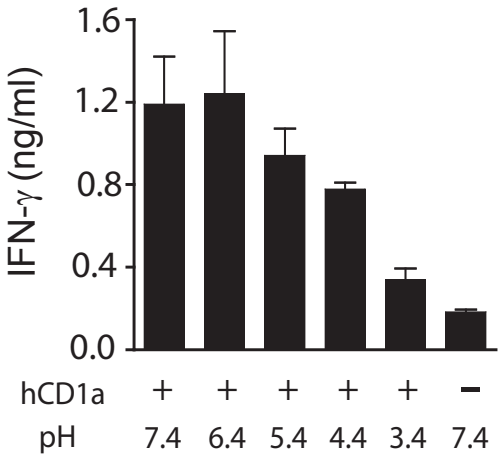
Supplementary Information

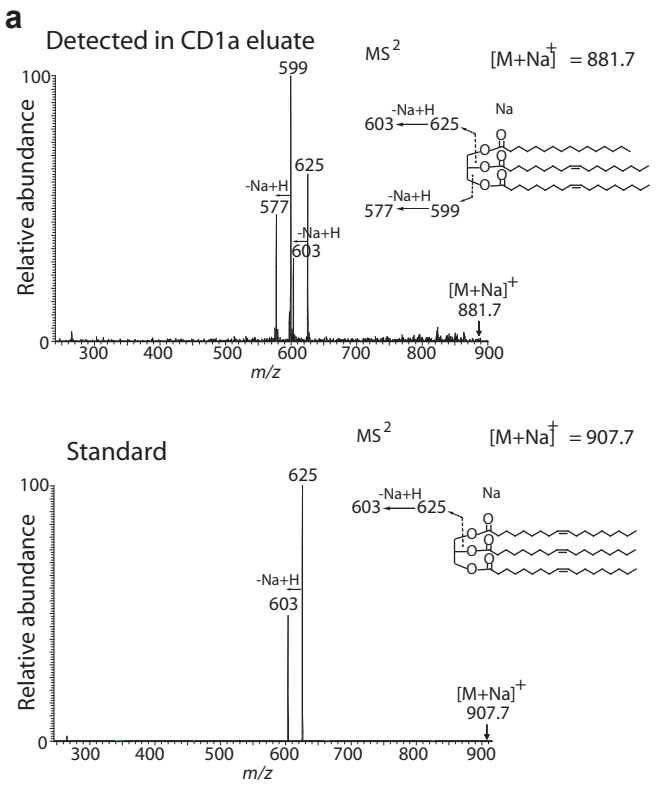
for the manuscript

CD1a autoreactive T cells recognize natural skin oils that function as headless antigens

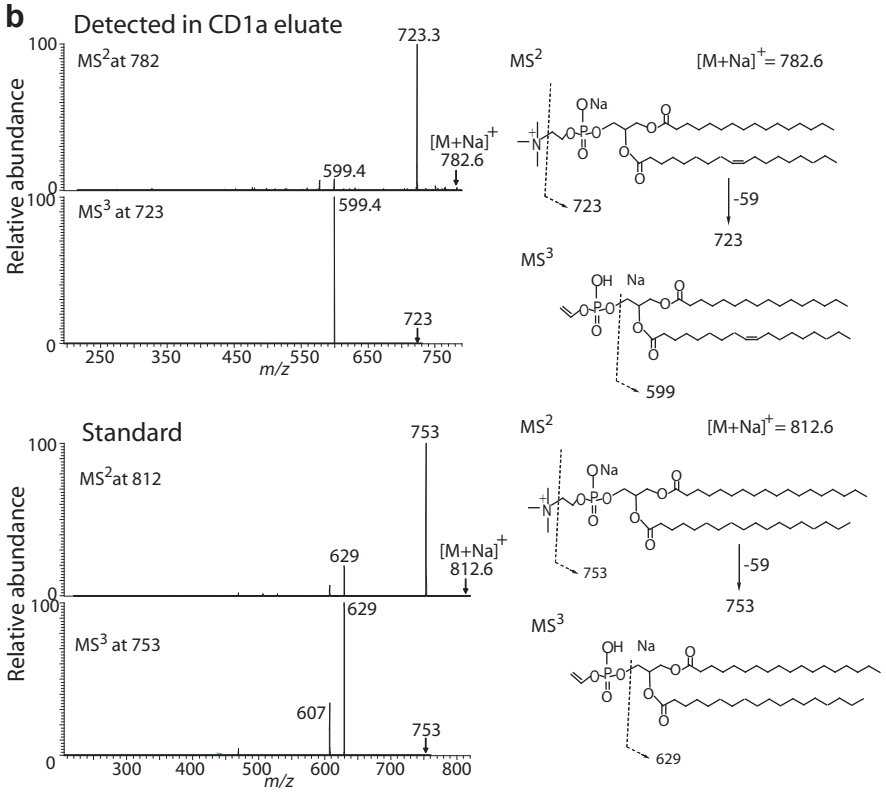
Annemieke de Jong,Tan-Yun Cheng, Shouxiong Huang, Stephanie Gras, Richard W. Birkinshaw, Anne Kasmar, Ildiko van Rhijn, Victor Peña-Cruz, Daniel T. Ruan, John D. Altman, Jamie Rossjohn, D. Branch Moody



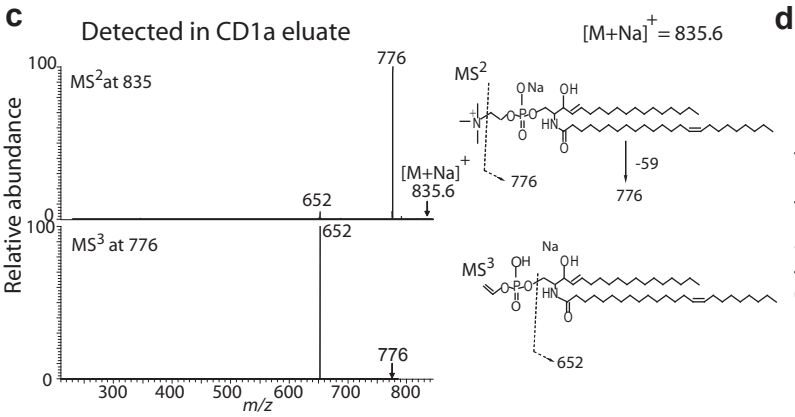
Supplementary Figure 1. Low pH promotes unloading of lipids from CD1a protein.
Plate-bound CD1a protein was treated with citrate buffers of indicated pH. 24h after co-incubation of BC2 T cell line with pH-treated CD1a protein, T cell activation was measured in the supernatant by IFNγ ELISA

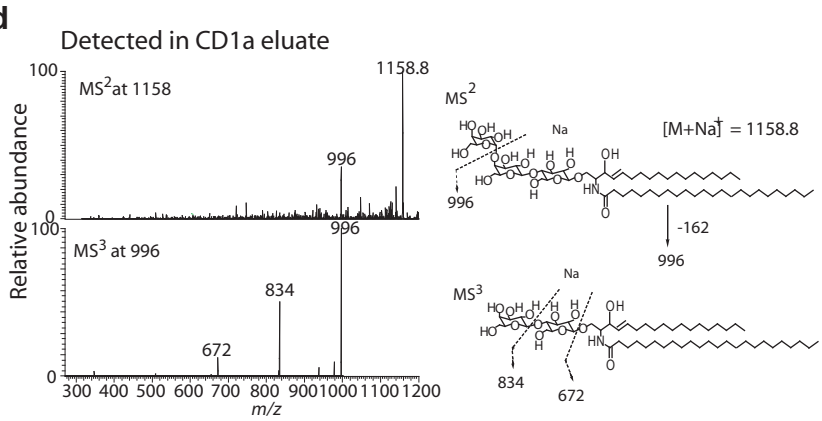


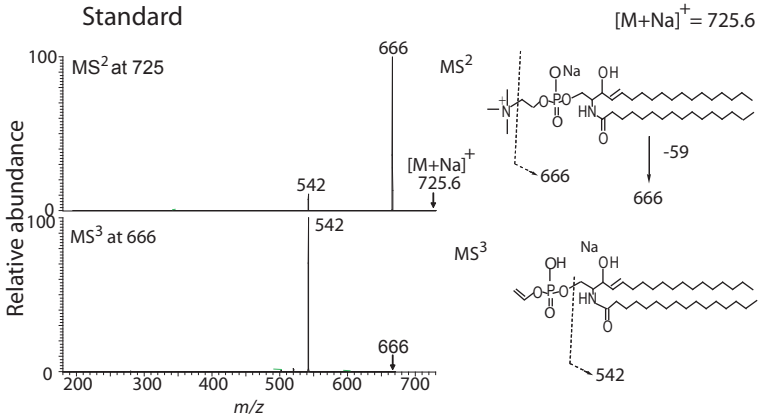
Positive mode analysis of MS^2 spectra of (top) m/z= 881.7, detected in CD1a eluates and (bottom) triacylglycerol standard, m/z = 907.7



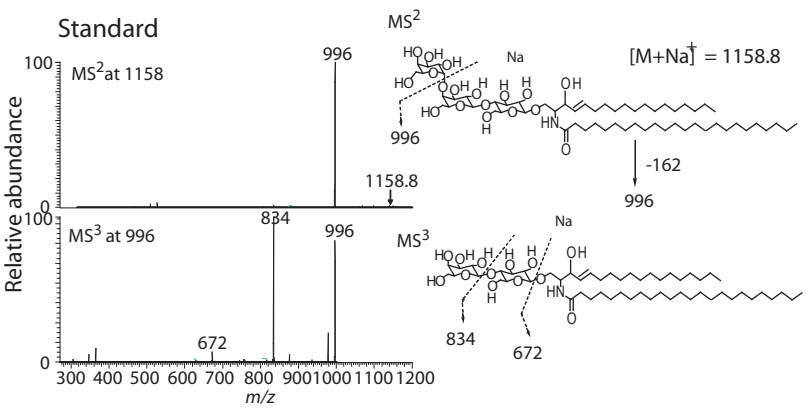
Positive mode analysis of $MS^2$and MS^3 spectra of (top) m/z= 782.6, detected in CD1a eluates and (bottom) phosphatidylcholine standard, m/z = 812.6





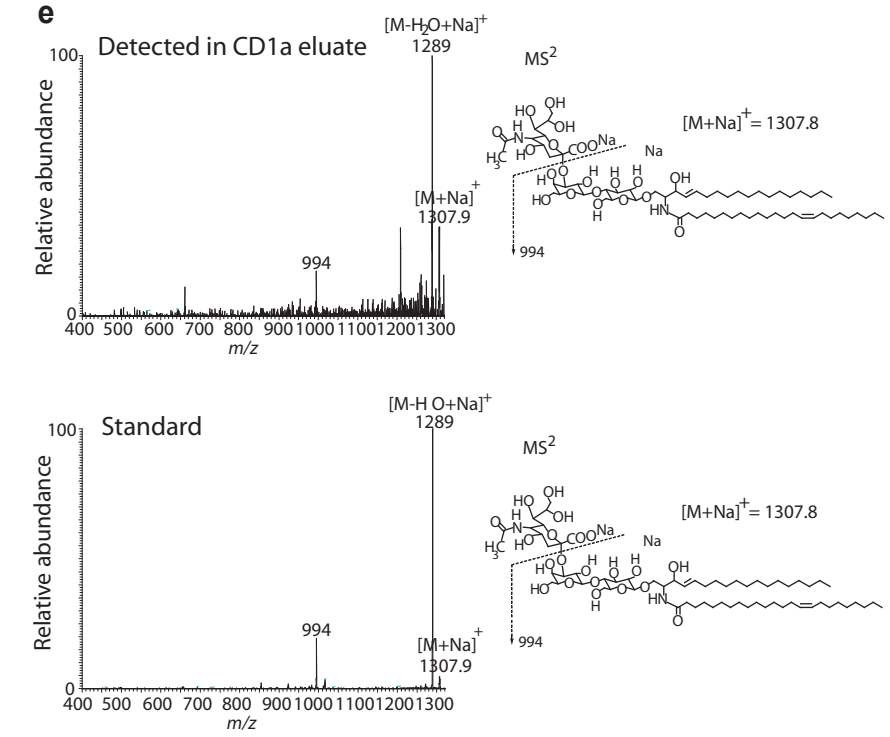


Positive mode analysis of $MS^2$and MS^3 spectra of (top) m/z= 835.6, detected in CD1a eluates and (bottom) sphingomyelin standard, m/z = 725.6

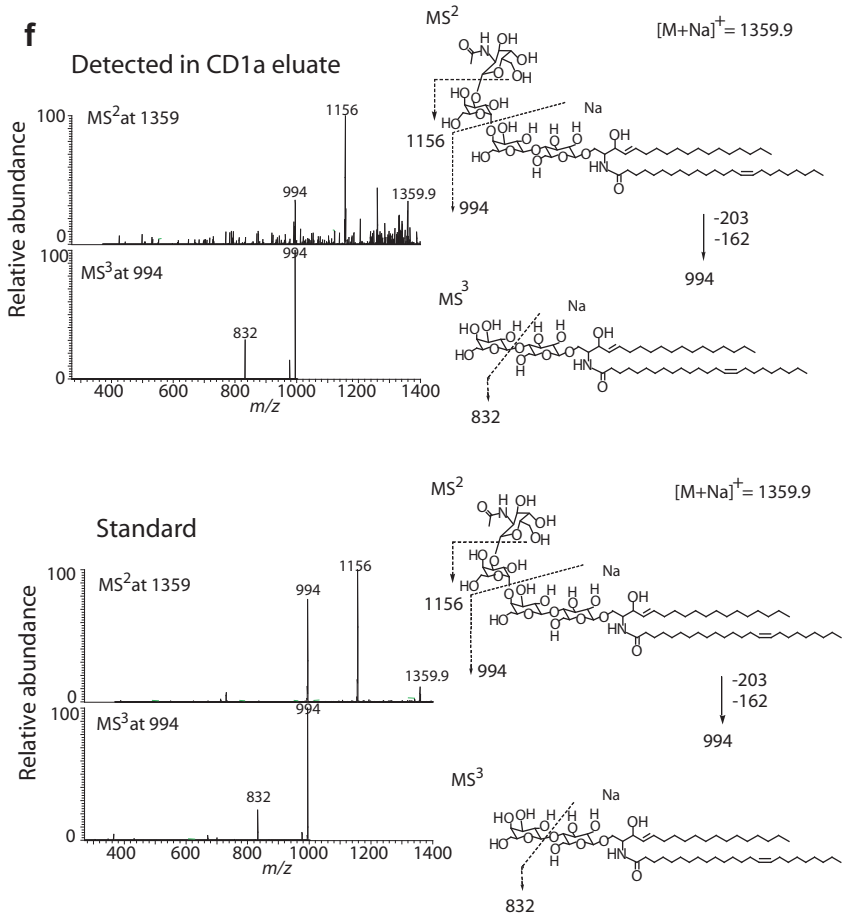


Positive mode analysis of $MS^2$and MS^3 spectra of (top) m/z= 1158.8, detected in CD1a eluates and (bottom) trihexosylceramide standard, m/z = 1158.8

Supplementary Figure 2 Identification of lipids eluted from recombinant CD1a proteins by collision induced dissociation mass spectrometry and comparison with lipid standards (positive mode analysis)

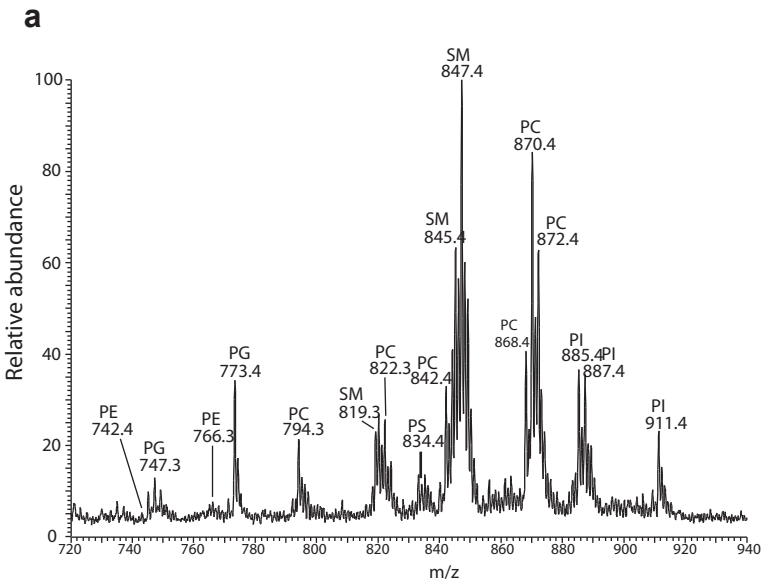


Positive mode analysis of MS^2 spectra of (top) m/z= 1307.8, detected in CD1a eluates and (bottom) monosialoganglioside GM3 standard, m/z = 1307.8

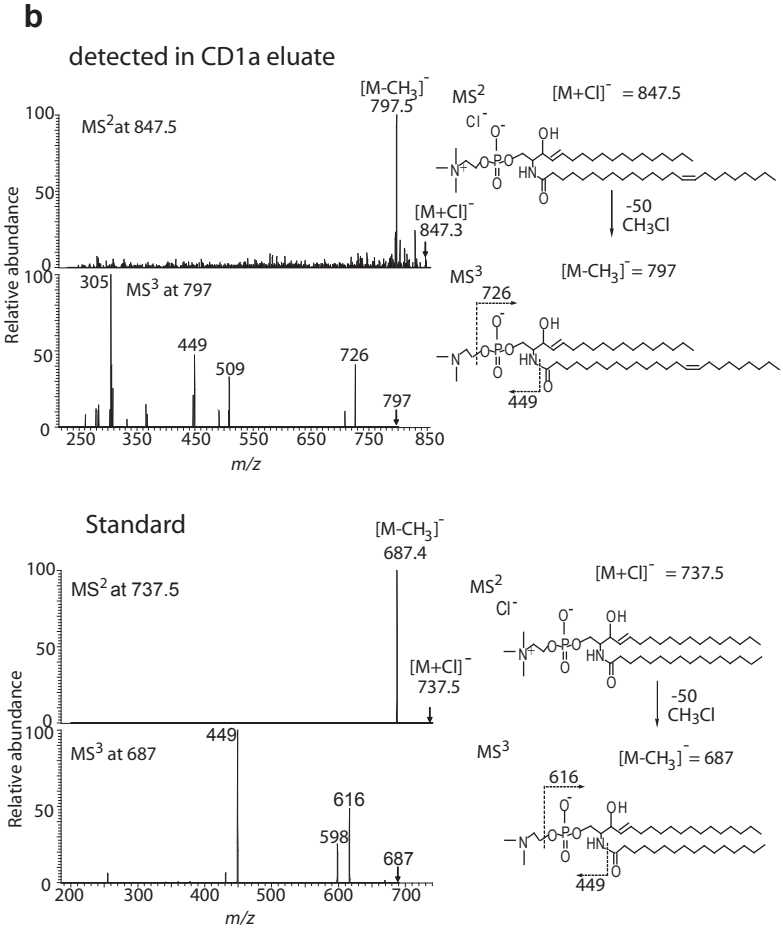


Positive mode analysis of MS^2 and MS^{3}spectra of (top) m/z= 1359.9, detected in CD1a eluates and (bottom) globotetrahexosylceramide (Gb4) standard, m/z = 1359.9

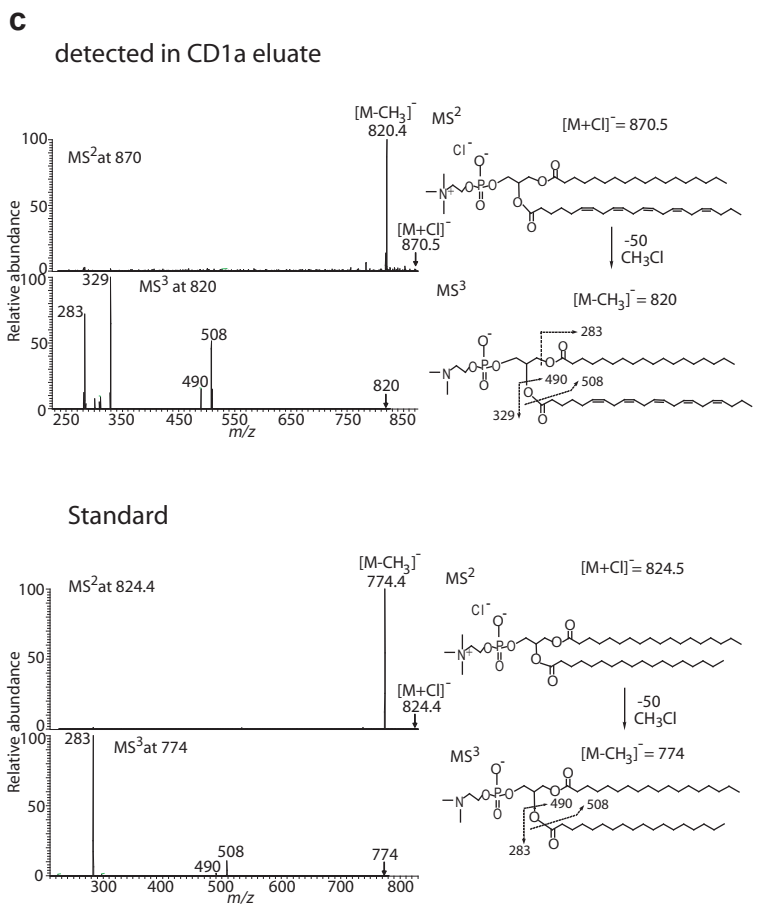
Supplementary Figure 2 Identification of lipids eluted from recombinant CD1a proteins by collision induced dissociation mass spectrometry and comparison with lipid standards (positive mode analysis)



Negative-mode electrospray ionization mass spectrometry of CD1a eluate.

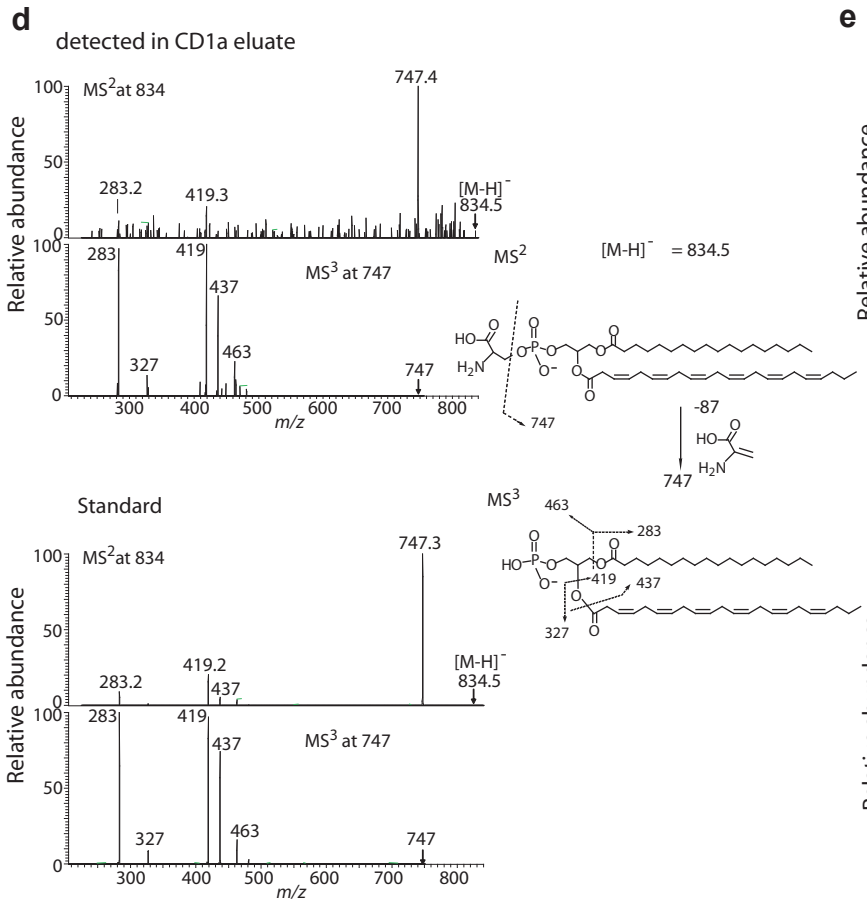


Negative mode analysis of $MS^2$and MS^3 spectra of (top) m/z= 847.3, detected in CD1a eluates and (bottom) sphingomyelin standard, m/z = 737.5

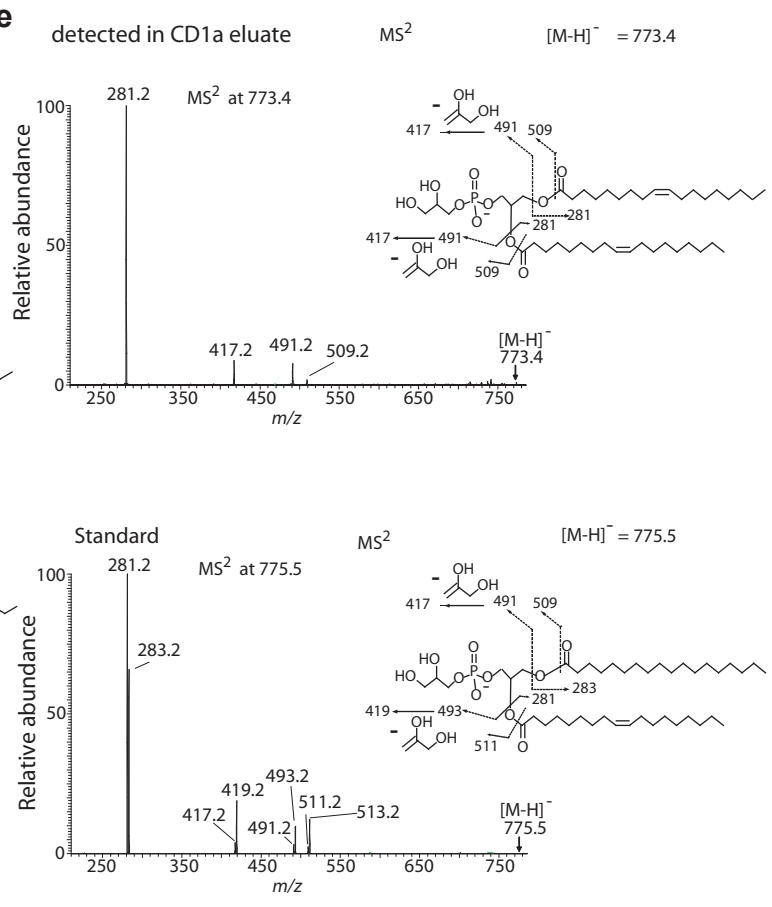


Negative mode analysis of $MS^2$and MS^3 spectra of (top) m/z= 870.5, detected in CD1a eluates and (bottom) phosphatidylcholine standard, m/z = 824.4

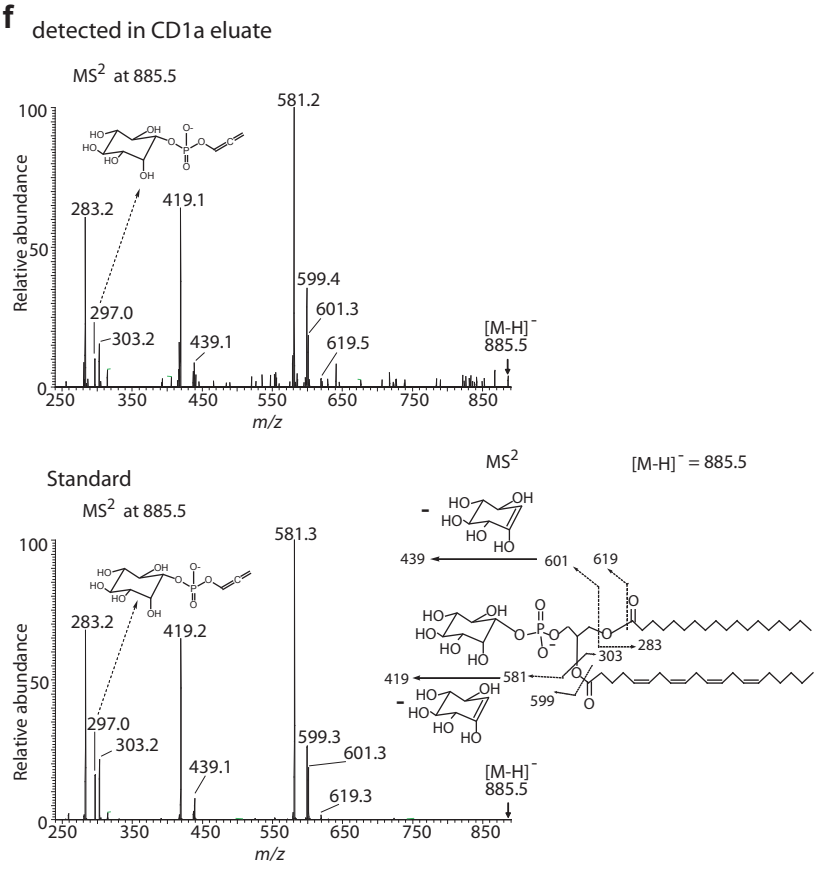
Supplementary Figure 3 Identification of lipids eluted from recombinant CD1a proteins by collision induced dissociation mass spectrometry and comparison with lipid standards (negative mode analysis)



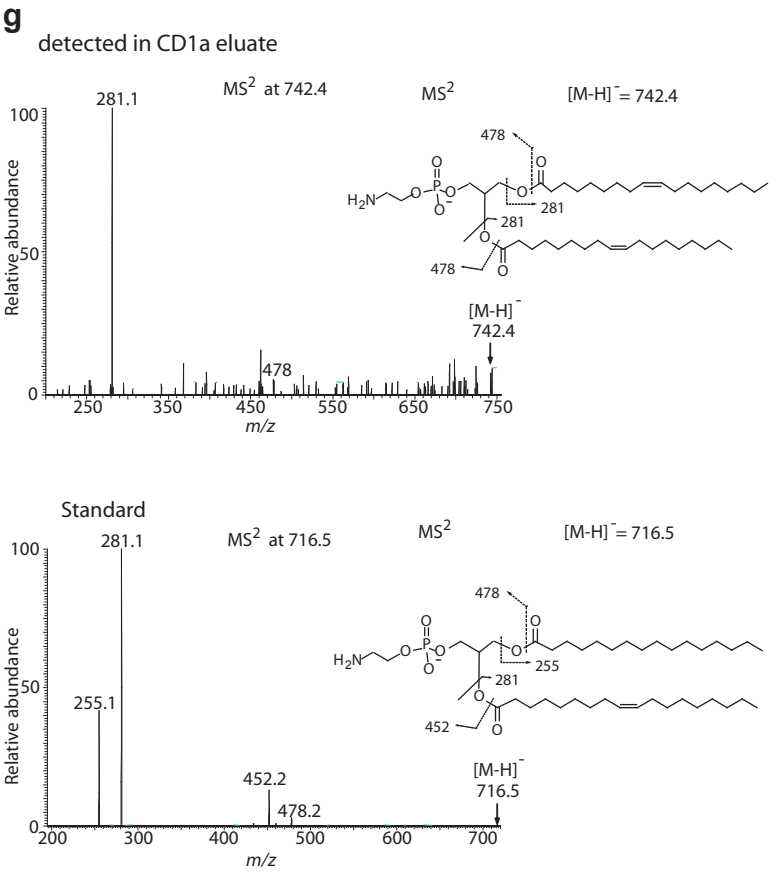
Negative mode analysis of $MS^2$and MS^3 spectra of (top) m/z= 834.5, detected in CD1a eluates and (bottom)phosphatidylserine standard, m/z = 834.5



Negative mode analysis of MS^2 spectra of (top) m/z= 773.4, detected in CD1a eluates and (bottom) phosphatidylglycerol standard, m/z = 775.5

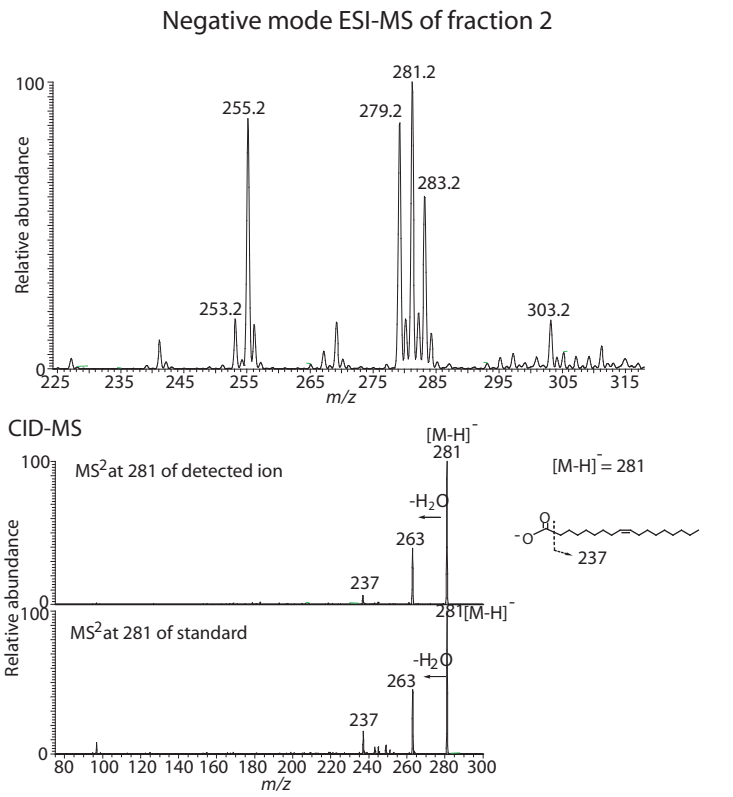


Negative mode analysis of MS^2 spectra of (top) m/z= 885.5, detected in CD1a eluates and (bottom) phosphatidylinositol standard, m/z = 885.5

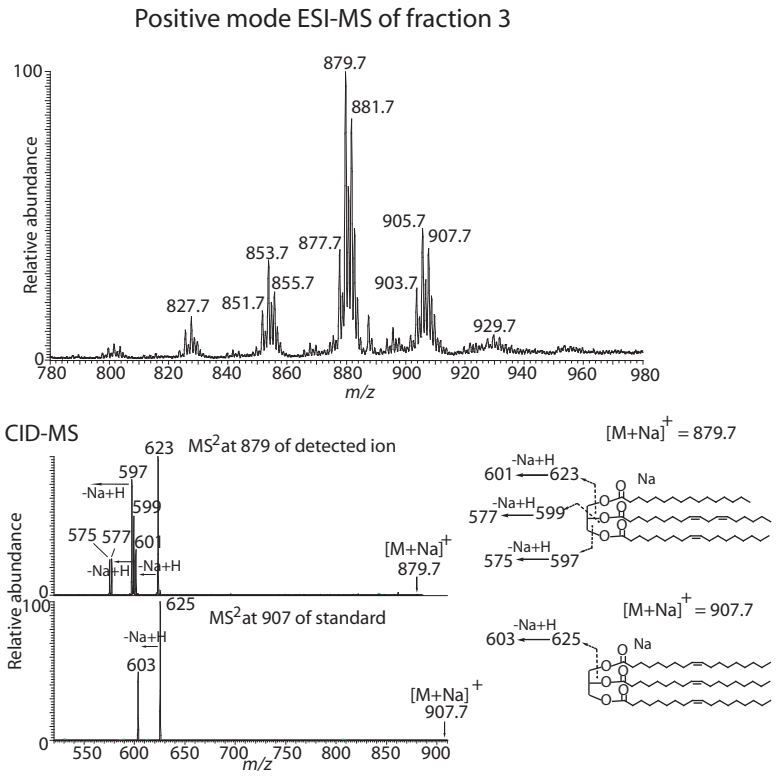


Negative mode analysis of MS^2 spectra of (top) m/z= 742.4, detected in CD1a eluates and (bottom) phosphatidylethanolamine standard, m/z = 716.5

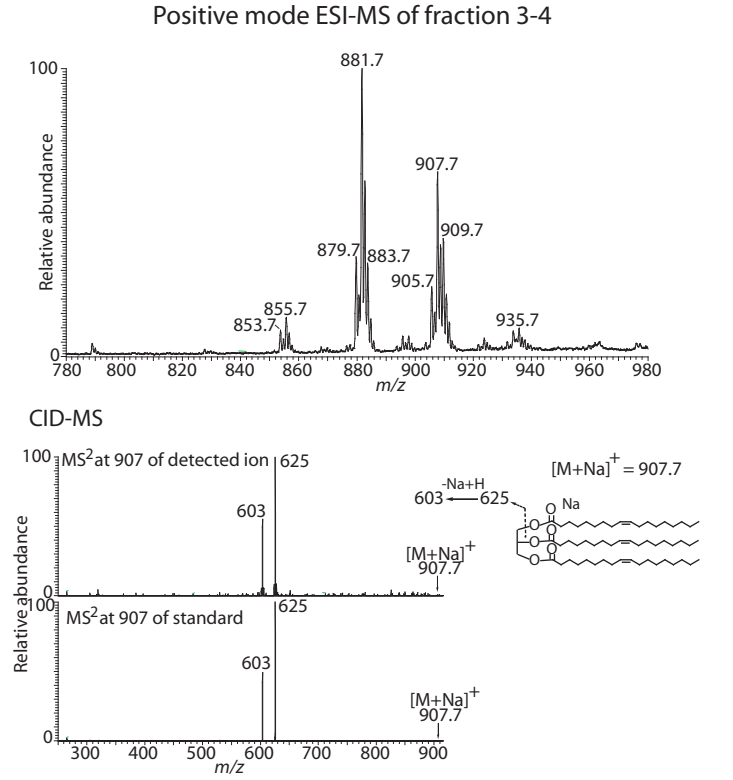
Supplementary Figure 3 Identification of lipids eluted from recombinant CD1a proteins by collision induced dissociation mass spectrometry and comparison with lipid standards (negative mode analysis)



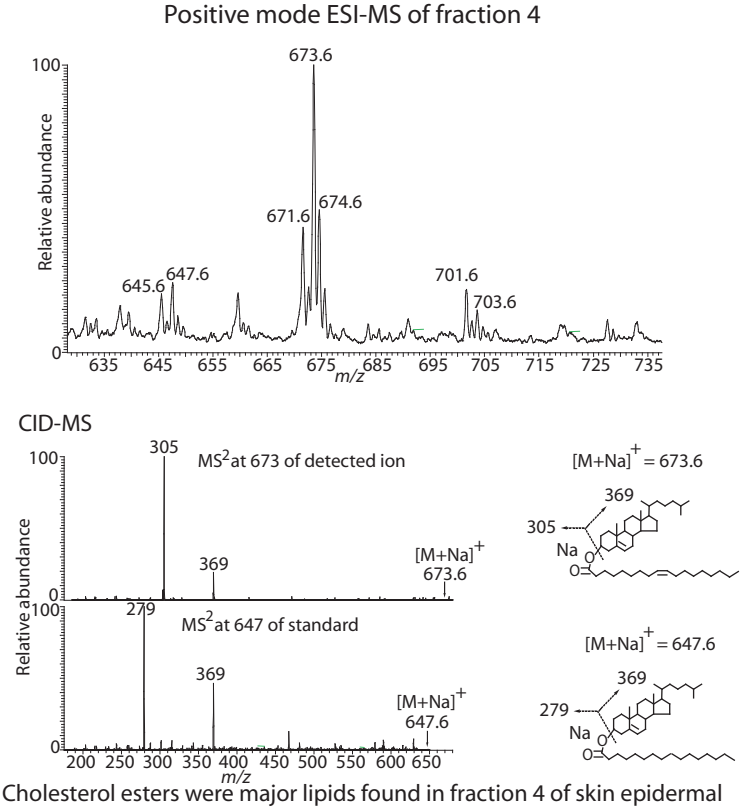
Fatty acids were major lipids found in fraction 2 of skin epidermal lipids purified by 1D TLC. (Top) Negative mode EIC-MS detected ions at m/z 255.2, 279.2, 281.2, 283.2 etc., correspond to masses of C16:0, C18:2, C18:1, and C18:0 fatty acid, respectively. (Bottom) CID-MS of m/z=281.2 (detected in fraction 2) vs. m/z=281.2 (C18:1 fatty acid standard)



Triacylglycerols were major lipids found in fraction 3 of skin epidermal lipids purified by 1D TLC. (Top) Positive mode EIC-MS detected ions at m/z 853.7, 879.7, 881.7, 905.7 etc., correspond to homologous series of triacylglycerol. (Bottom) CID-MS of m/z=879.7 (detected in faction 3) vs. m/z= 907.7 (triacylglycerol standard)



Triacylglycerols were major lipids found in fraction 3-4 of skin epidermal lipids purified by 1D TLC. (Top) Positive mode EIC-MS detected ions at m/z 855.7, 879.7, 881.7, 907.7 etc., correspond to homologous series of triacylglycerol. (Bottom) CID-MS of m/z=907.7 (detected in faction 3-4) vs. m/z= 907.7 (triacylglycerol standard)



Cholesterol esters were major lipids found in fraction 4 of skin epidermal lipids purified by 1D TLC. (Top) Positive mode EIC-MS detected ions at m/z 647.7, 671.6, 673.6, 701.6 etc., correspond to homologous series of cholesterol ester. (Bottom) CID-MS of m/z=673.6 (detected in faction 4) vs. m/z= 647.6 (cholesterol ester standard)

Supplementary Figure 4 Eletron spray Ionization mass spectrometry of lipids eluted from silica fraction of TLC plate. Comparison of collision induced dissociation mass spectrometry profiles with lipid standards

	TRAV	TRAJ	CDR3α	TRBV	TRBD	TRBJ	CDR3β
BC2	26-1	34	CIVPPPDKLIF	29-1	1	1-2	CSVEDIGQGAFDYGYTF
Bgp	21	33	CAVLHSNYQLIW	11-3	1	2-2	CASSLDLGVLDEGTGELFF

Supplementary table 5 T cell receptors of CD1a autoreactive T cell clones
To determine the sequence of the BC2 TCR α and β chains, PCR was performed using the following primers: TRAV26-1LongF: atgaggctggtggcaagag; TRBV29LongF: catctctcaaaagccaagcagg; CalphaShortRev: catgtctagcacagttttg; CbetaConstRevForSeq: ggtggcagacaggacccttgc.
PCR products were sequenced from both sides using the PCR primers.
To determine the sequence of the Bgp TCR α and β chain, a published method was used, based on circularization of double stranded cDNA and amplification using constant region primers (Uematsu, Y. A novel and rapid cloning method for the T cell receptor variable region sequences. 1991, Immunogenetics)
Genbank: TCR α and β chain sequences of BC2 and Bgp clones: KF751594, KF751595, KF751596, KF751597