SUPPLEMENTAL MATERIAL

Serhan et al., http://www.jem.org/cgi/content/full/jem.20081880/DC1

Human MΦ incubations. Human peripheral blood monocytes were isolated from healthy donors by positive selection using CD14 microbeads and a MACS column (Miltenyi Biotec). After isolation, the cells were plated in 10% FBS RPMI 1640 in the presence of 10 ng/ml GM-CSF for 7 d to allow for differentiation to mature MΦs. MΦs were then incubated with 5 μg 14-hydroperoxydocosahexaenoic acid or 5 μg DHA in the presence of 100 μg zymosan for 30 min at 37°C in $DPBS^{+/+}$. Incubations were terminated by the addition of 2 vol cold methanol, and the samples were taken for solid-phase extraction.

GC-MS analysis. GC-MS analysis was performed with an HP6890 system (Agilent Technologies) equipped with an HP5973 mass selective detector (Agilent Technologies). Individual trimethylsilyl derivatives were prepared after the isolated compounds were treated with diazomethane. The ionization voltage was 70 eV, and the ion source temperature was 230°C. An HP-5MS capillary column (30 mm × 0.25 mm × 0.25 μm; Agilent Technologies) was used with a temperature program; the initial temperature was 150°C for 2 min, ramped to 230°C for 8 min and 280°C for 10 min, and maintained at 280°C for 10 min with a helium flow rate of 1 ml/min.

Chiral HPLC-MS/MS analysis. Chiralpak AD-RH (150 × 2 × 5 μm; Chiral Technologies, Inc.) was connected to an Applied Biosystems 3200 QTRAP, pumped by an Agilent Technologies 1100 series HPLC system. The mobile phase of acetonitrile/water/acetic acid (70:30:0.01, vol/vol/vol) was eluted at a flow rate of 200 μl/min for 7 min, followed by a gradient to 100:0:0.01, which was applied for the next 5 min.

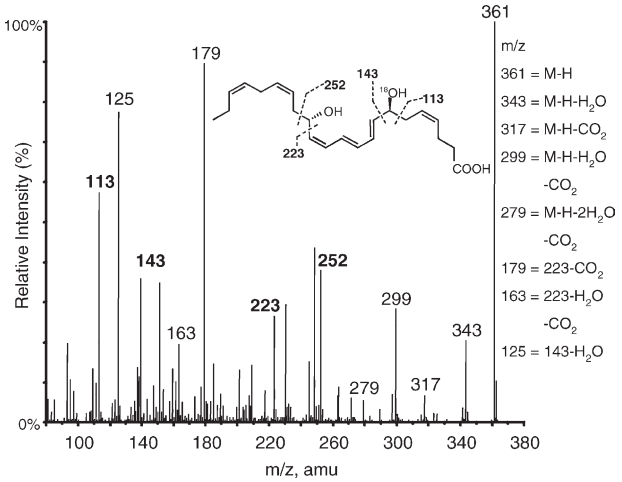

Figure S1. MS–MS spectrum of ^{18}O-containing product. ^{18}O incorporation was assessed with 0.45 ml $H_2{}^{18}O$ added to peritoneal macrophages (5×10^6 cells/incubation). Incorporation of ^{18}O in the carbon 7 position of MaR1 (Fig. 5 and see Results and discussion) provides evidence that 7 position alcohol was derived from $H_2{}^{18}O$ rather than molecular oxygen (O_2). If the 7 position alcohol was inserted via a double LOX mechanism, the alcohol at carbon 7 would have been obtained from O_2. Thus, in these experiments the MaR1 would not carry ^{18}O in the 7 position. amu, atomic mass unit.

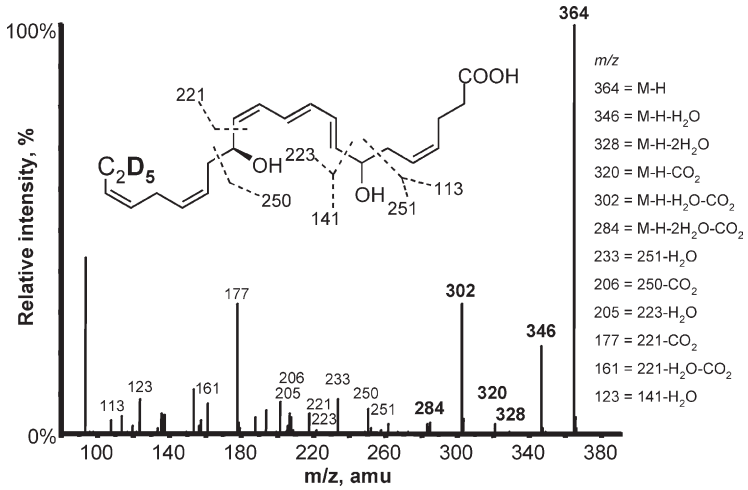

Figure S2. MS–MS spectrum of d_5-containing MaR1. Mouse MΦs (15.5×10^6 cells/3 ml) were incubated for 30 min at 37°C with zymosan and 10 µM of deuterium-labeled DHA-d_5. The five deuterium atoms were at positions C21, 21, 22, 22, and 22 of the omega end of the DHA precursor. amu, atomic mass unit.

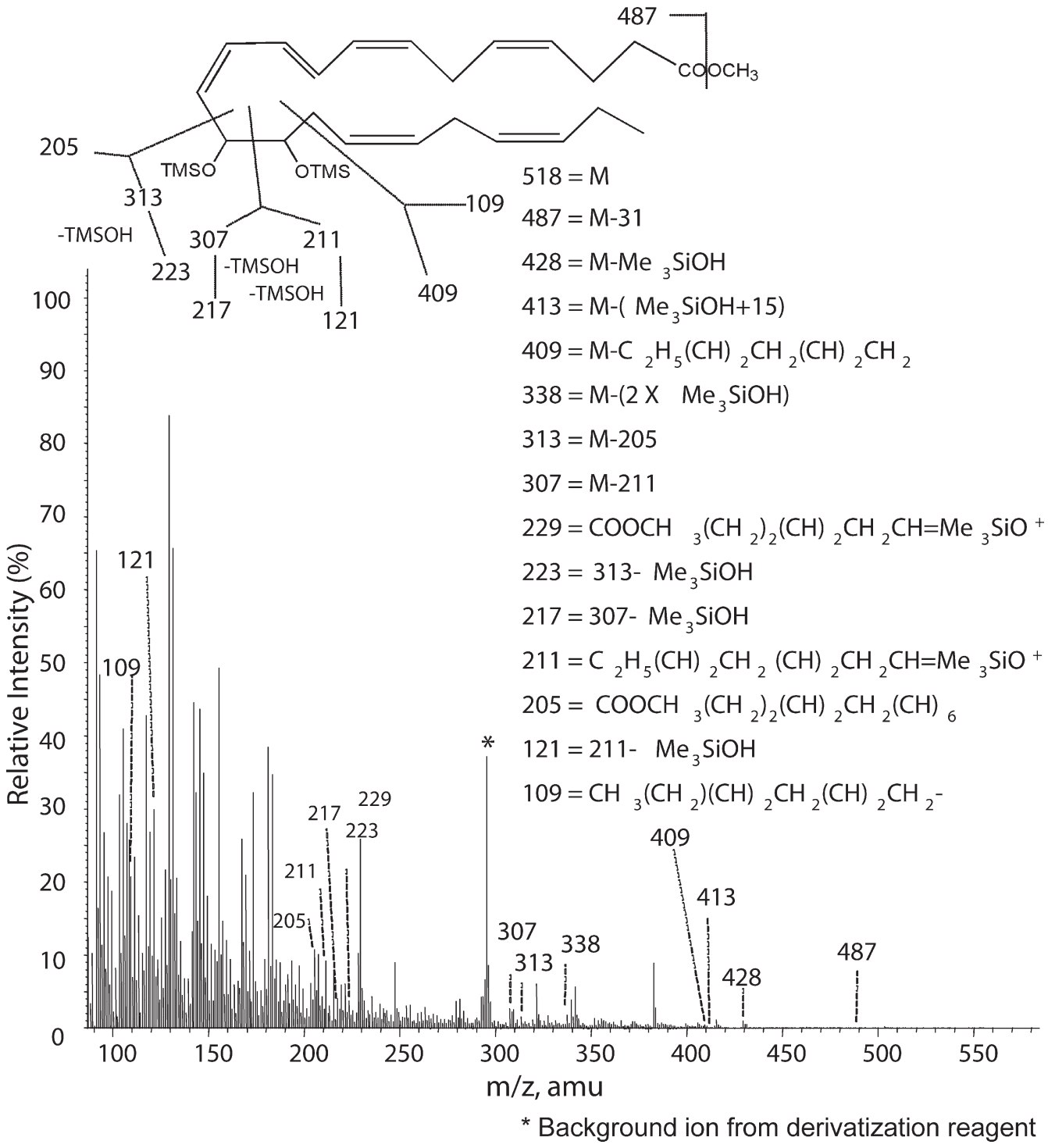

Figure S3. GC-MS spectrum of the 13,14-dihydroxy-containing product vicinal diol. GC-MS was performed with an HP6890 system equipped with an HP5973N mass detector using an HP-5MS column (see Table I). The isolated product was treated with diazomethane and the trimethylsilyl derivative was prepared. Identification of this vicinal diol from DHA and 14-hydroperoxydocosahexaenoic acid with MΦs provides evidence for an epoxide intermediate, which is also supported by identification of the epoxide trapping product and the results of ^{18}O incorporation (see Results and discussion). amu, atomic mass unit.