SUPPLEMENTAL DATA

The Precise Structures and Stereochemistry of Trihydroxy-Linoleates Esterified in Human and Porcine Epidermis and Their Significance in Skin Barrier Function: IMPLICATION OF AN EPOXIDE HYDROLASE IN THE TRANSFORMATIONS OF LINOLEATE

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Fig. S1. Analytical procedures for the identification of free and esterified products in epidermis

Fig. S2. Analytical procedures for the quantitation of free and esterified 9-HODE, epoxyalcohol (EpOH) and triols in epidermis.

Fig. S3 LC-MS analysis of the free (non-esterified) 9-HODE in human epidermis

Fig. S4 Chiral analysis of 9-HODE in human epidermis

Fig. S5 LC-MS analysis of the free (non-esterified) linoleate epoxyalcohol in human epidermis

Fig. S6 Chiral column LC-MS analysis of the stereochemistry of the major epoxyalcohol esterified in human epidermis

Fig. S7 LC-MS analysis of the free (non-esterified) linoleate triol in human epidermis

Fig. S8 The profile of protein-bound triols in pig epidermis

Figure S1. **Analysis of free and esterified products in epidermis**
Purification scheme for analysis of triols in human and pig epidermis. After extraction from epidermis using $CHCl_3$/MeOH and open-bed silica fractionation, separate aliquots were analyzed for free triols (left-hand arm) and esterified triols (middle and right-hand arms). Esterified triols were released by alkaline hydrolysis overnight. After Bligh & Dyer partitioning to remove neutral and non-polar lipids in the $CHCl_3$ phase, samples were either extracted on an Oasis HLB cartridge at pH 6 (to avoid hydrolysis of epoxyalcohols to triols), or after standing at room temperature at pH 3 for 30 min to allow acid hydrolysis of epoxyalcohols. The recovered triols were converted to the PFB DMP derivative and analyzed by HPLC, GC-MS and LC-MS.

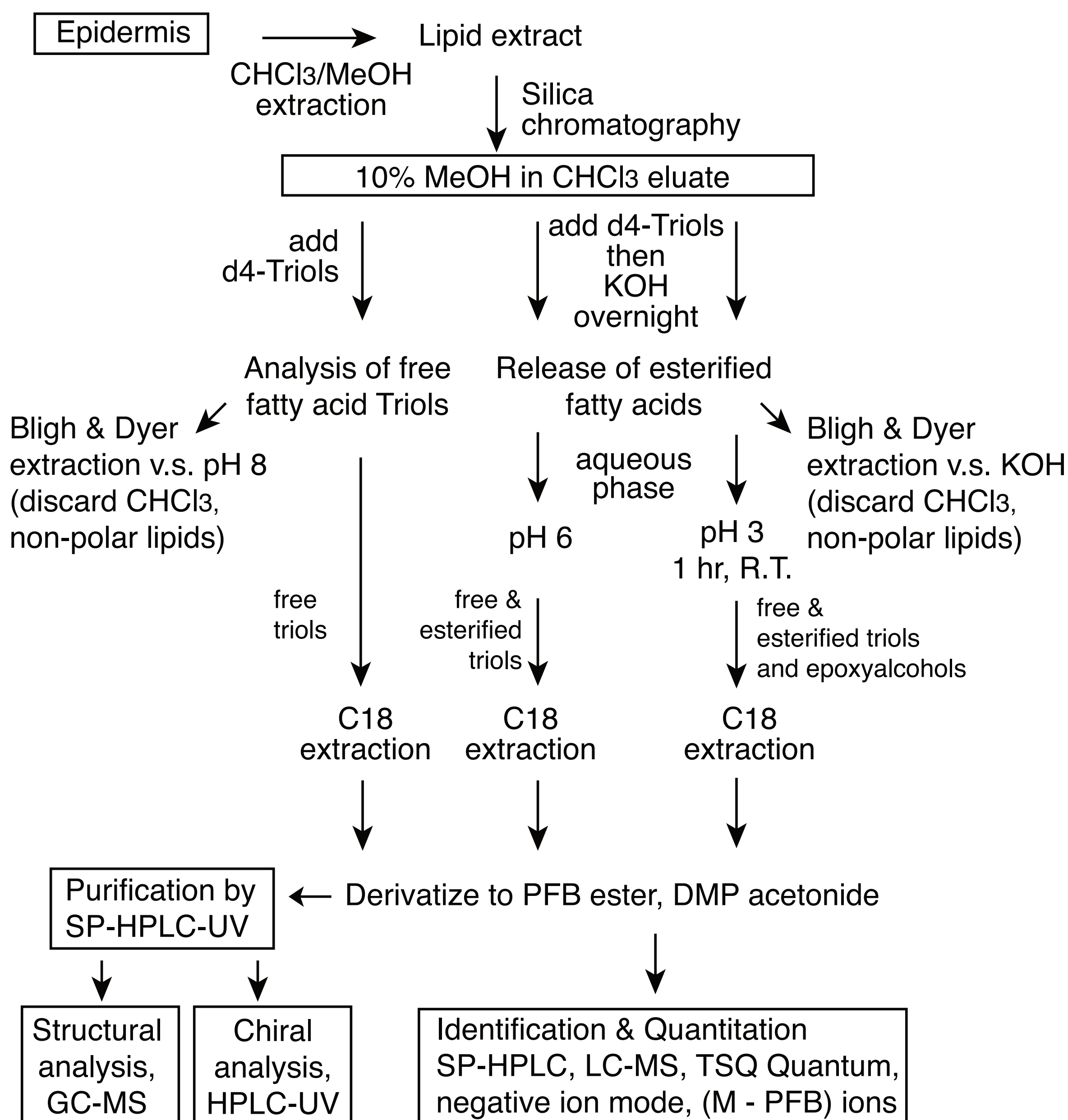

Figure S2. Quantitation of free and esterified 9-HODE, epoxyalcohol (EpOH) and triols in epidermis.
After the initial extraction and open-bed silica column, free fatty acid products are taken through the left-hand arm of the scheme; Bligh and Dyer partition using 0.1 M K_2HPO_4 as the aqueous phase allows removal of neutral and non-polar lipids in the $CHCl_3$ while retaining the ionized fatty acids in the aqueous layer; the free acids in the mildly alkaline solution are subsequently retained on an Oasis HLB cartridge, eluted with EtOAc and derivatized for LC-MS analyses. The right-hand arm follows a similar protocol after esterified products are released by KOH treatment overnight.

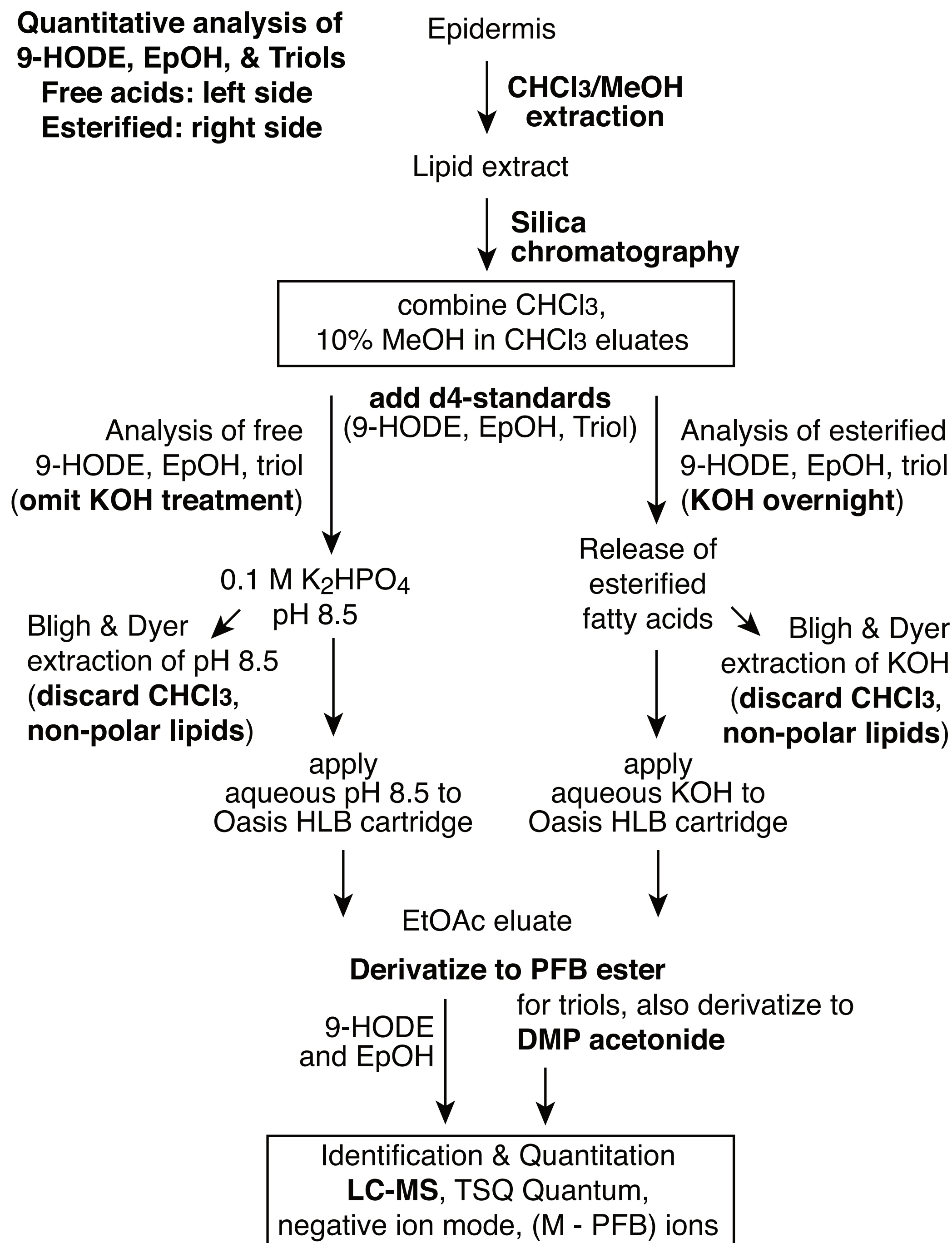

Fig S3: LC-MS analysis of the free (non-esterified) 9-HODE in human epidermis
The analyses used a Thomson Scientific Advantage 5µ silica column (25 x 0.46 cm), with a solvent of solvent hexane/IPA (100:0.5 v/v), flow rate 1 ml/min, and LC-MS detection of the PFB ester (M – PFB ion) at m/z 295 (do) and 299 (d4). The early peak at ~6.6 min in the m/z 295 channel (d0) is 13-HODE.
The chromatogram is representative of the epidermis from three different subjects.

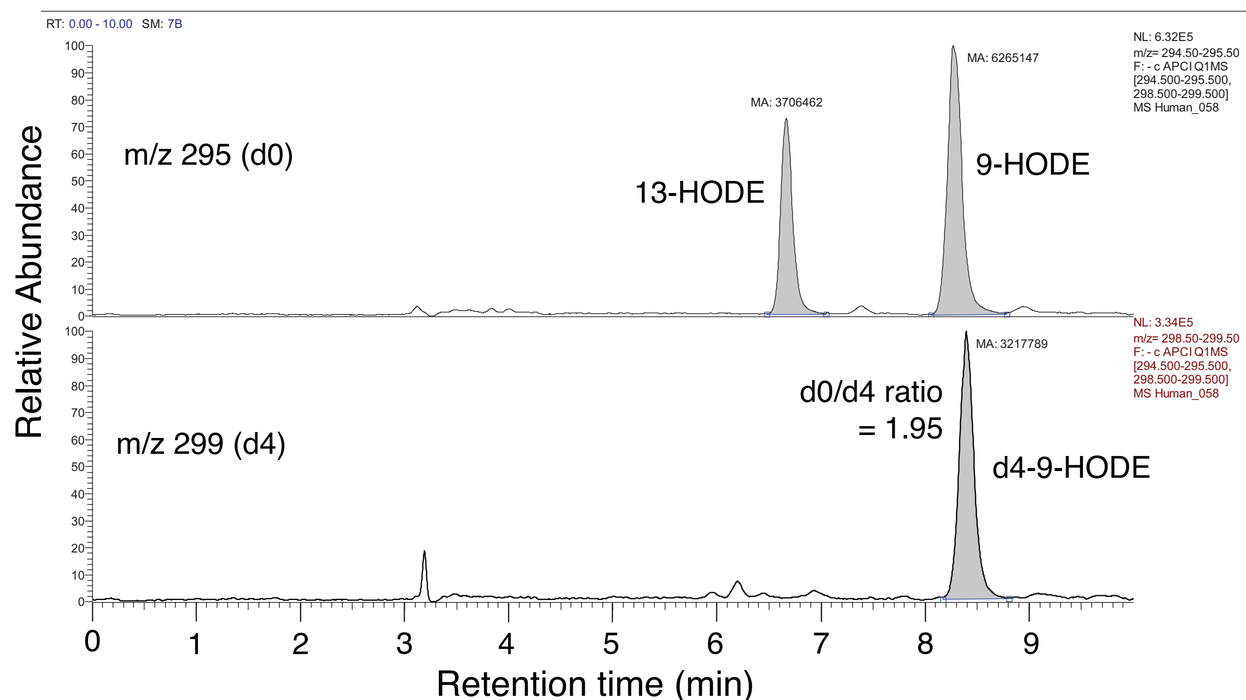

Fig. S4: Chiral HPLC analysis of 9-HODE from human epidermis
The analyses used a Chiralcel OD-H column (25 x 0.46 cm), a solvent of hexane/IPA (100:5 v/v) and a flow rate of 1 ml/min with UV-detection of the conjugated diene chromophore at 235 nm. Epidermis from three different individuals were analyzed: the percentage of 9*R* enantiomer was 79.8%, 85.5% (shown above), and 85.7%.
The analyses were conducted on the total 9-HODE (free plus esterified).

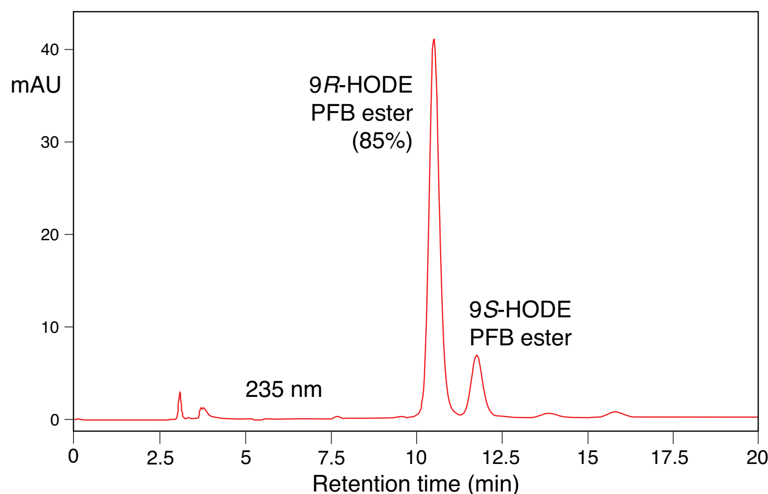

Fig. S5: LC-MS analysis of the free (non-esterified) epoxyalcohol in human epidermis

The ratio of d0/d4 epoxyalcohol in this analysis is 0.0025, corresponding to only ~2% of the esterified epoxyalcohol.
(By comparison, the d0/d4 ratio measured for esterified epoxyalcohol is approximately 0.5, Fig. 10B).

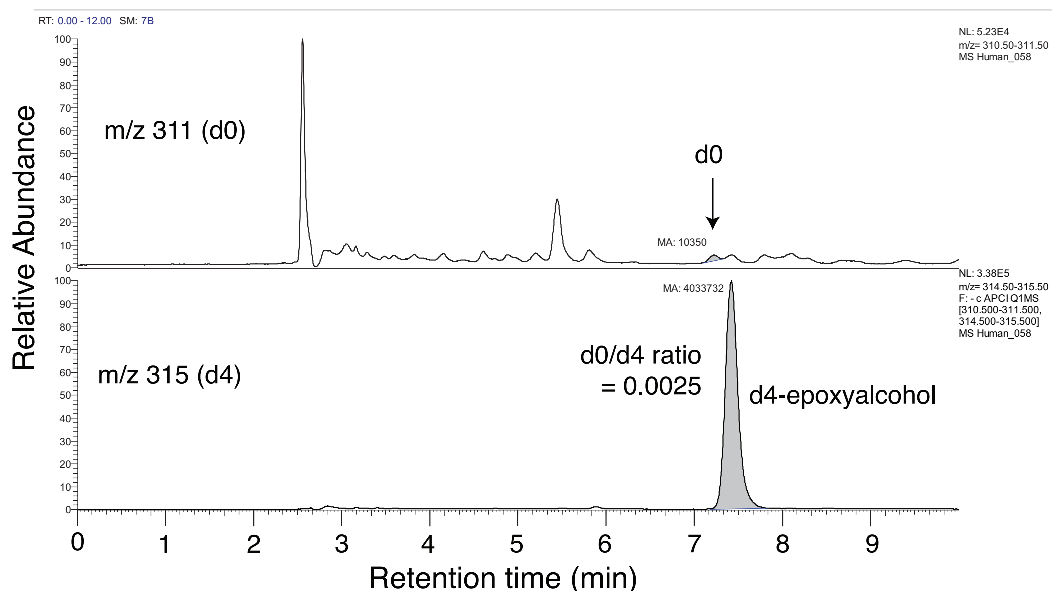

Fig S6: Chiral column LC-MS analysis of the chirality of the major epoxyalcohol esterified in human epidermis as 9*R*,10*R*-trans-epoxy-13*R*-hydroxy-octadec-11*E*-enoate.
The analysis used a Chiralcel OJ column (25 x 0.46 cm), a solvent of hexane/IPA (100:10 v/v), a flow rate 1 ml/min, with LC-MS detection of PFB ester monitoring the [M – PFB]- ion at m/z 311.
Epidermis from three different individuals were analyzed: the percentage of *RRR* enantiomer was 88%, 97% (shown above), and 98%.

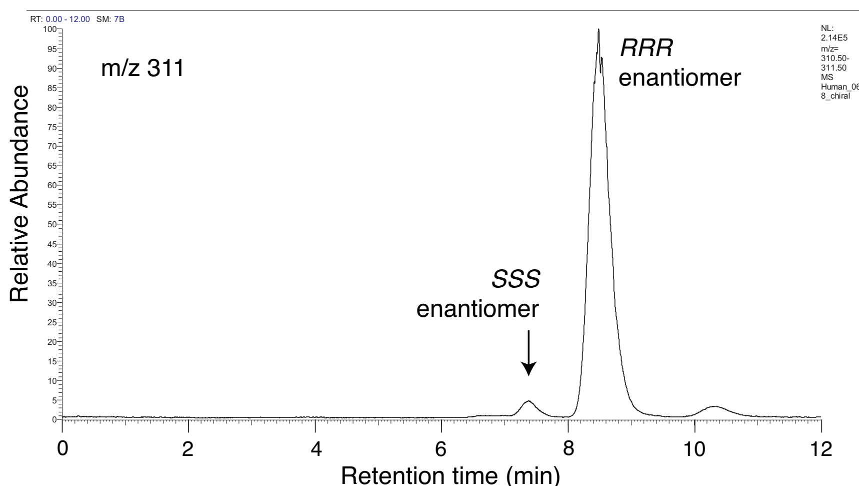

Fig. S7 LC-MS analysis of the free (non-esterified) linoleate triols in human epidermis

The samples were analyzed for free (non-esterified) linoleate triols using the method summarized in Fig 2B of the main text. Detection of the PFB ester, DMP derivative for unlabeled triols (m/z 369) and the d4-triol-3 and d4-triol-4 detected at m/z 373.

(By comparison, the d0/d4 ratio measured for esterified triol-3 is approximately 220 parts per thousand, Fig. 10C).

The peak at ~ 7.7 min below in the d0 channel is a contaminant of pinellic acid (triol-2) used to check the derivatization.

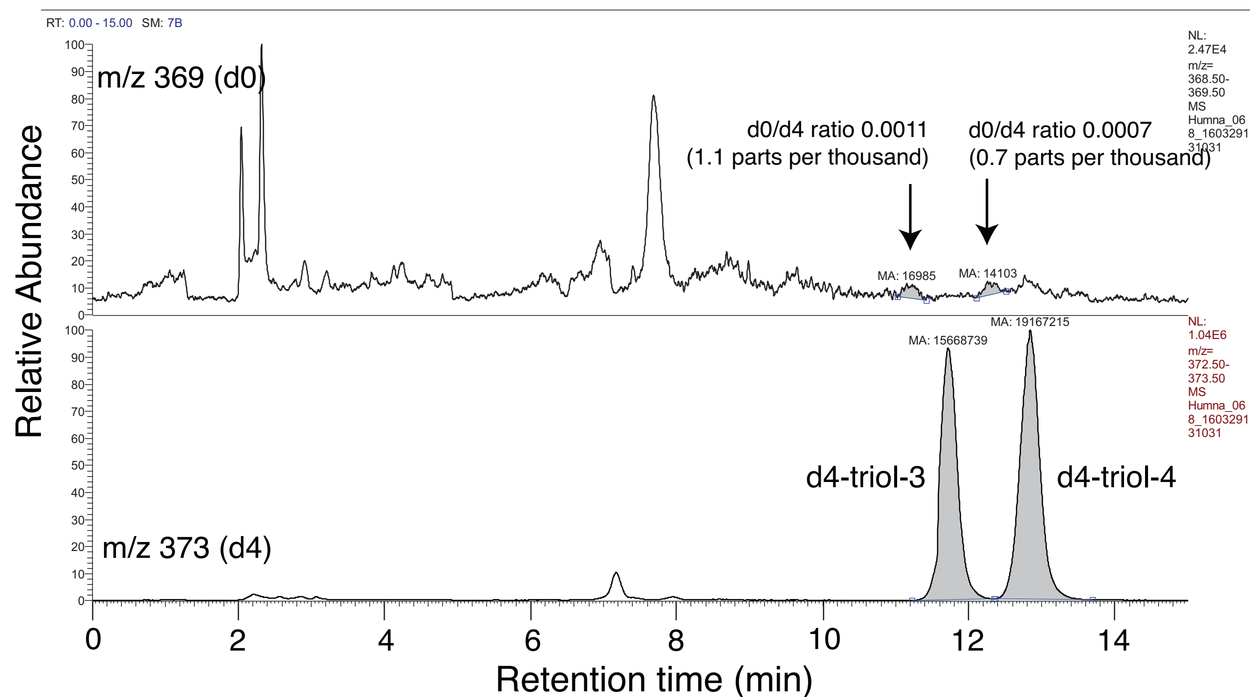

Fig. S8 (A, top) The profile of protein-bound triols in pig epidermis compared with $CHCl_3$/MeOH soluble esterified triols (B, below).

Normal-phase LC-MS analysis of the PFB ester DMP acetonide derivative using a Thomson Advantage 5 μ silica column with a solvent of hexane/IPA (100:1, v/v), a flow rate of 1 ml/min with monitoring of the $[M – PFB]^-$ ion at m/z 369.

The numbers correspond to the linoleate triols shown in Fig. 3 of the main text. The intensity of the major esterified triol-3 (lower panel) was over 10-fold higher than the corresponding protein-bound triol.

We conclude that non-specific oxidation accounts for these comparatively low levels of protein bound triols and that coupling of EOS-triol to protein (the a hydroxyl on the triol moiety) is of minor consequence compared to protein binding via the omega-OH of ceramide OS.