Supporting Information

Human 15-lipoxygnenase-2 role in the biosynthesis of the lipoxin intermediate, 5S,15S-diHpETE, implicated with altered positional specificity of human 15-lipoxygenase-1

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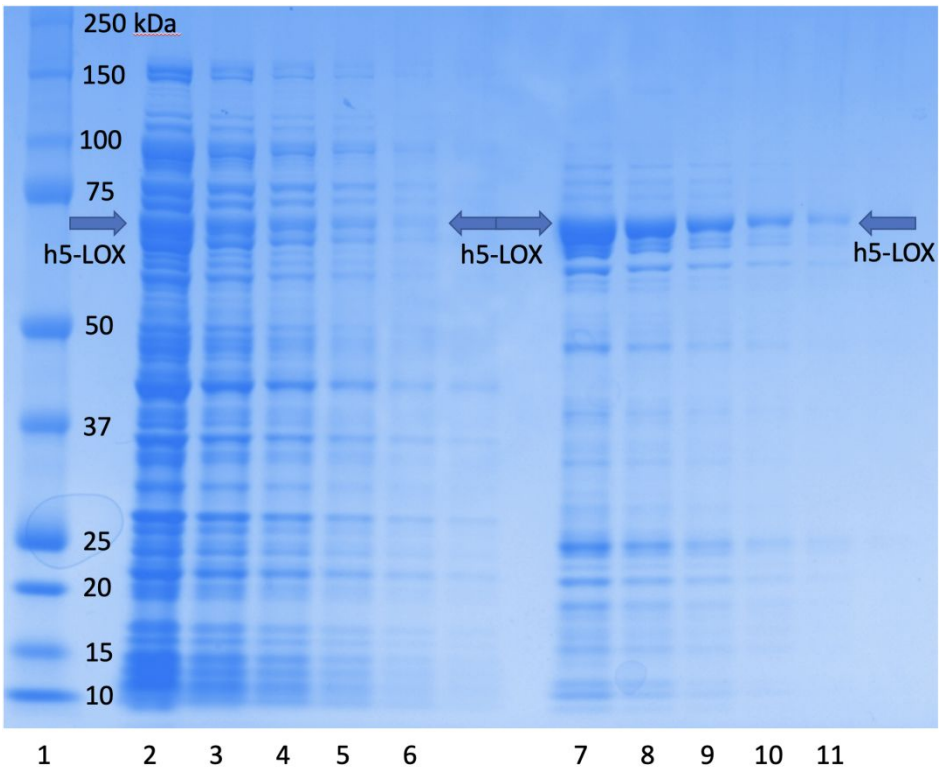

Figure S1. SDS-PAGE
Lane 1: Protein standards.
Lane 2: 380 ugs total protein ammonium sulfate h5-LOX fraction.
Lane 3: 190 ugs total protein ammonium sulfate h5-LOX fraction.
Lane 4: 95 ugs total protein ammonium sulfate h5-LOX fraction.
Lane 5: 48 ugs total protein ammonium sulfate h5-LOX fraction.
Lane 6: 24 ugs total protein ammonium sulfate h5-LOX fraction.
Lane 7: 5 ugs Hig-tag purified Stable h5-LOX.
Lane 8: 2.5 ugs Hig-tag purified Stable h5-LOX.
Lane 9: 1.3 ugs Hig-tag purified Stable h5-LOX.
Lane 10: 0.61 ugs Hig-tag purified Stable h5-LOX.

The h5-LOX protein band is indicated on the SDS-PAGE. Measuring band density with ImageJ software, h5-LOX was estimated to be approximately 1% of the total protein based on a Stable h5-LOX standard. From this estimation, the kinetic parameters were calculated. It should also be emphasized that we assumed that 100% of the h5-LOX was loaded with iron, therefore, the estimation of active h5-LOX concentration could be lower.

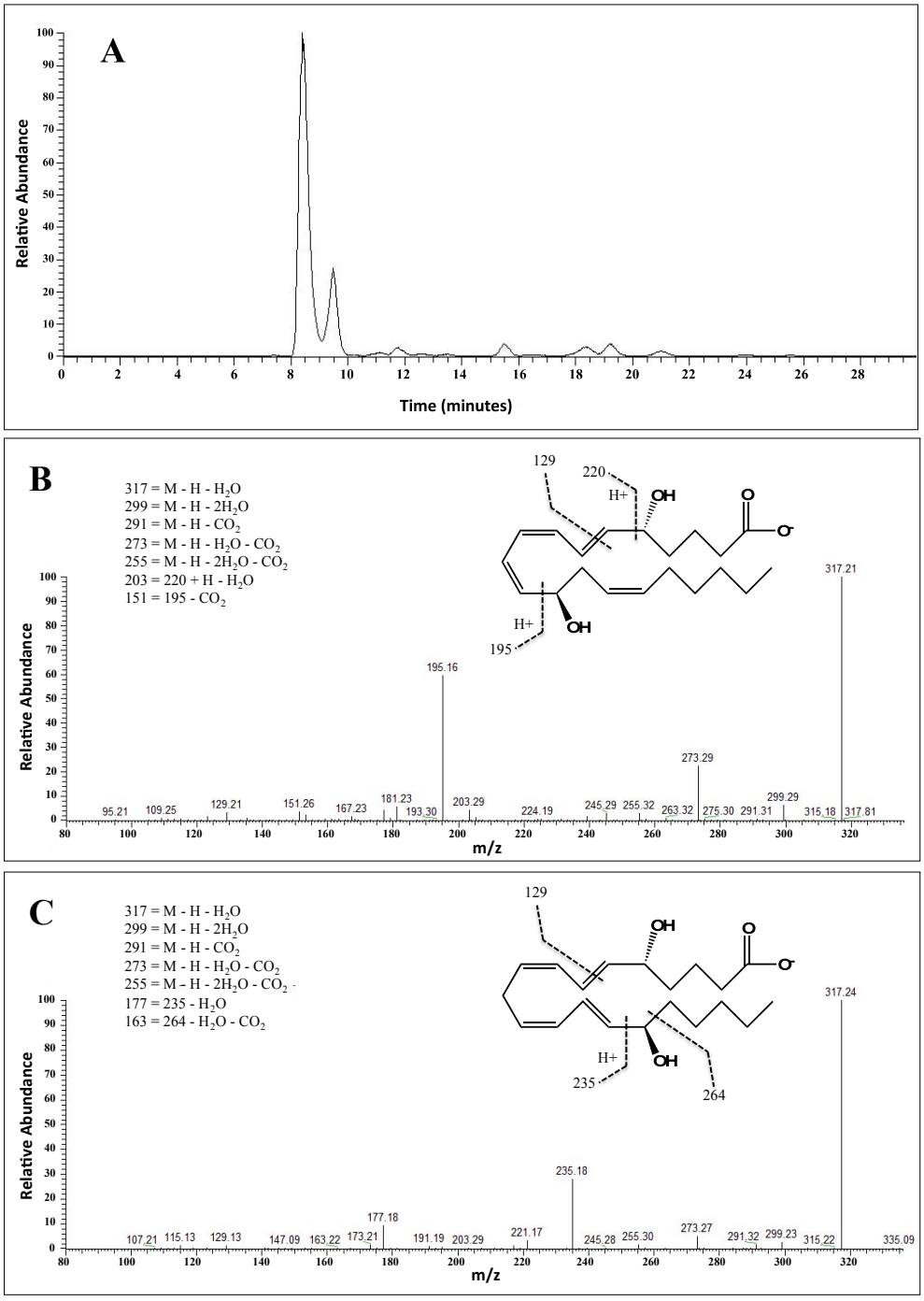

Figure S2. h15-LOX-1 primarily synthesizes 5S,12S-diHETE from 5S-HETE. (A) Selected ion chromatogram at m/z of 335. Larger peak at 8.5 min is 5S,12S-diHETE. Smaller peak at 9.5 min is 5S,15S-diHETE. (B) MS/MS spectra of 5S,15S-diHETE prepared from reaction of h15-LOX-1 with 5S-HETE. (C) MS/MS spectra of 5S,15S-diHETE prepared from reaction of h15-LOX-1 with 5S-HETE. Samples were reduced to form the di-alcohol products

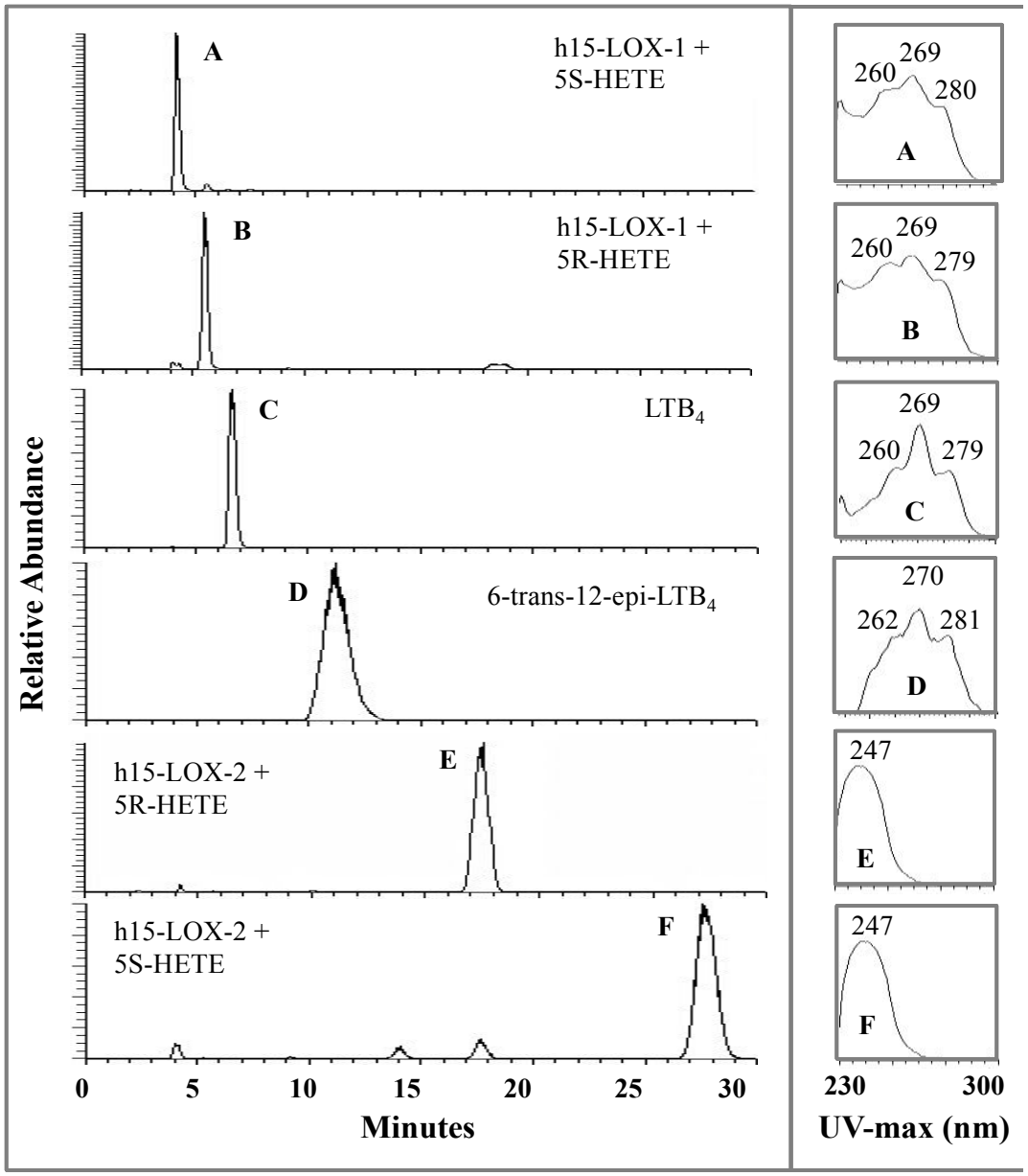

Figure S3. Chiral chromatograms and UV-maxima of 5,12-diHETE and 5,15-diHETE isomers. The products formed by h15-LOX-1 and h15-LOX-2 from 5S-HETE and 5R-HETE were analyzed using Chiral LC-MS/MS and UV-vis spectroscopy and compared to LTB_4 and 6-trans-7-epi-LTB_4 standards. All 5,12-diHETE isomers contained a central peak at ~270 nm, flanked by shoulders at ~260 nm and ~280 nm, consistent with the presence of a conjugated triene. Shoulders of equal intensity at 281 nm and 261 nm are indicative of an EEZ configuration, while a more intense shoulder at 260 nm compared to 280 nm indicates the EZE configuration. 5,15-diHETE isomers contain two conjugated dienes separated by a methylene, indicated by a UV-maxima of 247 nm.

compound	stereochemistry	source	RT
5S,15S-diHETE	5(S),15(S)-6E,8Z,11Z,13E	standard	27.5 min
	5(S),15(S)-6E,8Z,11Z,13E	h5-LOX +15S-HETE	27.2 min
	5(S),15(S)-6E,8Z,11Z,13E	h12-LOX + 5S-HETE	27.9 min
	5(S),15(S)-6E,8Z,11Z,13E	h15-LOX-1+ 5S-HETE	27.9 min
	5(S),15(S)-6E,8Z,11Z,13E	h15-LOX-2+ 5S-HETE	27.8 min
5R,15S-diHETE	5(R),15(S)-6E,8Z,11Z,13E	h15-LOX-2+ 5R-HETE	17.5 min
6-trans-12-epi-LTB_4	5(S),12(S)-6E,8E,10E,14Z	standard	11.6 min
LTB_4	5(S),12(R)-6Z,8E,10E,14Z	standard	6.6 min
5R,12S-diHETE	5(R),12(S)-6E,8Z,10E,14Z	h15-LOX-1+ 5R-HETE	5.5 min
5S,12S-diHETE	5(S),12(S)-6E,8Z,10E,14Z	standard	4.2 min
	5(S),12(S)-6E,8Z,10E,14Z	h5-LOX +12S-HETE	4.2 min
	5(S),12(S)-6E,8Z,10E,14Z	h12-LOX + 5S-HETE	4.3 min
	5(S),12(S)-6E,8Z,10E,14Z	h15-LOX-1+ 5S-HETE	4.2 min

Figure S4. 5,15-diHETE and 5,12-diHETE isomers produced through different LOX pathways were compared to 5S,15S-diHETE, 5S,12S-diHETE, LTB_4 and 6-*trans*-12-epi-LTB_4 standards using LC-MS/MS with a reverse-phase chiral column.