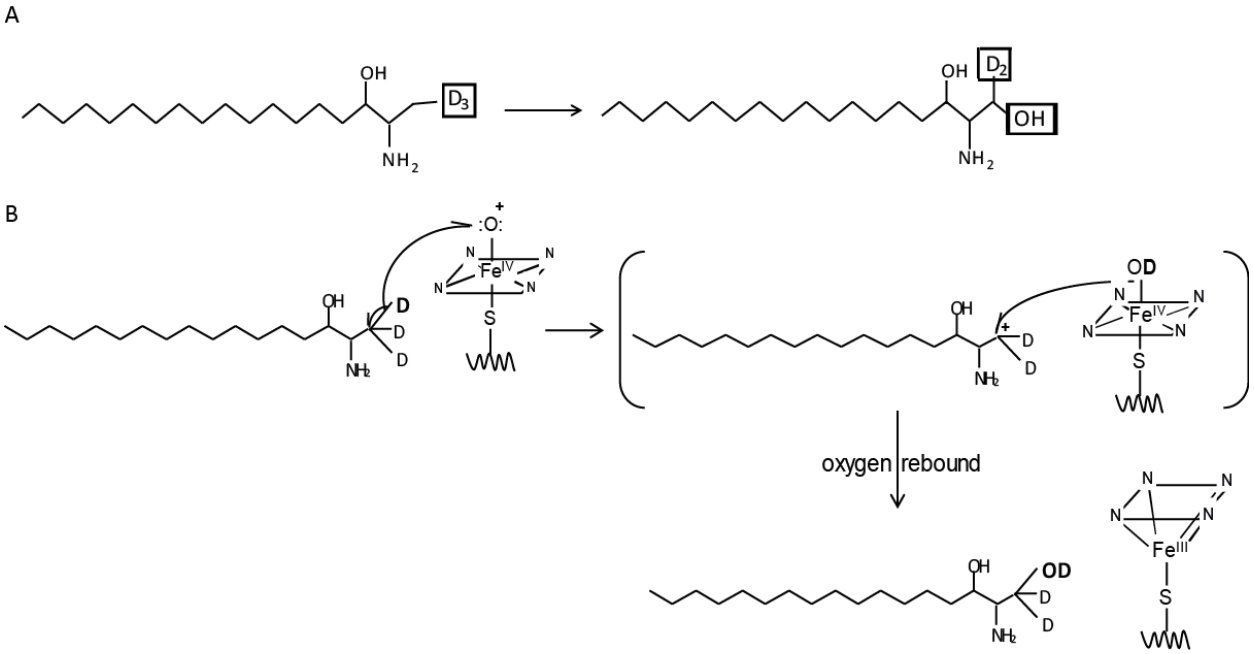
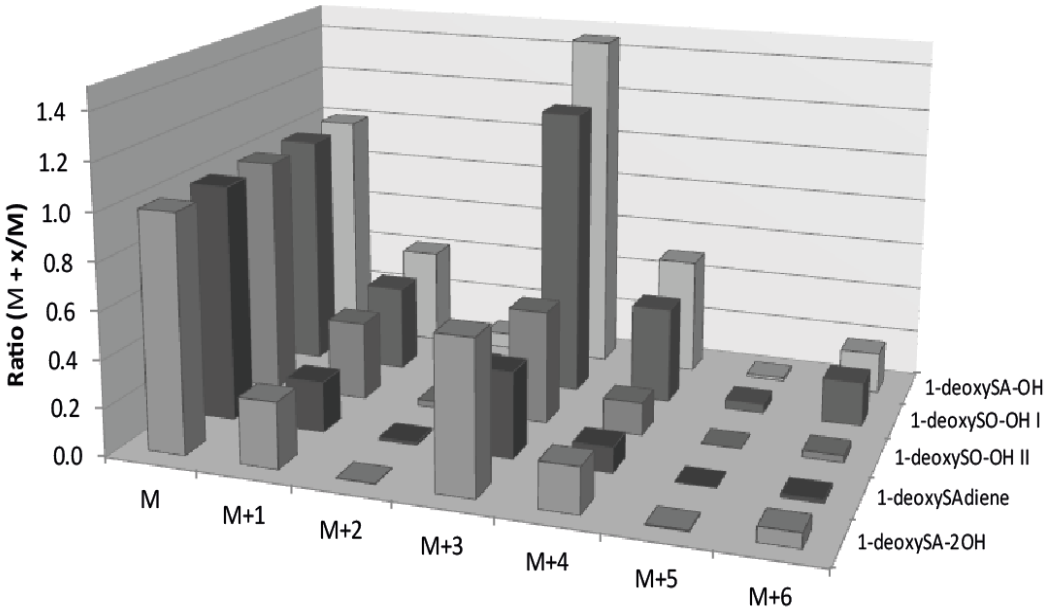
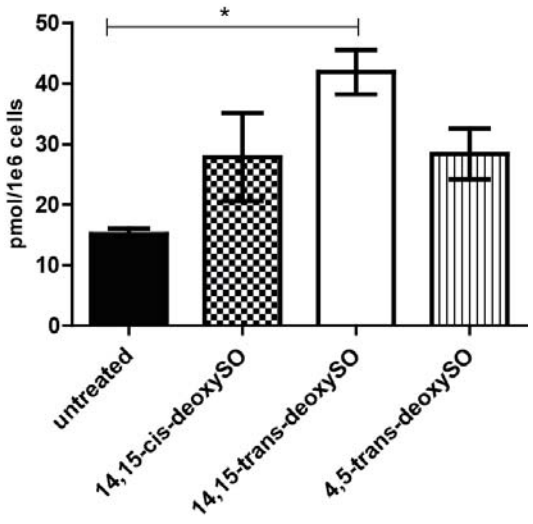
Supplemental Figures



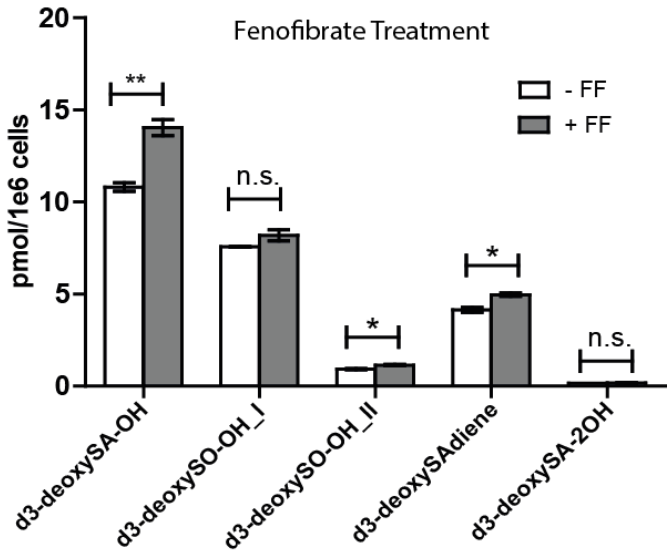
Supplemental Figure S1. Putative reactions for the conversion of d3-deoxySA to canonical SA. A.) Conversion to d2-labelled SA via replacement of a deuterium with an OH at C1. B.) Conversion to d3-labelled SA via nucleophilic attach of an oxygen by a heme-dependent mono-oxygenase.



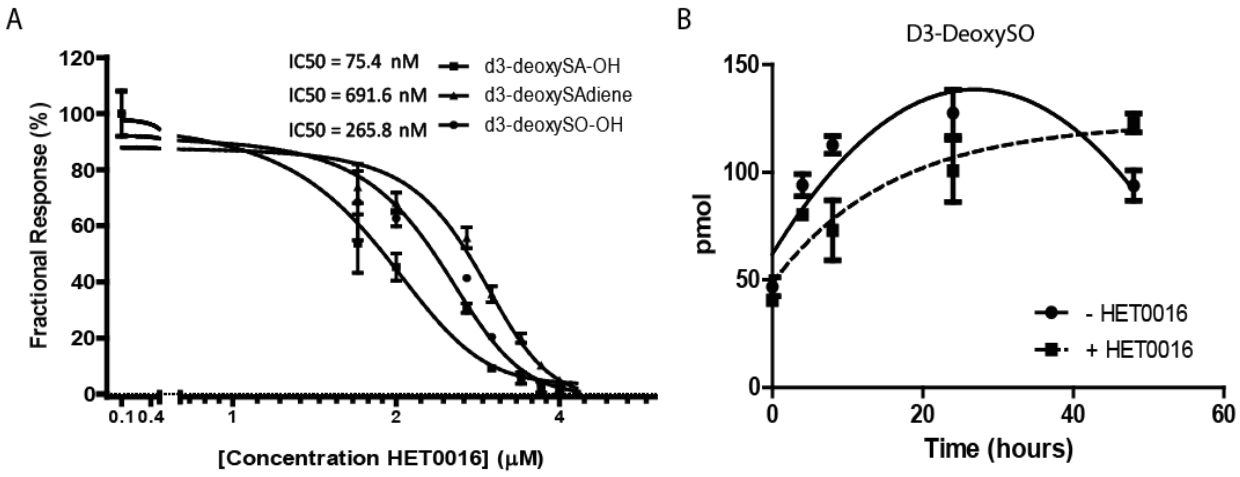
Supplemental Figure S2. All of the identified 1-deoxySL metabolites downstream of 1-deoxySA are produced *de novo* from d3-methyl-palmitic acid and d4-alanine. MEF cells overexpressing the SPTLC1-C133W mutation were treated with methyl-d3-palmitic acid and d4-alanine for 48 hours, after which lipids were acid/base hydrolyzed and measured by LC/MS. The values shown are a ratio of each possible mass offset (+1, +2, +3, etc.) to the unlabeled M (M+x/M), where M represents the mass of the unlabeled lipid. This is to account for the natural isotopologues. We have used M+1, +2, +3, etc. to refer to both the isotopologues of the lipids incorpotating ^{13}C, as well as to the deuterated lipids that would result from the use of the labelled substrates (d4-alanine and d3-palmitoyl CoA) in the *de novo* synthesis reaction. The use of the labelled alanine or the labelled palmitoyl CoA would result in a d3-labelled metabolite (and therefore a mass of M+3), while the use of both substrates would result in a d6 label (M+6 mass). The increased relative amounts of M+3 and M+6 metabolites indicate that both the labelled amino acid and the labelled fatty acid were used in the *de novo* synthesis of the 1-deoxySL metabolites. The increased relative amount of the M+4 is coming from the M+1 isotope (^{13}C) of the d3-labelled lipids.



Supplemental Figure S3. Levels of 1-deoxySA in cells treated with different 1-deoxySO synthetic standards. MEF cells were treated with 14,15-*cis*-deoxySO, 14,15-*trans*-deoxySO, or 4,5-*trans*-deoxySO for 48 hours and the 1-deoxySA levels were measured by LC/MS (after acid-base hydrolysis). The levels of 1-deoxySA were not significantly different amongst the three 1-deoxySO treatments, nor between the 14,15-*cis*-deoxySO or the 4,5-*trans*-deoxySO-fed cells. However, the levels of 1-deoxySA in 14,15-*trans*-deoxySO-treated cells were significantly higher than in untreated cells. Statistical significance was calculated using one-way ANOVA followed by Bonferroni correction. Data are represented as mean ± SEM. *$p < 0.05$



Supplemental Figure S4. Fenofibrate treatment increases the levels of some of the downstream 1-deoxySL metabolites. Cells were treated with 1 µM d3-deoxySA and 20 µM fenofibrate for 48 hours. The downstream metabolites d3-deoxySA-OH, d3-deoxySO-OH II, and d3-deoxySAdiene were significantly increased upon fenofibrate treatment. Bars represent averages ± SEM; N = 3. Statistical significance of the differences between untreated and treated cells was calculated using the Student's t-test. * $p<0.05$, ** $p<0.01$, n.s. not significant. FF, fenofibrate



Supplemental Figure S5. HET0016 inhibits the formation of the downstream 1-deoxySL metabolites in a dose-dependent manner and prevents the decrease of 1-deoxySO over time. A.) MEF cells were treated with d3-deoxySA and different concentrations of HET0016 for 24 hours. The inhibition of the enzyme is shown as a % of the amount of the metabolites d3-deoxySA-OH, d3-deoxySAdiene, and d3-deoxySO-OH I produced. The levels of d3-deoxySO-OH II and d3-deoxySA-2OH were not quantifiable at the 6 highest concentrations, and therefore the curves are not shown. B.) MEF cells were treated with d3-deoxySA for a 2 hour pulse, followed by a chase in unsupplemented growth medium. For the HET0016-treated cells, the inhibitor was present during the entire duration of both the pulse and chase periods. Time point 0 represents the start of the chase period. In the presence of HET0016, d3-deoxySO levels no longer decreased after 24 hours. All points represent averages ± SEM; N = 3.