Supporting Information

Stereospecificity of Fatty Acid 2-Hydroxylase and Differential Functions of 2-Hydroxy Fatty Acid Enantiomers

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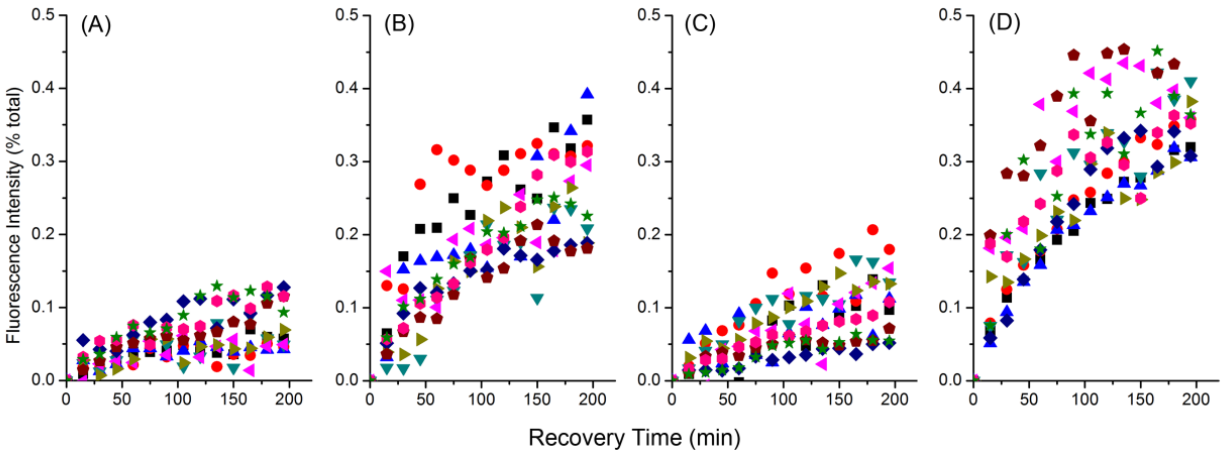

Figure S1: Stereospecific reverse effect on accelerated raft fluidity induced by FA2H depletion. 3T3-L1 adipocytes were treated with a negative control siRNA (A) or an siRNA recognizing FA2H (B-D). 24 h post tranfection, 50 μM (*R*)-2-OH palmitic acid (C) or (*S*)-2-OH palmitic acid (D) were added. Kinetics of recovery for Alexa 488-CTxB of each experiment was shown.

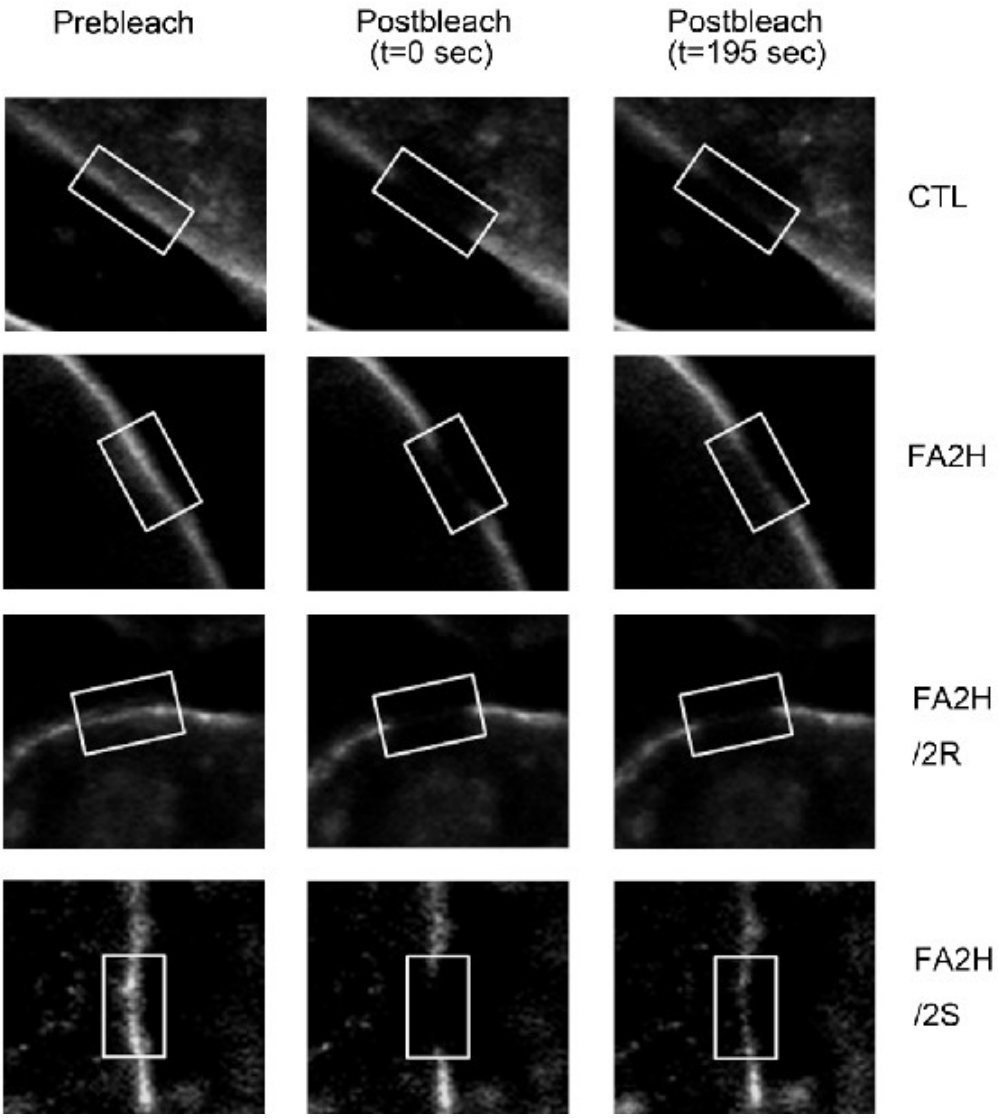

Figure S2: Amplified images of figure 3A.

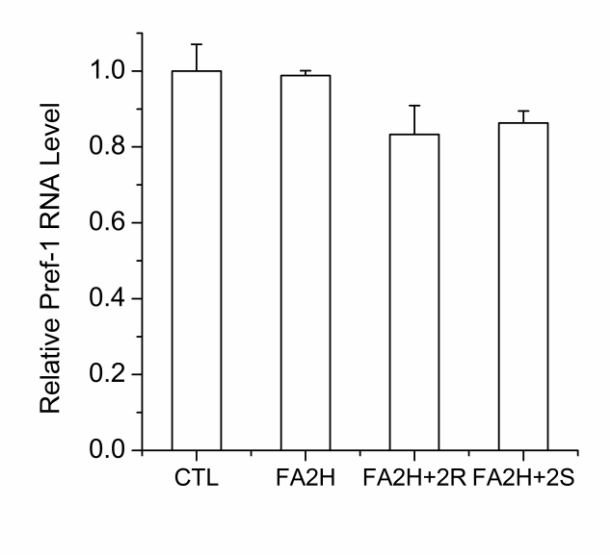

Figure S3: Pref-1 mRNA levels in FA2H knockdown adipocytes and in adipocytes treated with 2-OH PA enantiomers. 3T3-L1 adipocytes were treated with a negative control siRNA (CTL) or an siRNA recognizing FA2H (FA2H). 24 h post tranfection, 50 μM (R)-2-OH PA (2R) or (S)-2-OH PA (2S) were added as indicated. mRNA samples were prepared and the Pref-1 level was analyzed by RT-PCR. The data represent the means ± S.E. of three independent experiments.

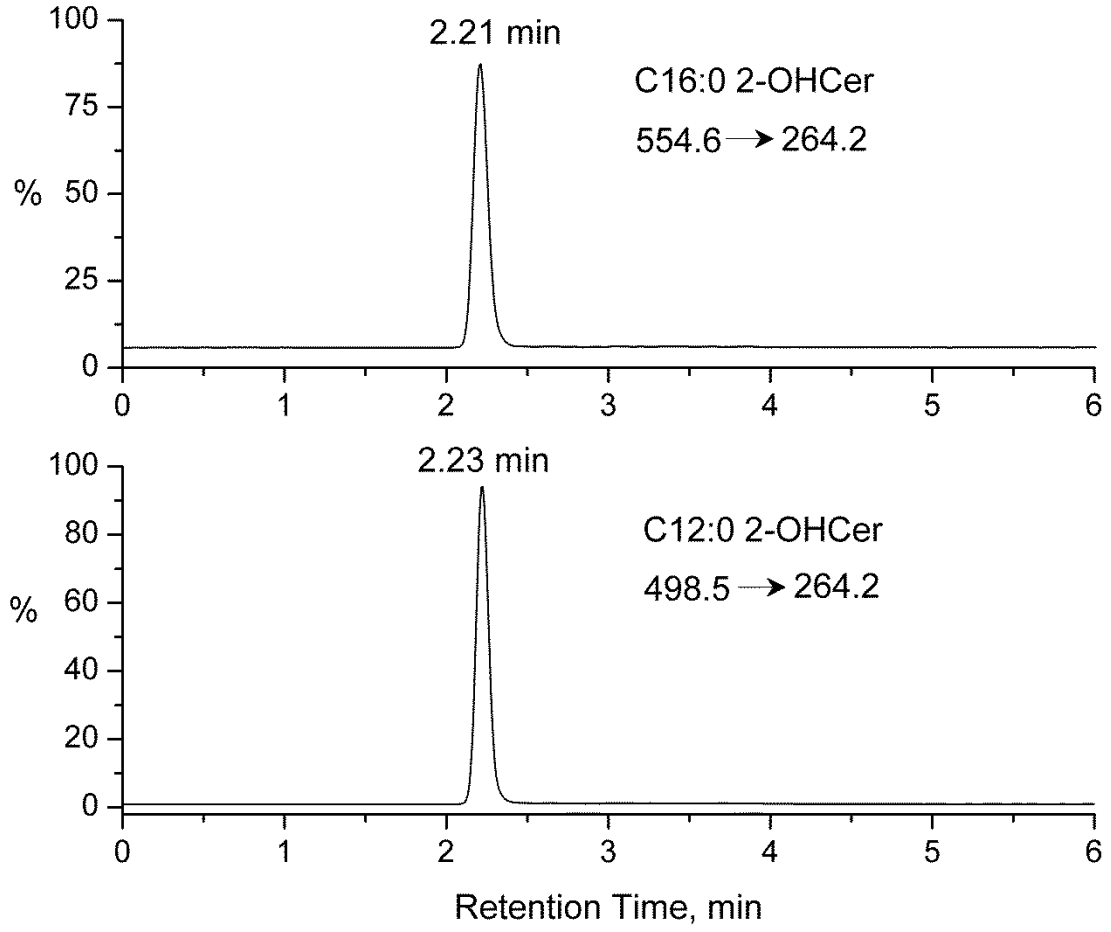

Figure S4: MRM chromatograms obtained from the analysis of C16:0 2-OHCer in adipocyte extracts (top) and authentic standard C12:0 2-OHCer (bottom) spiked in the lipid extracts.

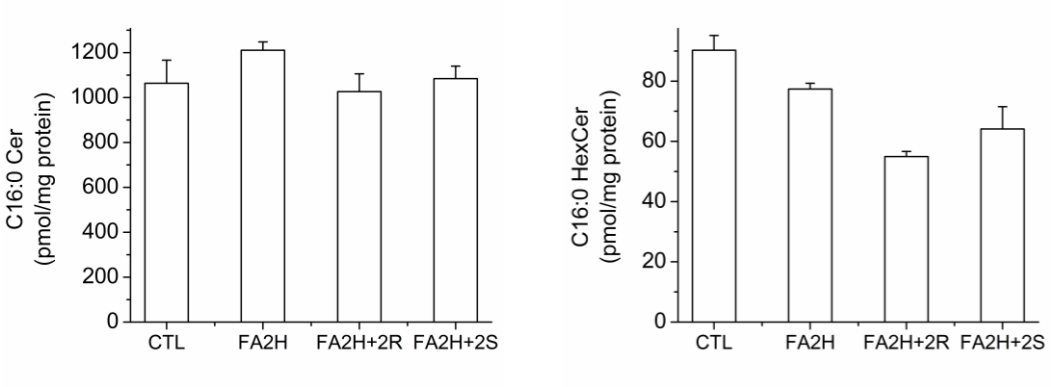

Figure S5: Regulation of C16:0 Cer and HexCer by FA2H and 2-OH PA enantiomers. 3T3-L1 adipocytes were treated with a negative control siRNA (CTL) or an siRNA recognizing FA2H (FA2H). 24 h post tranfection, 50 μM (*R*)-2-OH PA (2R) or (*S*)-2-OH PA (2S) were added as indicated. C16:0 Cer and C16:0 HexCer were quantified by LC ESI-MS/MS and results are the means ± S.E. of three independent experiments.

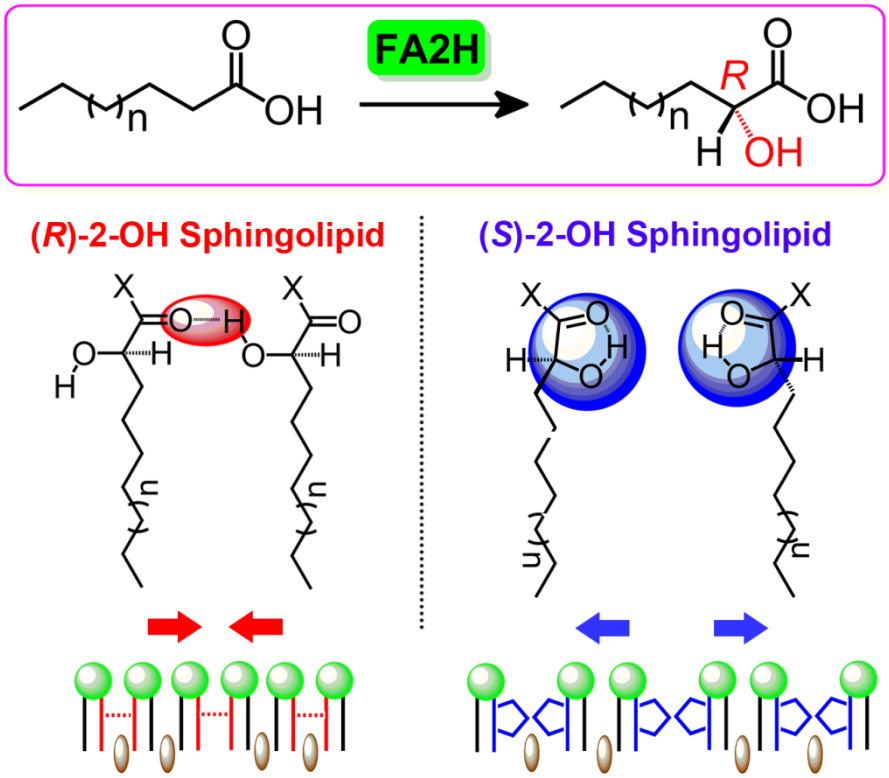

Figure S6. A proposed model for differential interaction of sphingolipids containing (*R*)-2-OH FA or (*S*)-2-OH FA. The (*R*)-2-hydroxyl group is preferred to participate in intermolecular hydrogen bonding, favoring lipid-lipid interaction whereas the 2-hydroxyl group of (*S*)-form is usually involved in an intramolecular hydrogen bonding with adjacent carbonyl oxygen. Accordingly, raft domains enriched in (*R*)-2-OH sphingolipids are more tightly packed and become less mobile, as compared to those containing more (*S*)-2-OH sphingolipids, where the steric hindrance caused by the branched loop of intramolecular hydrogen bond attenuates the lipid-lipid interaction and thus promotes membrane fluidity.