

Supplementary Data for

Comprehensive profiling of prostaglandins in human ovarian follicular fluid using mass spectrometry

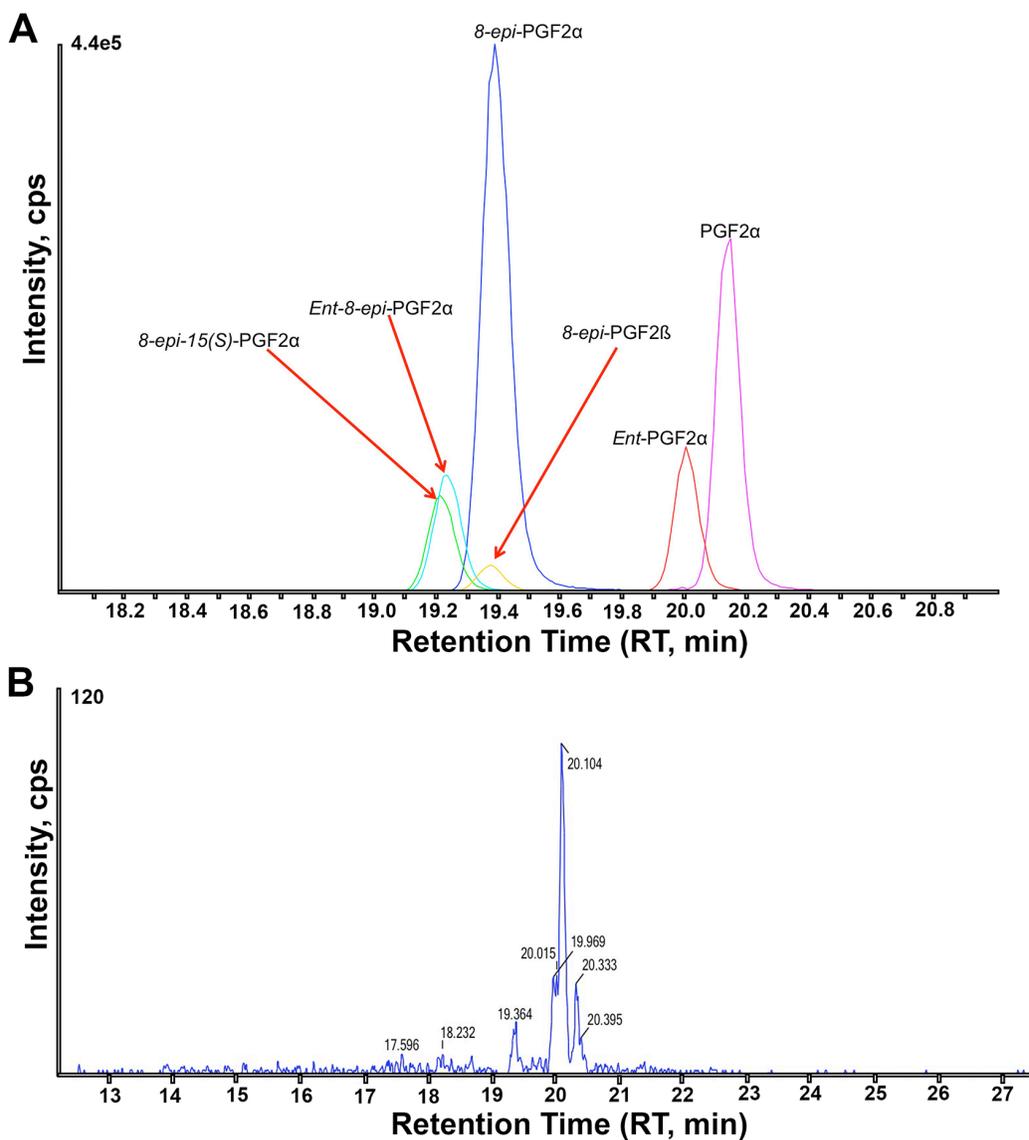
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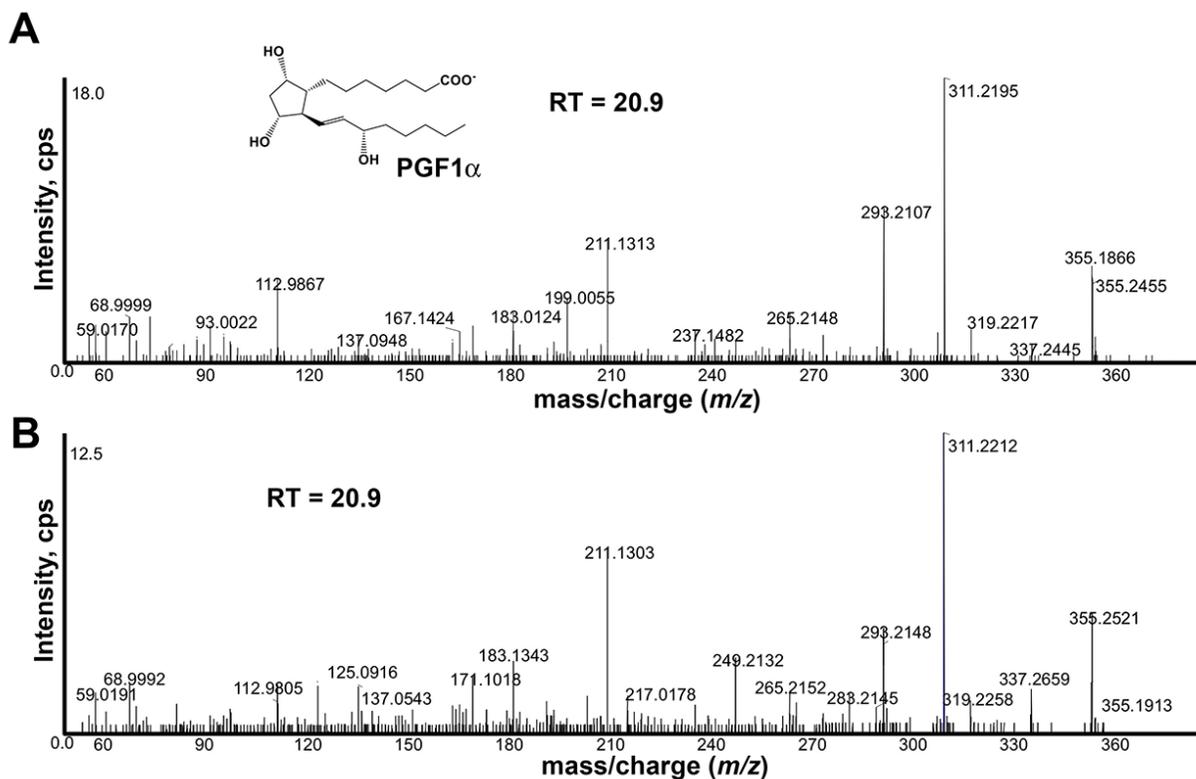
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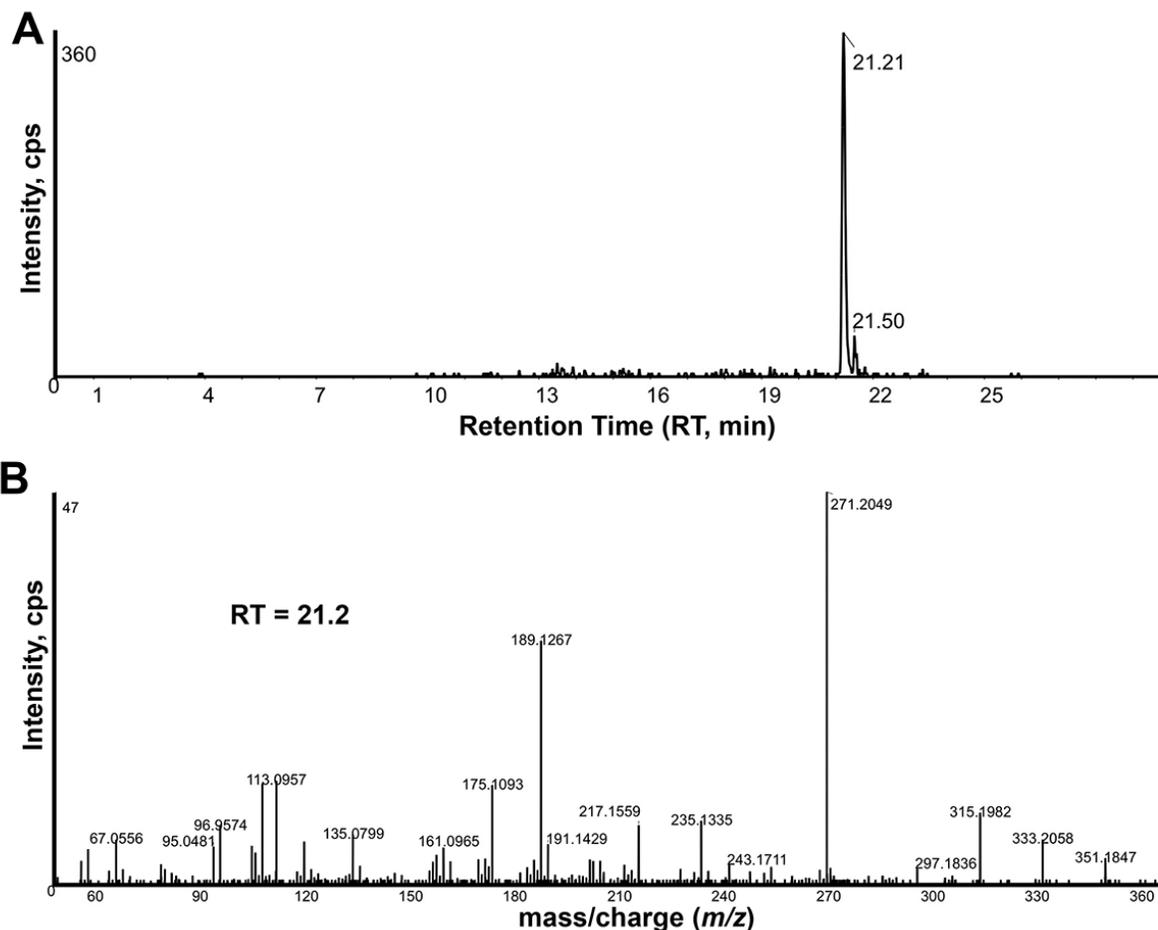
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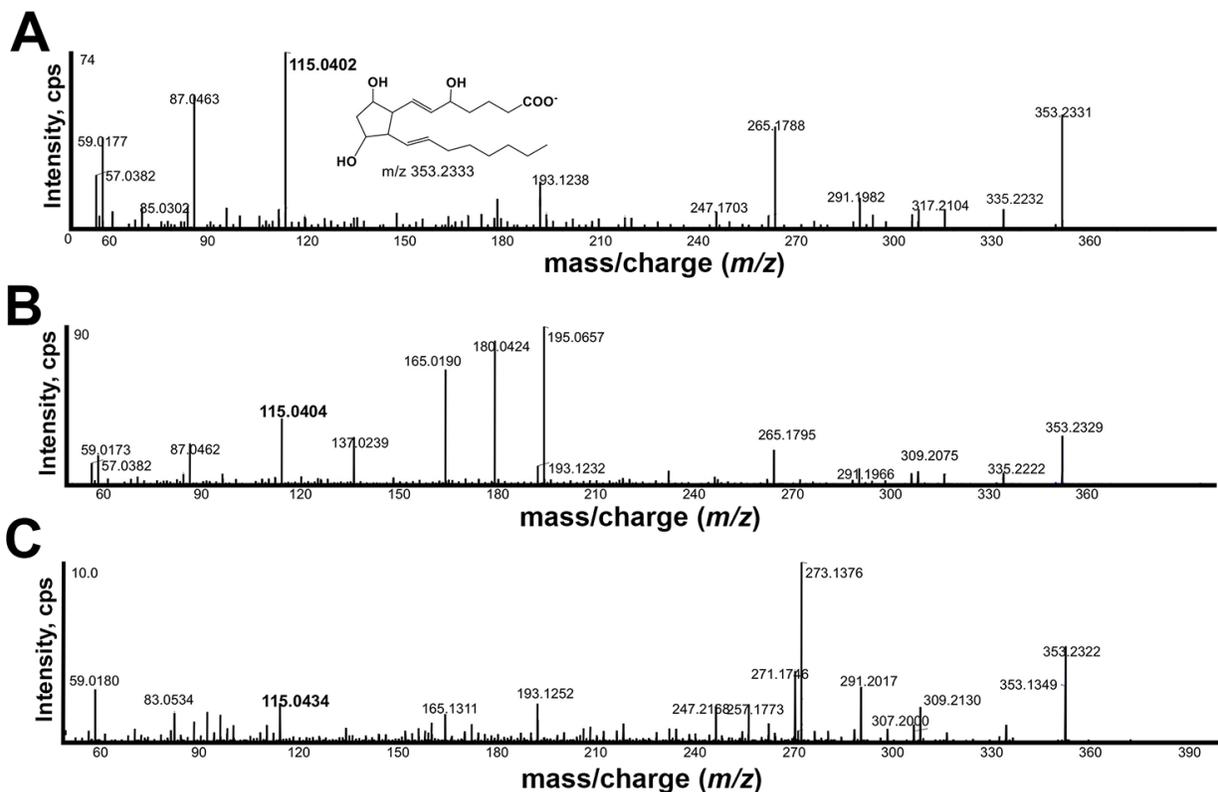
Supplementary Figure 1. Nano-LC Q-TOF chromatograms of F2-isoprostane standards and HFF. An XIC generated using the precursor ion m/z 353.233 using 6 F2-isoprostane standards (A). An XIC generated from HFF using the product ion m/z 193.1218 that is characteristic of 15-F2-isoprostanes (B). Panel B is from a control patient's HFF that is different than the XIC in Figure 1A. Cps, counts per second.



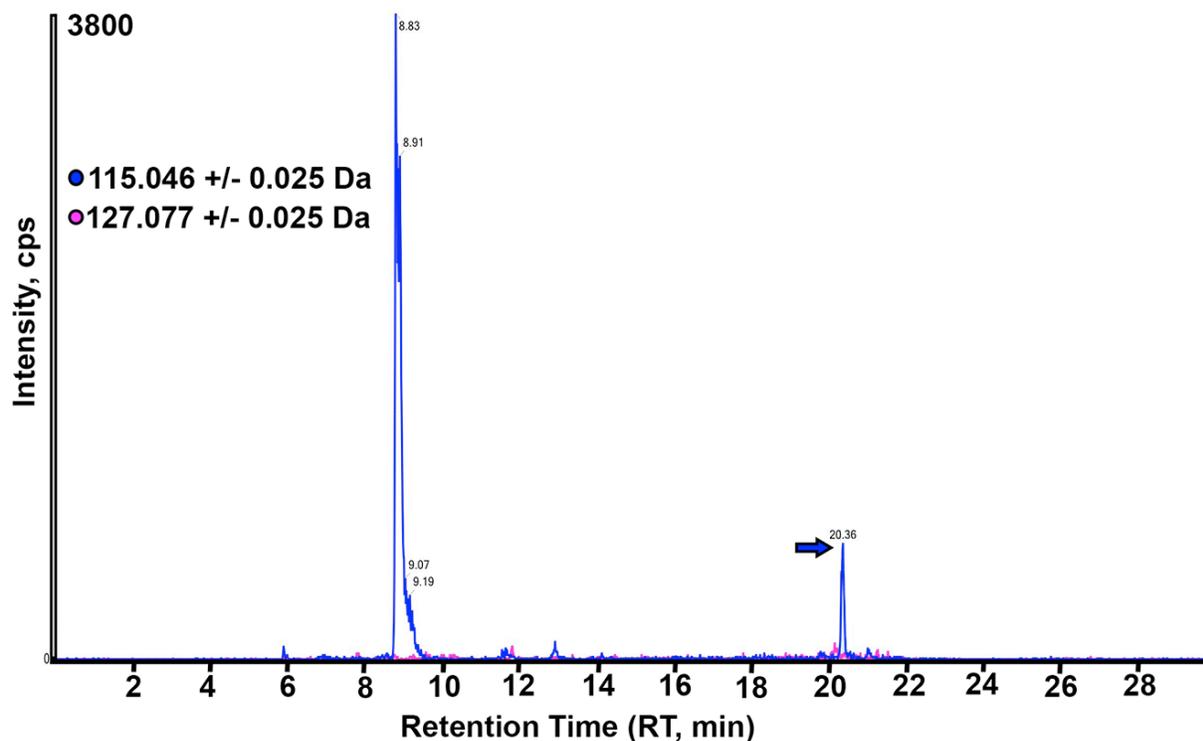
Supplementary Figure 2. PGF1 α in HFF. MS/MS spectra of the PGF1 α standard (A) and m/z 355.245 at RT = 20.9 minutes (B). A single major peak at RT 20.9 minutes was observed in an XIC for m/z 355.242. Panel B is from a single control patient. The few product ions not seen in PGF1 α spectra are low in intensity and could be from co-eluting lipids. Cps, counts per second.



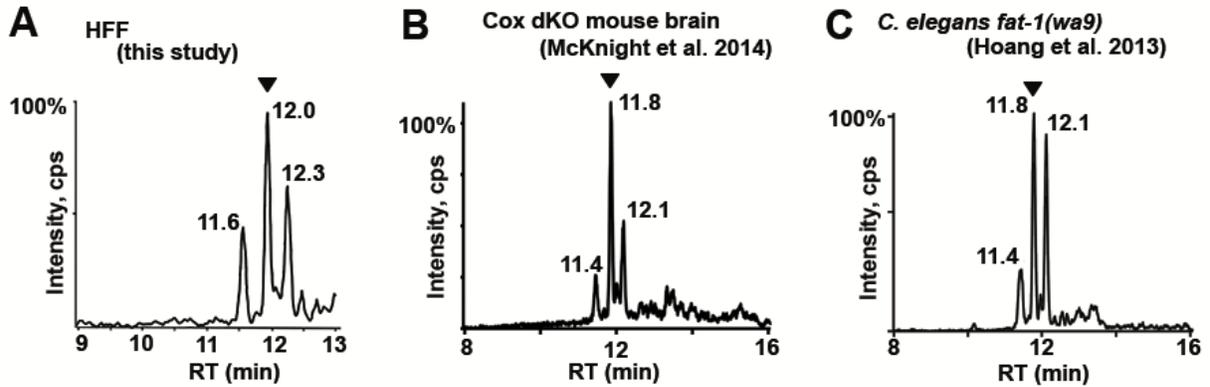
Supplementary Figure 3. PGE2 isomers in HFF. An XIC generated from the product ion m/z 189.127 that is characteristic of PGE2 isomers (A). Authentic PGE2 eluted at RT 21.2 minutes and authentic PGD2 eluted at RT 21.5 minutes. Note that only one major (PGE2) and one minor peak (PGD2) are observed. MS/MS product ion spectra of m/z 351.216 at RT 21.2 minutes in the extracted HFF sample (B). The product ions m/z 333, 315, 271, 235, 189, 175, and 113 are observed in authentic PGE2 and PGD2 [23, 27]. The few product ions not seen in PGE2 spectra are low in intensity and could be from co-eluting lipids. Standards also available online at <http://www.lipidmaps.org/data/standards/index.html>. Panels A and B are from a single control patient. Cps, counts per second.



Supplementary Figure 4. 5-F2-isoprostanes in HFF. MS/MS product ion spectra for three 5-F2-isoprostanes (also known as iPGF 2α -VI) observed in two patients (A-C). 5-F2-isoprostanes contain the characteristic product ion m/z 115.0402, representing C 5 H 7 O 3 ⁻ [21, 33]. A single major 5-F2-isoprostanes was detected in one patient (panel A, also see Supplemental Figure S5) and two 5-F2-isoprostanes were detected in the second patient (B-C). Cps, counts per second.



Supplementary Figure 5. 5-F2-isoprostanes and 8-F2-isoprostanes in HFF. XICs generated from the product ions m/z 115.046 and m/z 127.077 that are characteristic of 5-F2-isoprostanes and 8-F2-isoprostanes, respectively. The blue peak at RT = 20.3 minutes (blue arrow) is a 5-F2-isoprostanes whose MS/MS spectra is shown in Supplemental Figure 4A. The blue peak eluting at RT = 8.9 is not a PG. Note that 8-F2-isoprostanes (pink) are not detected. Cps, counts per second.



Supplementary Figure 6. 15-F2-isoprostanes in three species detected using MRM mass transition m/z 353/193. MRM chromatogram from the HFF of most patients shows three major peaks consisting of multiple 15-F2-isoprostanes (A). F2-isoprostanes in Cox-deficient adult mouse brain [26] (B) and Cox-deficient *C. elegans* [27] (C) are shown for reference. *C. elegans fat-1(wa9)* mutants synthesize specific F2-isoprostanes from arachidonic acid, exclusively [27]. Arrowhead points to the retention time (RT) of the PGF2 α standard in each chromatogram. Cps, counts per second.