

Supplementary data

Protectin Dx Production from Docosahexanoic Acid (DHA)-Enriched Fish Oil via 10-Hydroxydocosahexaenoic Acid using Recombinant Cells Expressing Lipoxygenases

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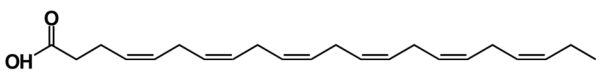
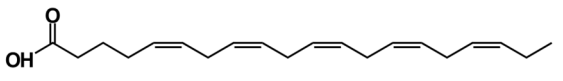
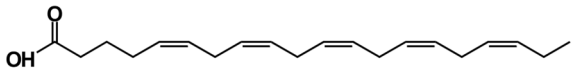
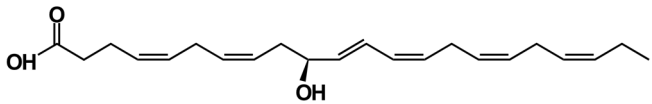
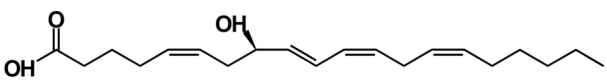
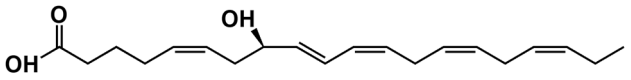
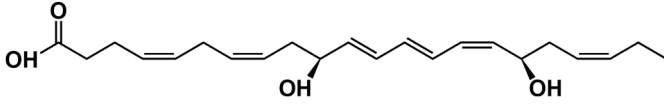
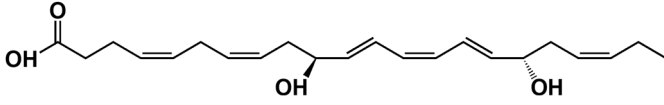
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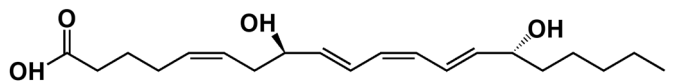
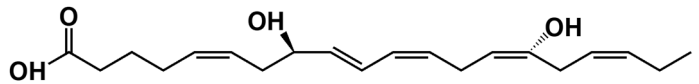
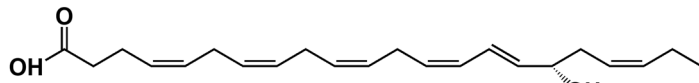
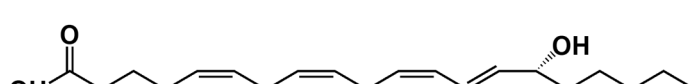
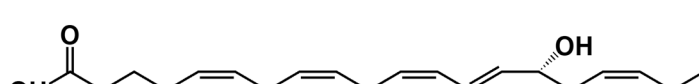
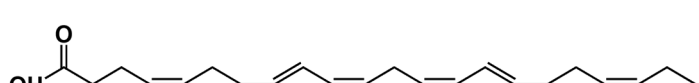

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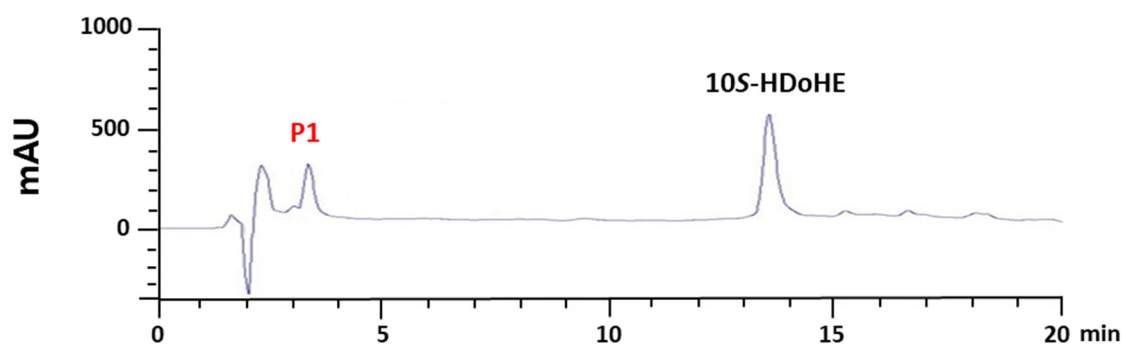
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Table S1. The chemical structures and abbreviations of SPMs and their analogs.

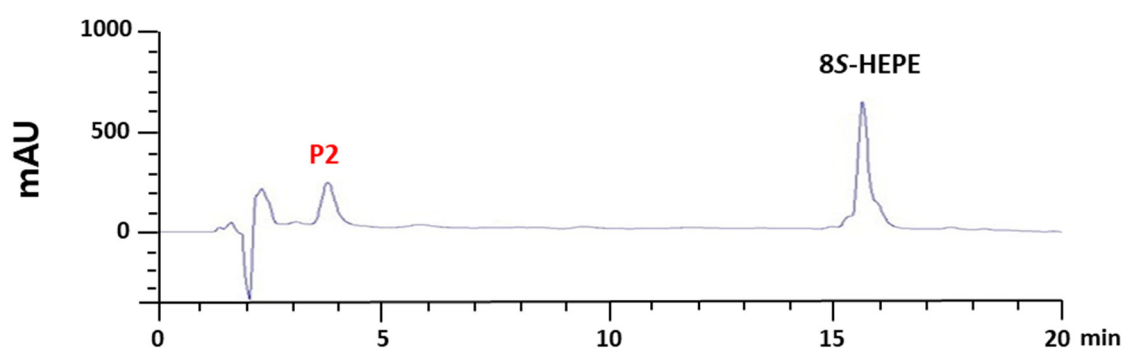
Abbreviation	Full name	Structure
DHA	Docosahexaenoic acid	
AA	Arachidonic acid	
EPA	Eicosapentaenoic acid	
10S-HDHA	10S-Hydroxydocosahexaenoic acid	
8S-HETE	8S-Hydroxyeicosatetraenoic acid	
8S-HEPE	8S-Hydroxyeicosapentaenoic acid	
10R,17S-DiDHA (or PD1)	10R,17S-Dihydroxydocosahexaenoic acid (or Protectin D ₁)	
10S,17S-DiDHA (or PDX)	10S,17S-Dihydroxydocosahexaenoic acid (or Protectin Dx)	

8 <i>S</i> ,15 <i>S</i> -DiHETE	8 <i>S</i> ,15 <i>S</i> -Dihydroxyeicosatetraenoic acid	
8 <i>S</i> ,15 <i>S</i> -DiHEPE	8 <i>S</i> ,15 <i>S</i> -Dihydroxyeicosapentaenoic acid	
17 <i>S</i> -HDHA	17 <i>S</i> -Hydroxydocosahexaenoic acid	
15 <i>S</i> -HETE	15 <i>S</i> -Hydroxyeicosatetraenoic acid	
15 <i>S</i> -HEPE	15 <i>S</i> -Hydroxyeicosapentaenoic acid	
7 <i>S</i> ,17 <i>S</i> -DiHDHA (or RvD5)	7 <i>S</i> ,17 <i>S</i> -Dihydroxydocosahexaenoic acid (or Resolvin D5)	
7 <i>S</i> ,14 <i>S</i> -DiHDHA	7 <i>S</i> ,14 <i>S</i> -Dihydroxydocosahexaenoic acid	

(a)



(b)



(c)

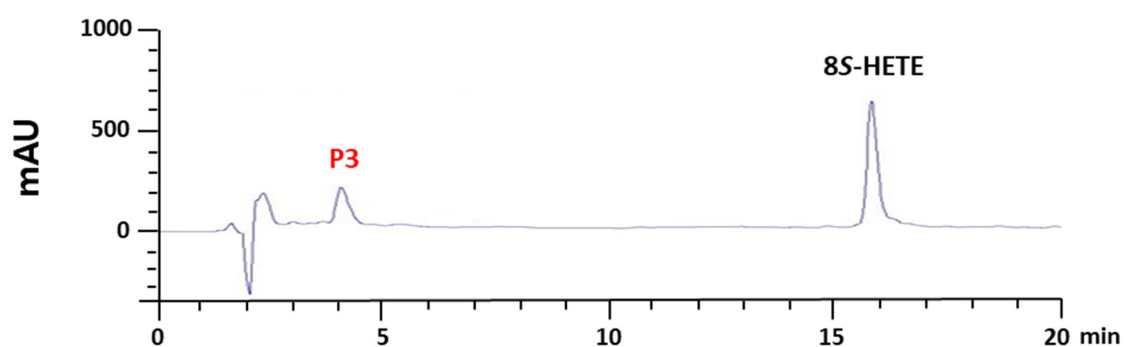
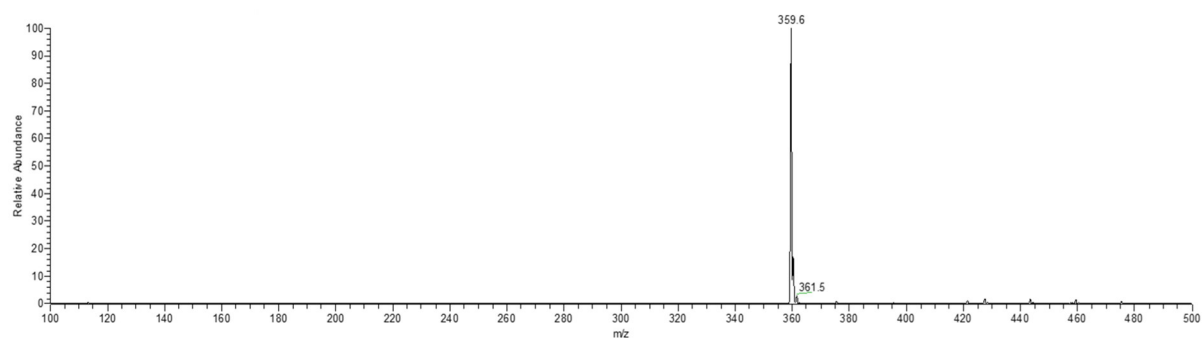
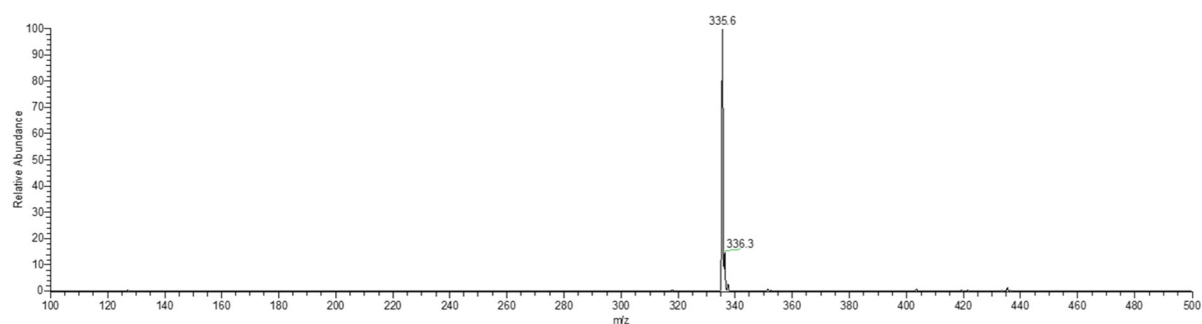


Figure S1. HPLC chromatograms for the products obtained from the conversion of (a) 10S-HDHA, (b) 8S-HETE, and (c) 8S-HEPE by BT 15SLOX. P1, P2, and P3 represent unknown products derived from 10S-HDHA, 8S-HETE, and 8S-HEPE, respectively.

(a)



(b)



(c)

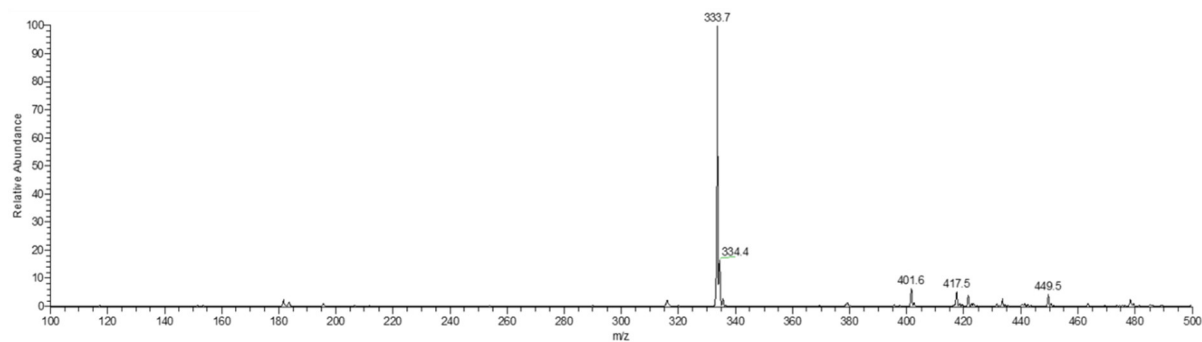
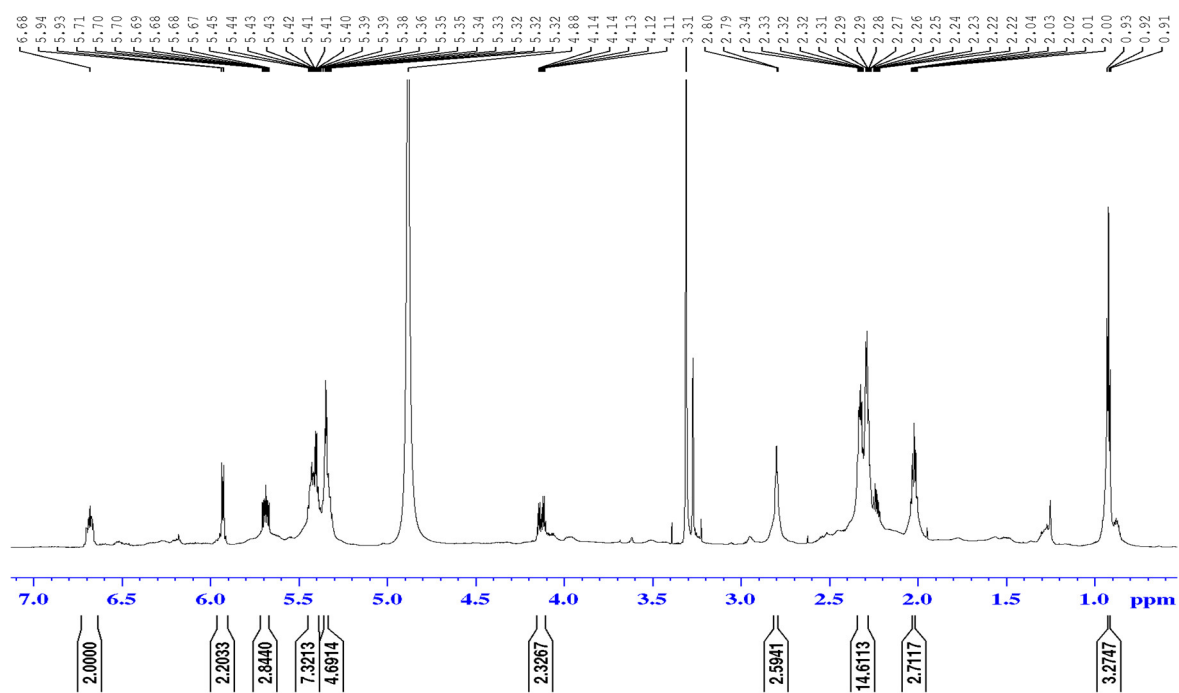
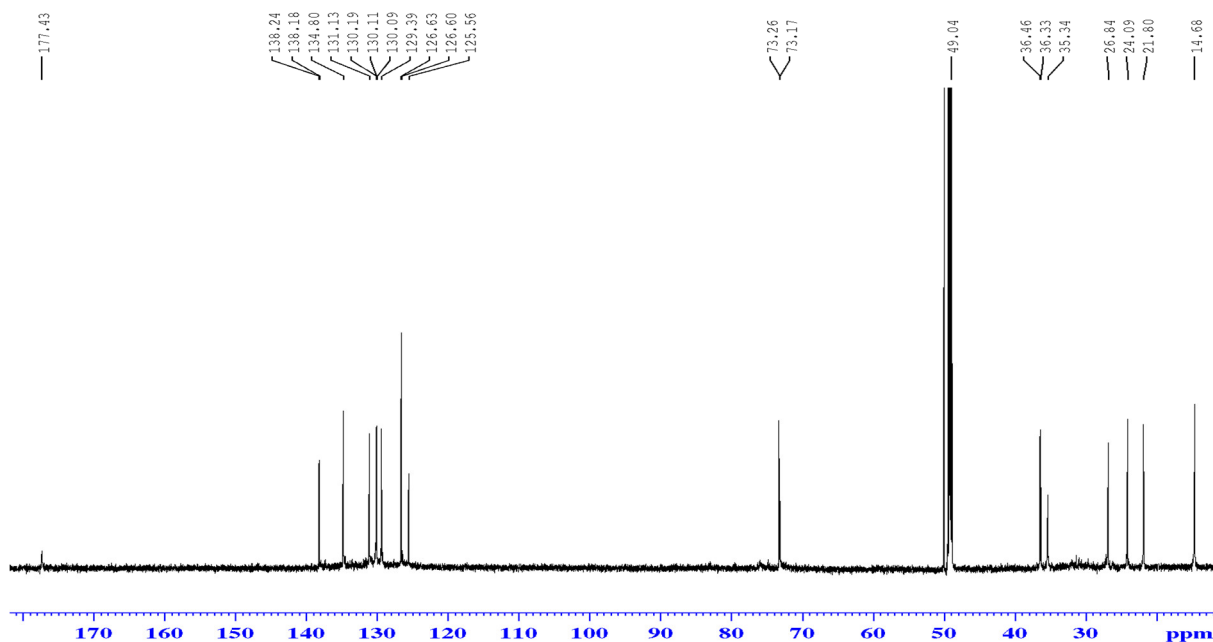


Figure S2. LC-MS spectra for the products obtained from the conversion of (a) 10*S*-HDHA, (b) 8*S*-HETE, and (c) 8*S*-HEPE by BT 15SLOX.

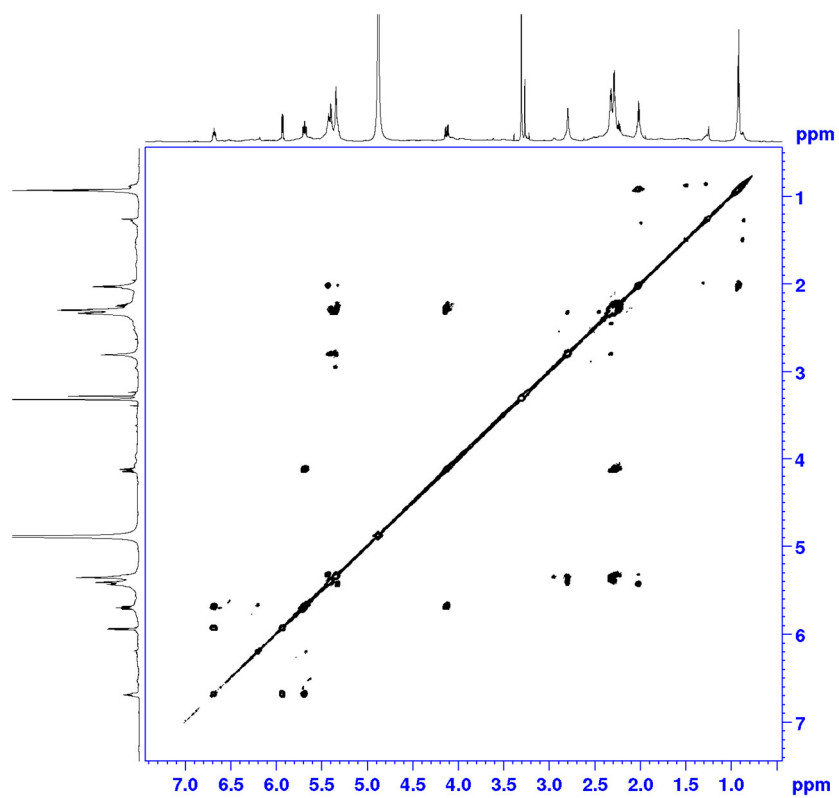
(a)



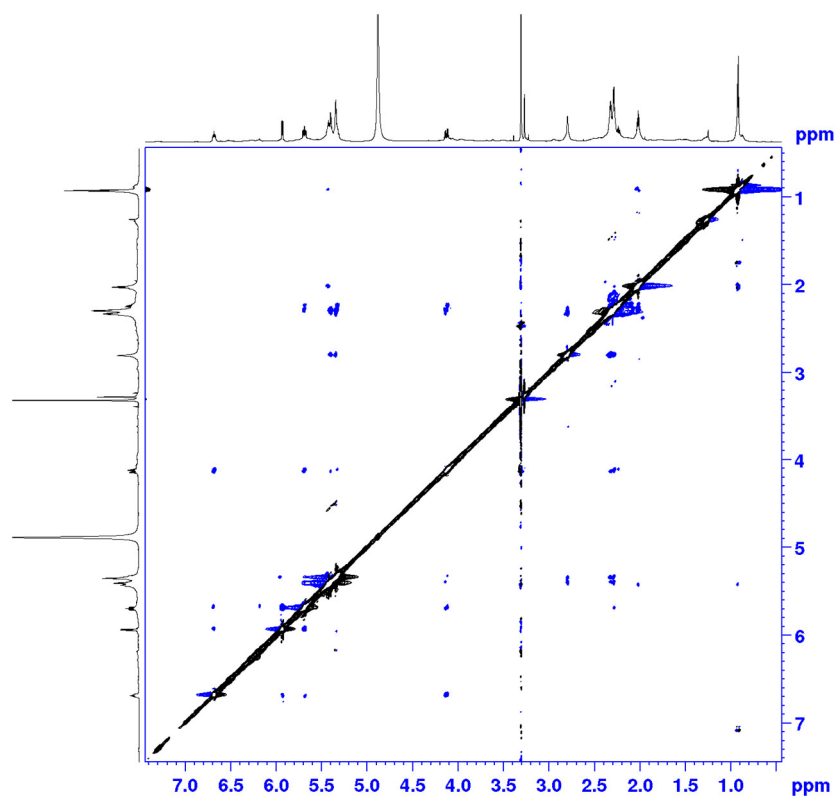
(b)



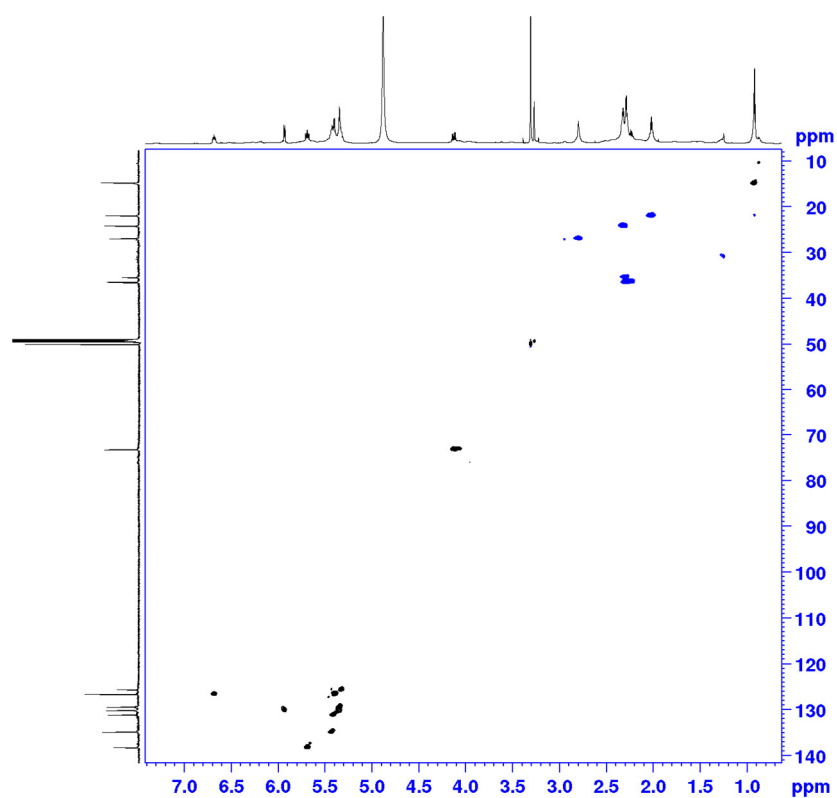
(c)



(d)



(e)



(f)

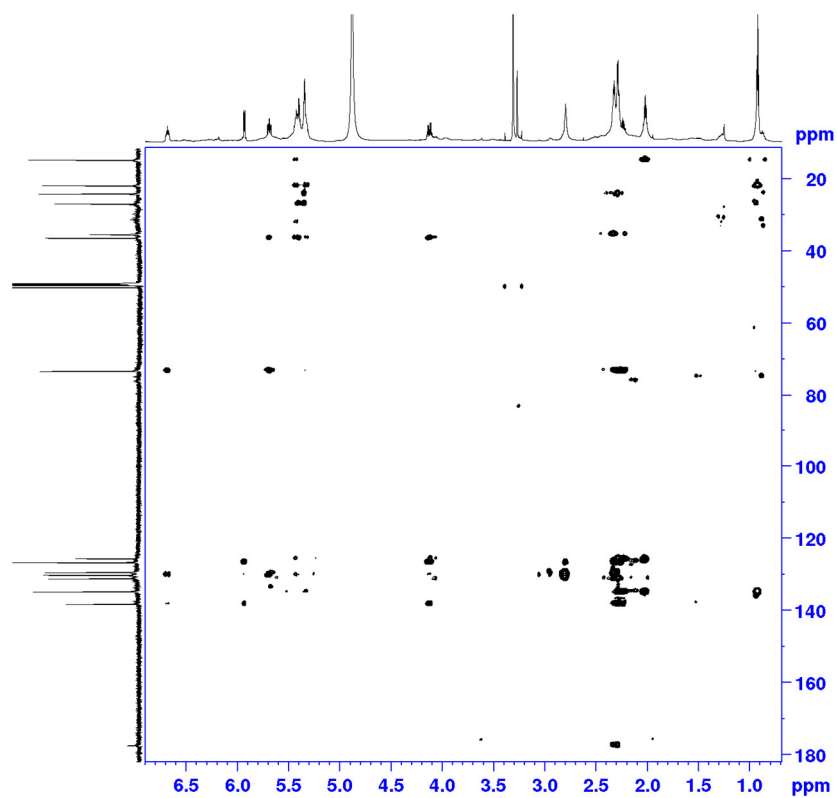
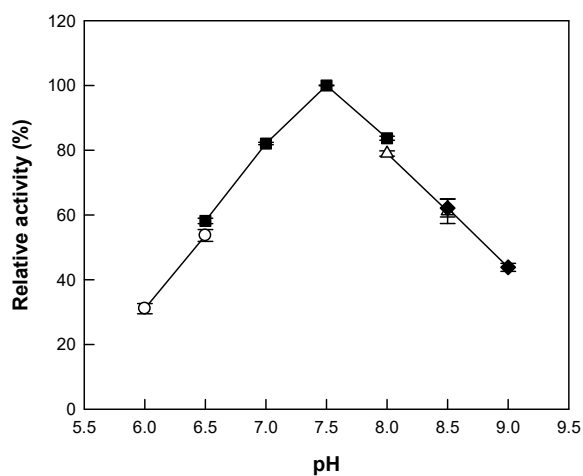


Figure S3. 1D (^1H and ^{13}C) and 2D (COSY, ROSEY, HSQC, and HMBC) NMR spectra of 10*S*,17*S*-DiHDHA (PDX) using NMR spectroscopy (850 MHz NMR, MeDO). (a) ^1H NMR spectrum of PDX. (b) ^{13}C NMR spectrum of PDX. (c) COSY spectrum of PDX. (d) ROSEY spectrum of PDX. (e) HSQC spectrum of PDX. (f) HMBC spectrum of PDX.

(a)



(b)

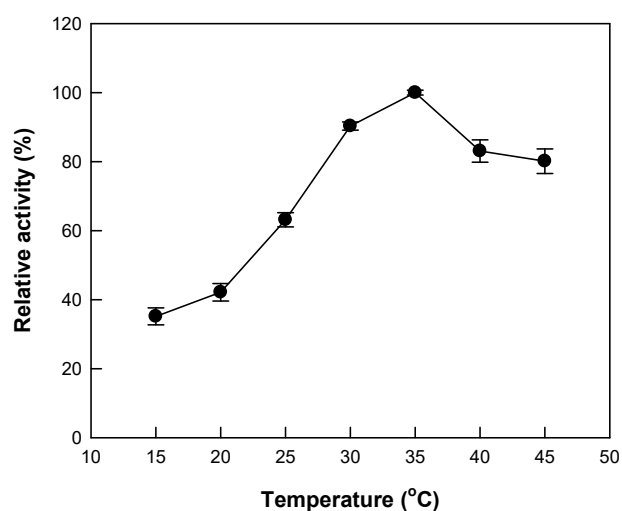
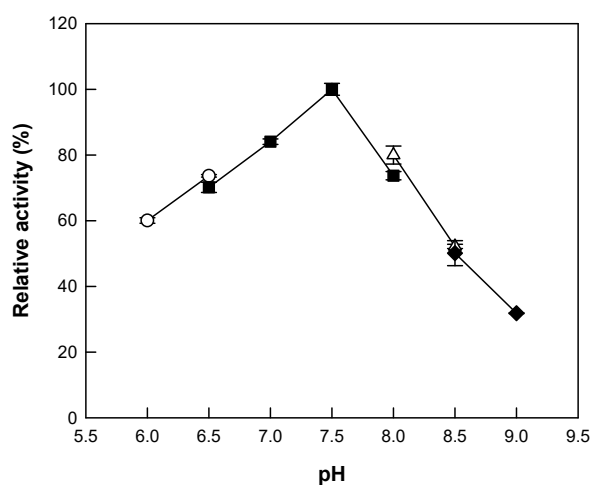


Figure S4. Effects of pH and temperature on the production of 10S-HDHA from DHA in DFOH using *E. coli* cells expressing MO 8SLOX. (a) Effect of pH. The reactions were performed in 50 mM MES (pH 6.0–6.5, ○), HEPES (pH 6.5–8.0, ■), EPPS (pH 8.0–8.5, △), and CHES (pH 8.5–9.0, ◆) buffers with 1 g/L of *E. coli* cells expressing MO 8SLOX and DFOH containing 1 mM DHA at 35 °C for 10 min. (b) Effect of temperature. The reactions were performed in 50 mM HEPES buffer (pH 7.5) with 1 g/L of *E. coli* cells expressing MO 8SLOX and DFOH containing 1 mM DHA by varying the temperature from 15 to 45 °C for 10 min. Data represent the means of three separate experiments, and error bars represent the standard deviation.

(a)



(b)

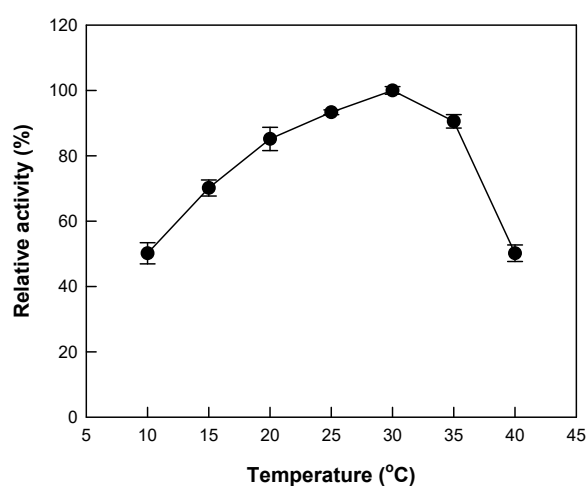


Figure S5. Effects of pH and temperature on the production of PDX from 10S-HDHA in 8SLOX-treated DFOH using *E. coli* cells expressing BT 15SLOX. (a) Effect of pH. The reactions were performed in 50 mM MES (pH 6.0–6.5, ○), HEPES (pH 6.5–8.0, ■), EPPS (pH 8.0–8.5, △), and CHES (pH 8.5–9.0, ◆) buffers with 1 g/L cells expressing BT 15SLOX and 8SLOX-treated DFOH containing 0.43 mM 10S-HDHA at 35 °C for 10 min. (b) Effect of temperature. The reactions were performed in 50 mM HEPES buffer (pH 7.5) with 1 g/L of *E. coli* cells expressing BT 15SLOX and 8SLOX-treated DFOH containing 0.43 mM 10S-HDHA by varying the temperature from 10 to 40 °C for 10 min. Data represent the means of three separate experiments, and error bars represent the standard deviation.