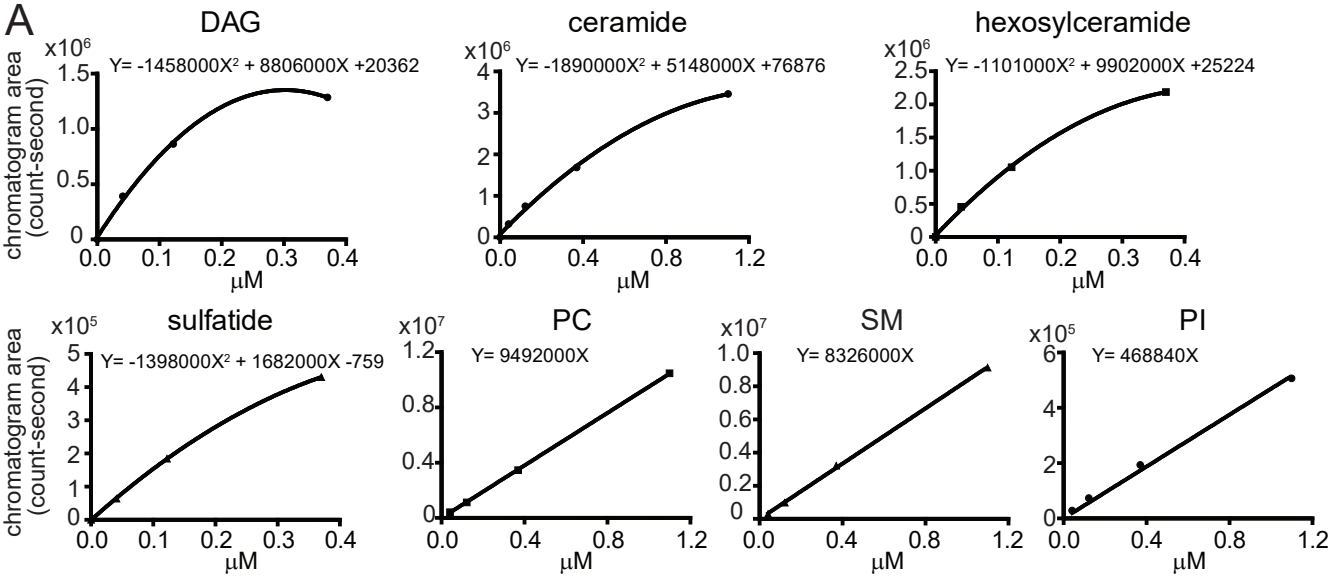
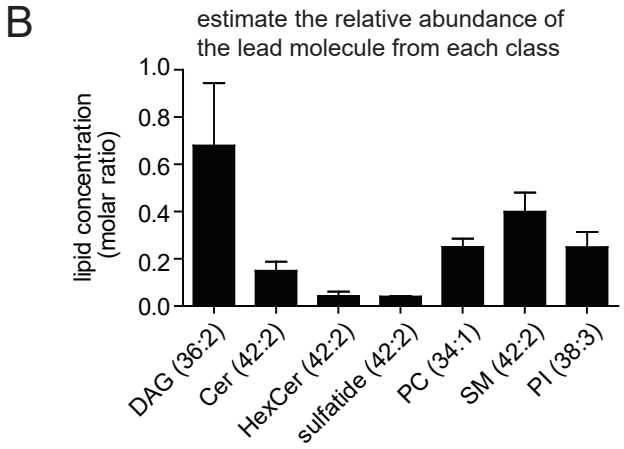


Figure S2. Skin T cell staining with 4 CD1a tetramer reagent formats.
Donor 37 polyclonal skin T cells (Pregated: Live, $CD3^{+}$, $Autofluoresence^{neg}$) stained in parallel with one of 4 different CD1a-endo tetramers assembled in parallel on streptavidin-PE in Boston from monomers produced at the NIH Tetramer Core Facility or Monash University. Red box highlights monomer and cells from which lipids were eluted and characterized by HPLC-MS in main figures 3 and 4.
Two dot plots are reproduced from donor 37 data shown in main figure 4.





lipid class	lead molecule chain: unsaturation	chromatogram area (count-second) mean ± SD
diacylglycerol (DAG)	C36:2	(6.11 ± 2.27) x 10^5
ceramide (Cer)	C42:2	(1.54 ± 0.195) x 10^5
hexosyl-ceramide (HexCer)	C42:2	(6.77 ± 1.82) x 10^4
sulfatide	C42:2	(6.05 ± 0.407) x 10^3
phosphatidyl-choline (PC)	C34:1	(2.38 ± 0.333) x 10^5
sphingomyelin (SM)	C42:2	(3.33 ± 0.670) x 10^5
phosphatidyl-inositol (PI)	C38:3	(1.17 ± 0.300) x 10^4

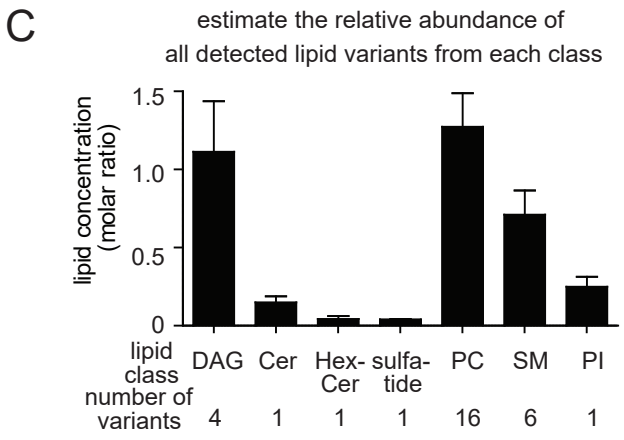


Fig. S3. Quantitative analysis of ligands eluted from CD1a proteins.
(A) For each standard, a series of known concentrations were prepared and analyzed by HPLC-MS. The ion chromatogram peak areas from known concentrations of lipid standards were used to generate external standard curves for determining the concentrations of the extracted lipids using the linear or non-linear curve fitting equations as indicated. (B) Lipid concentrations of the lead molecule from each class eluted from CD1a were estimated by fitting the detected chromatogram area to the external standard curve. The data are presented as mean ± standard deviation of triplicate measurements. (C) The total concentrations of all detected lipid variants from each class were estimated by separately measuring peak areas and calculating absolute mass for the indicated number of molecular species, which were then summed to get total lipid for each class.

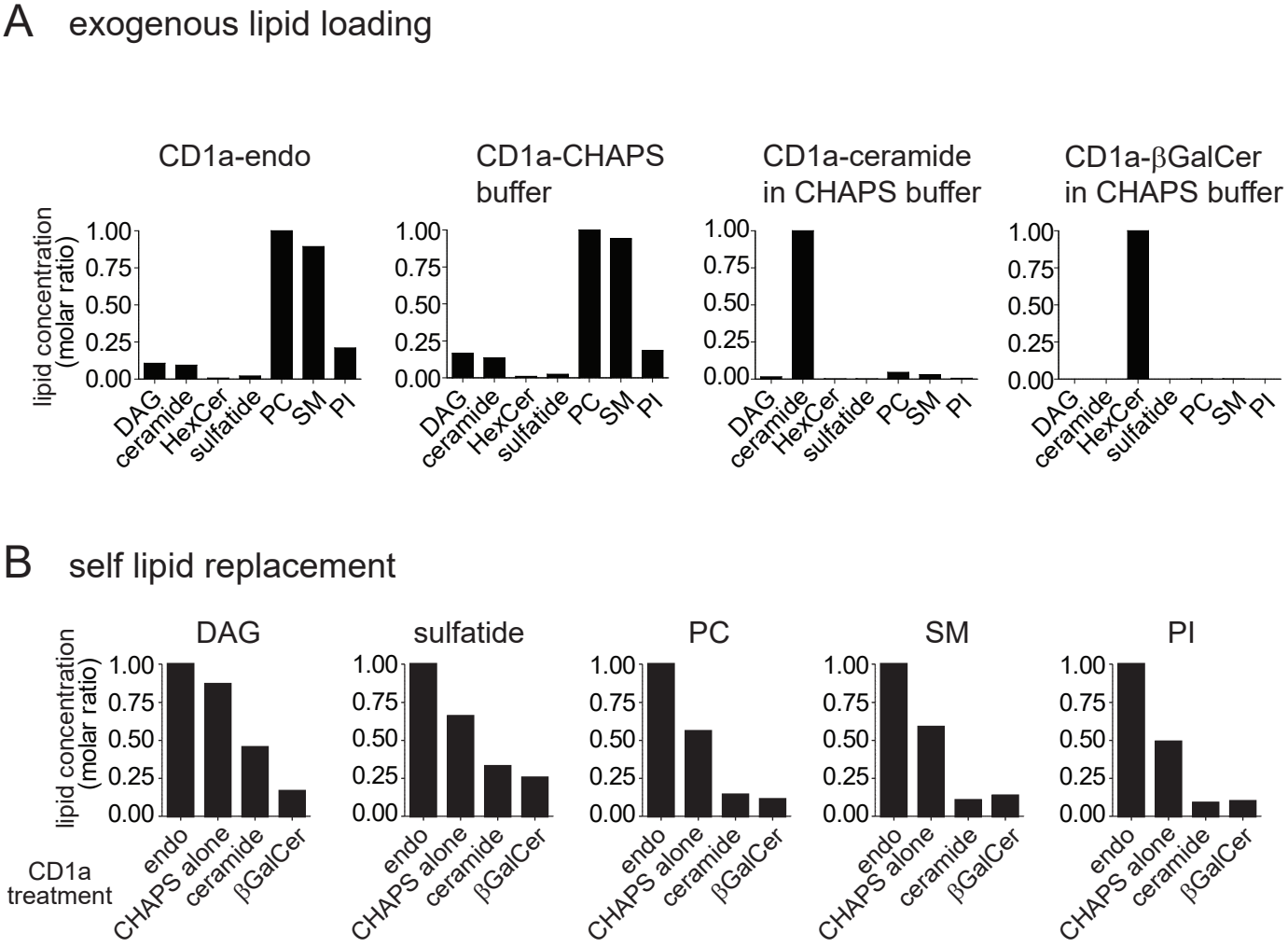


Fig. S4. Binding of exogenous ceramide lipids to CD1a proteins.
CD1a monomers were treated with lipid loading buffer (containing 0.5% CHAPS) as a control, ceramide (40-fold molar excess in the loading buffer), or βGalCer (40-fold molar excess in the loading buffer) under conditions used to make CD1a monomers that are tetramerized for T cell measurements. The lipid control treated monomers were washed with PBS three times by filtering through centrifugal filter units (Amicon Ultra-0.5 Centrifugal Filter 30K Devices, Millipore) to remove the excess exogenous lipids. The treated proteins recovered from the filter units and untreated CD1a-endo protein were subjected to lipid elution followed by HPLC-MS analysis. (A) Ceramide and βGalCer were largely enriched in the ceramide treated and βGalCer treated CD1a eluents, respectively. (B) The self-lipids, such as DAG, sulfatide, PC, SM, and PI were reduced by ceramide or βGalCer treatment.

Supplementary Table 1. Summary of human skin donor samples

Donor code	Skin site (if known)	Tested in:
A	unknown	IL-22 ELISPOT
B	unknown	IL-22 ELISPOT
C	unknown	IL-22 ELISPOT
1	face	IL-22 ELISPOT
2	breast	IL-22 ELISPOT
3	face	IL-22 ELISPOT
4	abdominal	IL-22 ELISPOT
7	face	IL-22 ELISPOT
9	abdominal	IL-22 ELISPOT
11	unknown	IL-22 ELISPOT
12	thigh	IL-22 ELISPOT
17	abdominal	IL-22 ELISPOT
18	abdominal	IL-22 ELISPOT
19	face	IL-22 capture
20	unknown	IL-22 capture
29	abdominal	IL-22 capture, CD1 tetramer
30	abdominal	IL-22 capture, CD1 tetramer
31	unknown	IL-22 capture, CD1 tetramer
32	unknown	IL-22 capture, CD1 tetramer
34	unknown	IL-22 capture
35	unknown	CD1 tetramer
36	abdominal	CD1 tetramer
37	abdominal	CD1 tetramer
38	abdominal	CD1 tetramer
39	abdominal	CD1 tetramer

Supplementary Table 2

	detected *m/z*	RT (min)	Formula	predicted *m/z*	lipid chain
diacylglycerol (DAG)	638.5721	2.524	C39H76NO5 [M+NH_4]+	638.5718	C36:2
ceramide	648.6223	2.546	C42H82NO3 [M+H]+	648.6290	C42:2
hexosyl-ceramide	810.6756	3.964	C48H92NO8 [M+H]+	810.6818	C42:2
sulfatide	890.6376	19.955	C48H92NO11S [M+H]+	890.6386	C42:2
phosphatidyl-choline (PC)	760.5846	21.268	C42H83NO8P [M+H]+	760.5851	C34:1
sphingomyelin (SM)	813.6836	21.734	C47H94N2O6P [M+H]+	813.6844	C42:2
phosphatidyl-inositol (PI)	906.6064	23.611	C47H89NO13P [M+NH_4]+	906.6067	C38:3

Supplementary Table 3. TCR sequences from DermT2 and line 36

Line	TRAV	CDR3α	TRAJ
DermT2	8-6	CAVSYGDYKLSFGAG	20
line 36	1-2	CAVRGPGGSYIPTFGRG	6
line 36	8-6	CAVSLSGNTPLVFGKG	29

Line	TRBV	CDR3β	TRBJ
DermT2	2	CASSEENQPQHFGDG	1-5
line 36	13	CASSLGWVAGVVETQYFGPG	2-5
line 36	27	CASPTAARWGNILYGYTFGSG	1-2