Figure S1. Related to Figure 1. Flow chart for oxylipin discovery. Flow chart showing experimental design of how directed non-targeted LC-MS/MS in conjunction with chemical networking was used to identify definitive known, putative known and putative novel oxylipins.

Figure S1

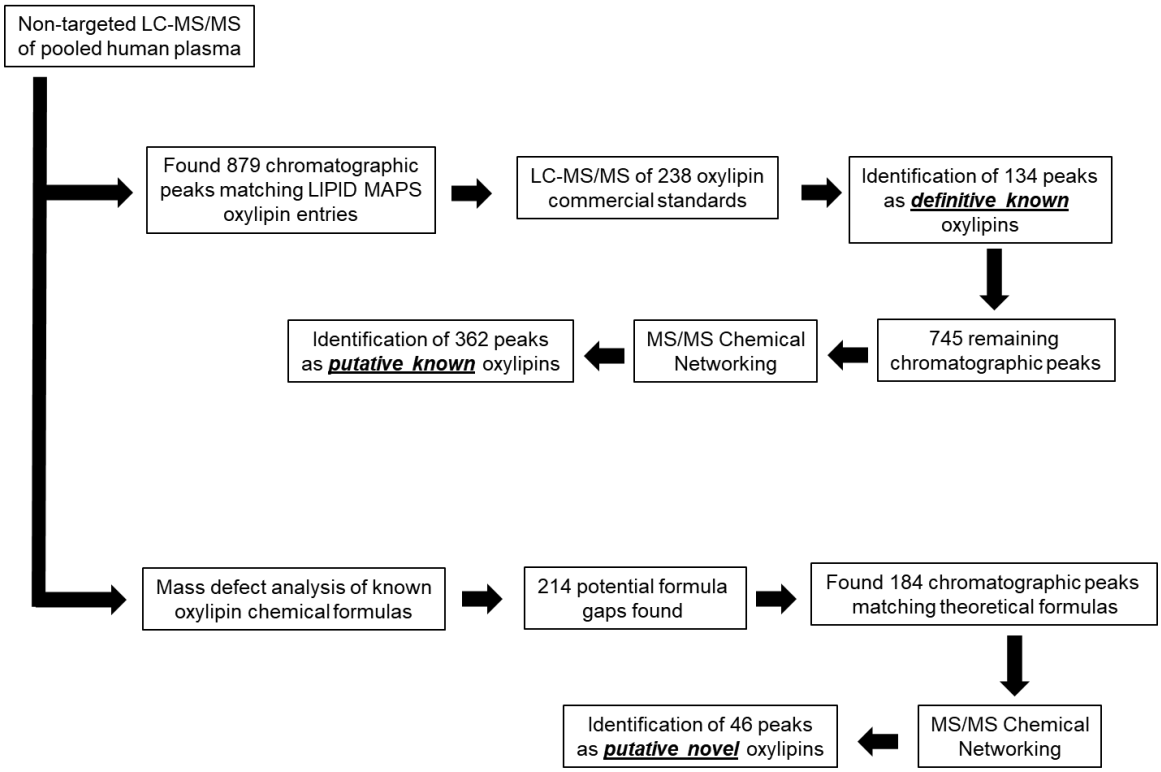
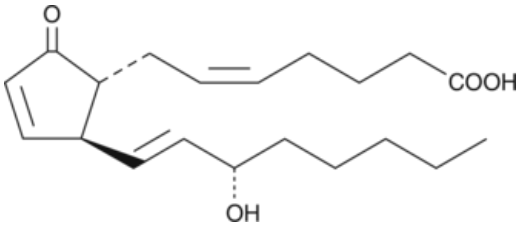
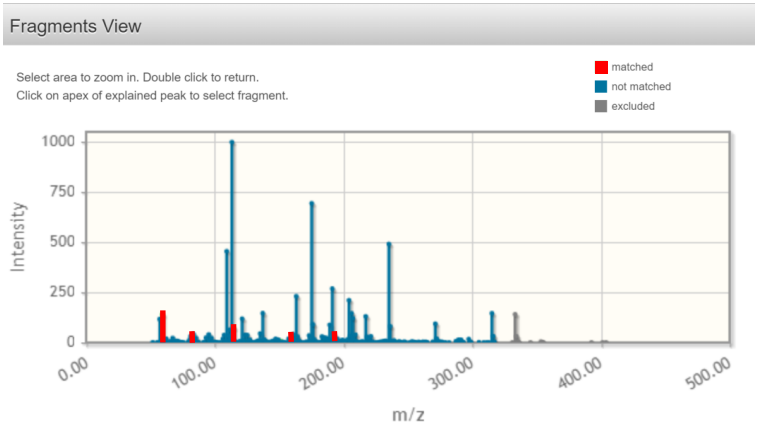

Non-targeted LC-MS/MS of pooled human plasma
Found 879 chromatographic peaks matching LIPID MAPS oxylipin entries
LC-MS/MS of 238 oxylipin commercial standards
Identification of 134 peaks as definitive known oxylipins
745 remaining chromatographic peaks
MS/MS Chemical Networking
Identification of 362 peaks as putative known oxylipins
Mass defect analysis of known oxylipin chemical formulas
214 potential formula gaps found
Found 184 chromatographic peaks matching theoretical formulas
MS/MS Chemical Networking
Identification of 46 peaks as putative novel oxylipins

Figure S2. Related to Figure 3. Performance of automated tandem MS fragment assignment tools. (Top)Tandem mass spectra from commercial standards were inputted into the MetFrag webtool with LIPID MAPS serving as the library. On average, Metfrag was only able to assign ~5% of fragment peaks to corresponding chemical structures. Here are two examples using a linear and a cyclic eicosanoid where both results showed poor ability to assign spectral fragments to chemical structures with almost all the dominant fragments not assigned. Note that matched peaks were re-colored red for visibility. (Bottom) *In sili*co prediction of tandem mass spectral fragments of commercial standards was also performed using CFM-id. Here is a typical example using a cyclic eicosanoid where many of the observed fragments fail to be predicted. CFM-id performed notably better at predicting fragments for linear oxylipins, however, many fragments were still left unpredicted.

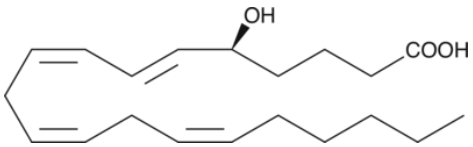
Figure S2

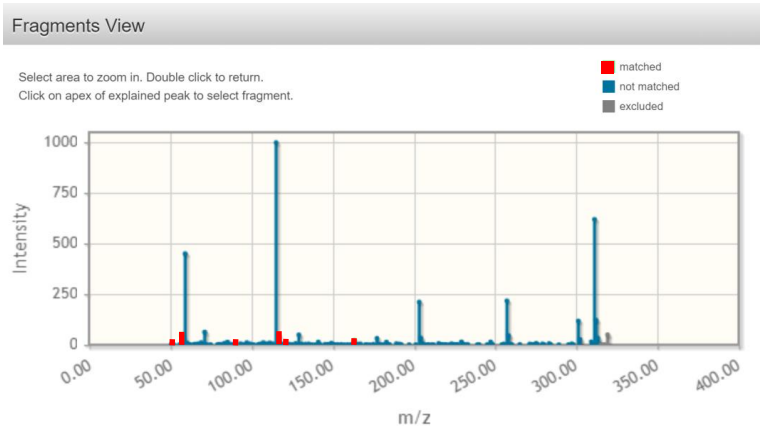
Prostaglandin A2
MS/MS peaks matched: 5 of 115



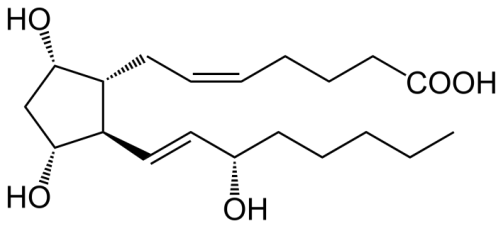


5-HETE
MS/MS peaks matched: 6 of 84

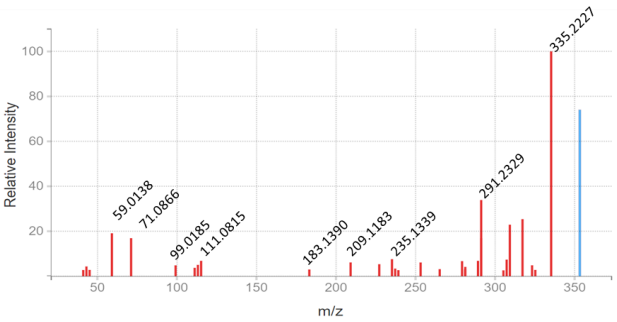




Prostaglandin F2α



Predicted Tandem MS Spectra



Experimental Tandem MS Spectra

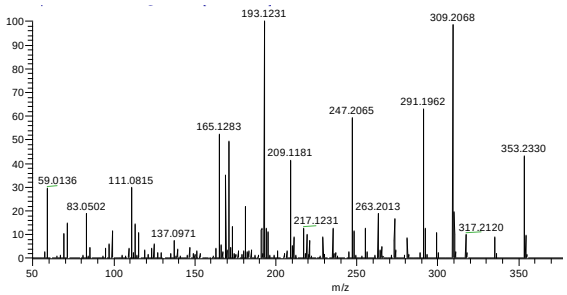


Figure S3. Related to Figure 4. Mass defect plots used for identification of chemical formulas potentially belonging to novel compounds. (Top) For each oxylipin entry in the LIPID MAPS library, the nominal mass was plotted against the mass defect to reveal patterns in chemical formulas which compose the 1149 unique compounds. (Middle) By searching for gaps in the chemical iterations within each chemical family, ‘missing’ entries were identified and plotted in red. (Bottom) Once data for each of the missing formulas was extracted and searched via chemical spectral networking, all formulas resulting in positive analog matches were left plotted in red relative to all entries from oxylipins in LIPID MAPS.

Figure S3

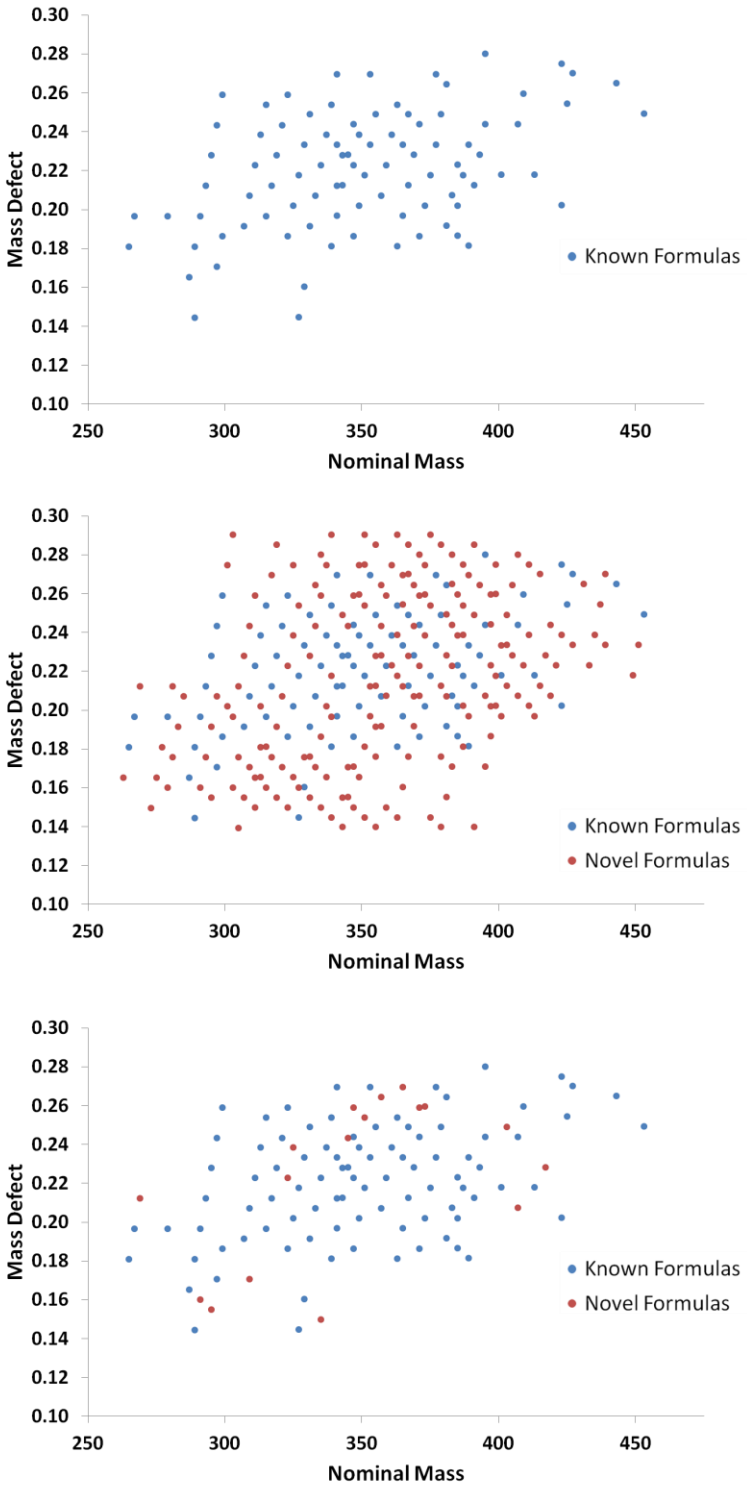
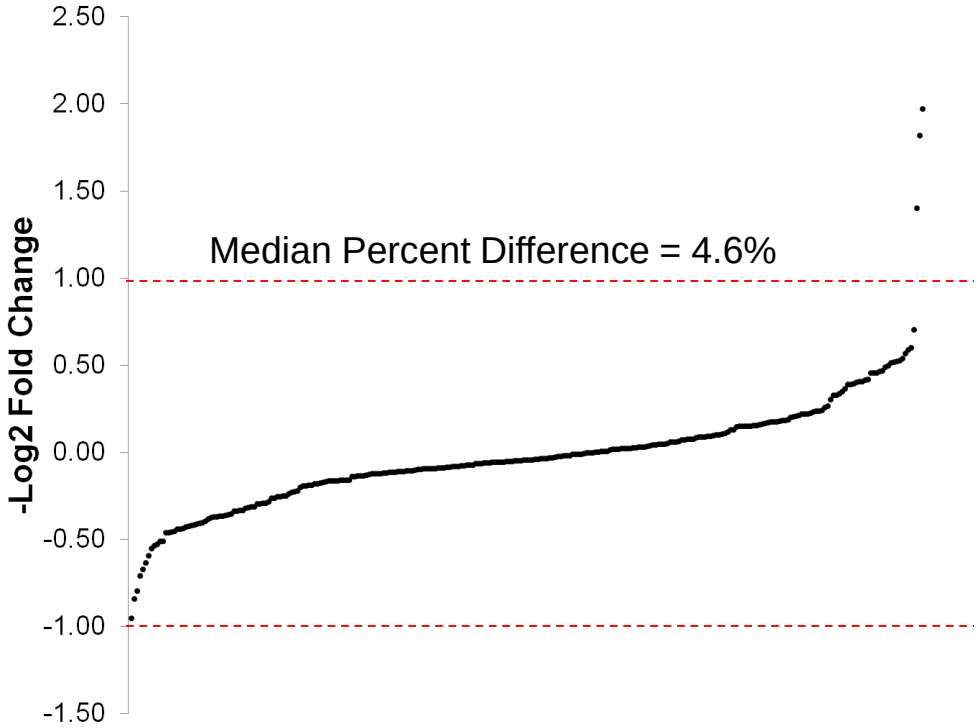

Mass Defect
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
250
300
350
400
450
Nominal Mass
Known Formulas
Novel Formulas

Figure S4. Related to Figures 3 and 5. Stability of oxylipins during blood processing and oxylipin extraction. (Top) Scatter plot showing the -$\log_2$ fold change of BHT and non-BHT treated samples of fresh human plasma (n=10 total samples) across observed known and putative oxylipins. Median percent difference in peaks height between BHT and non-BHT samples was 4.6%. Of the 313 observed known and putative oxylipins, four compounds were observed only in the non-BHT samples and eight were observed only in the BHT samples. (Bottom) LC-MS measurement profiles represented as relative chromatographic peak height of all observed known and putative oxylipins across 96 replicate sample preparations. Black line represents the median relative peak height for observed compounds while the shaded region shows the median min and max values (top and bottom 10%) for each injection. Median coefficient of variation for all observed compounds was 7.8%.

Figure S4


2.50
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
-Log2 Fold Change
Median Percent Difference = 4.6%

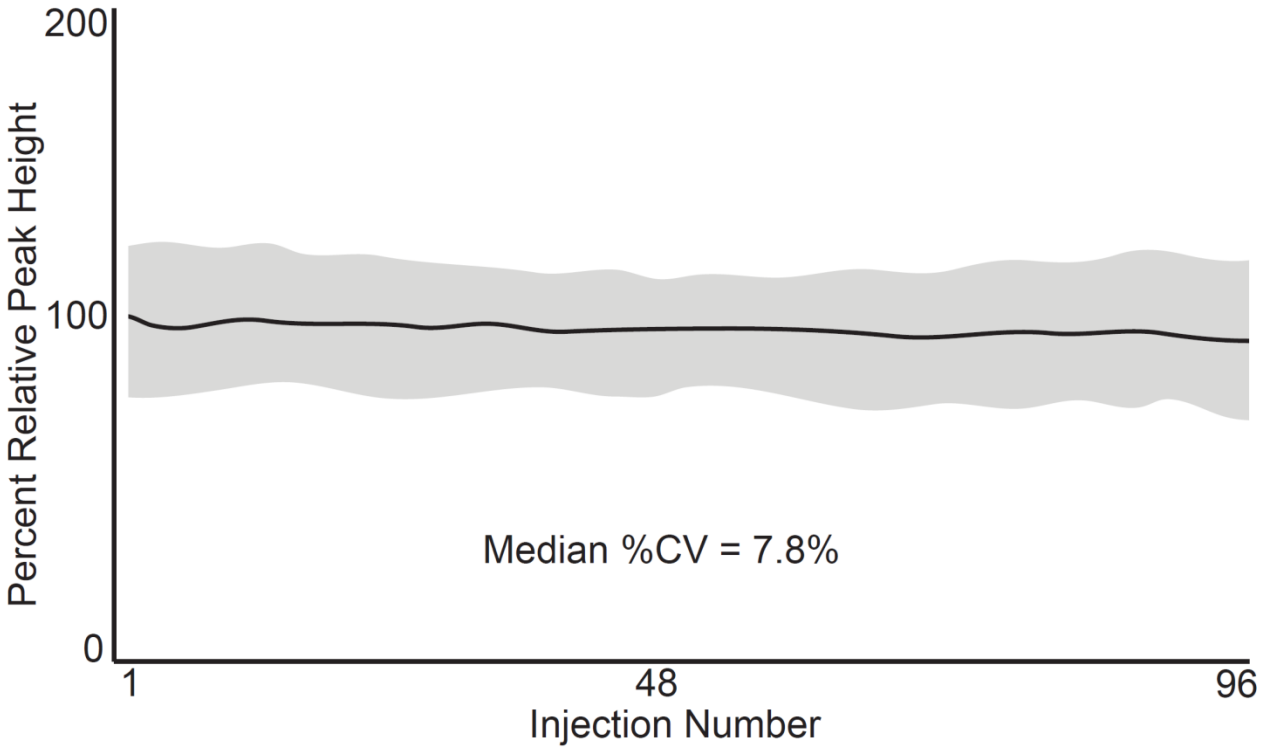
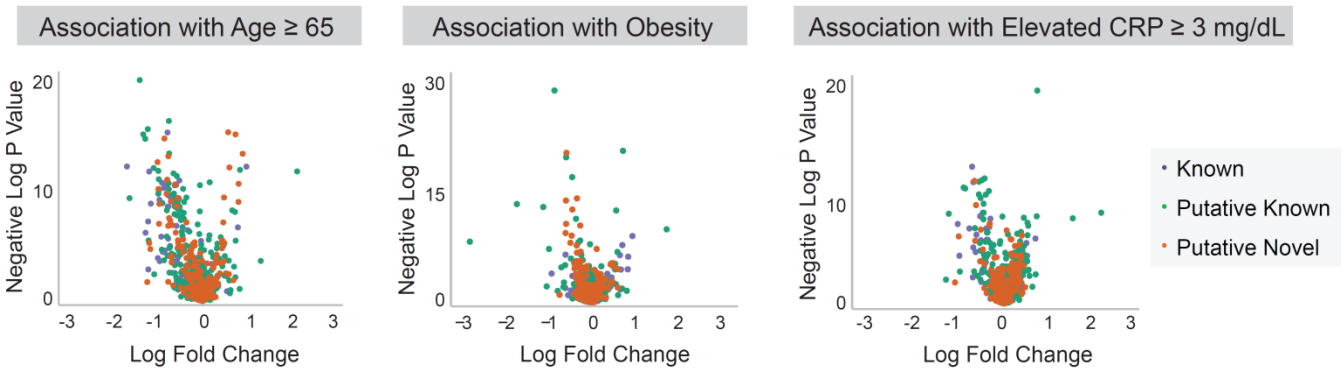

200
100
0
Percent Relative Peak Height
Median %CV = 7.8%
1
48
96
Injection Number

Figure S5. Related to Figure 5. Correlates of categorized age, BMI, and C-reactive Protein. Associations among known (blue), putative known (red), and putative novel (green) plasma oxylipins with dichotomized age (<65 versus ≥65 years), body mass index (<30 versus ≥ 30 kg/m^2), and C-reactive protein (<3 versus ≥3 mg/dL). Data points, visualized using volcano plots, represent the FDR-corrected negative Log_{10} p-values derived from linear regression models relating each oxylipin with given clinical variables.

Figure S5


Association with Age ≥ 65
Association with Obesity
Association with Elevated CRP ≥ 3 mg/dL
Negative Log P Value
Log Fold Change
Known
Putative Known
Putative Novel

SUPPLEMENTAL INFORMATION TABLE LEGENDS

Table S1. Related to Figure 1. List of 1125 LIPID MAPS database entries used as source database for mining non-targeted data for putative known and putative novel oxylipins. List of all commercial standards used for assignment of definite known oxylipins as well as identification of putative known and putative novel compounds through chemical networking.

Table S2. Related to Figure 3 and 4. Results for chemical networking for putative known and putative novel oxylipins.

Table S3. Related to Figure 5. Results from statistical analysis for Figure 5 and Figure S6. Top 20 associations of known, putative known and putative novel oxylipins with age, BMI and Log CRP and dichotomized age (<65 versus ≥65 years), BMI (<30 versus ≥30 kg/m2) and CRP (<3 versus ≥3 mg/dL).