Phospholipid esters from herring roe promotes SPM biosynthesis in human monocyte-derived macrophages and a keratinocyte/fibroblast co-culture with implications for the treatment of psoriasis

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Abstract

Immune-mediated inflammatory disease (IMID) is a major public health issue which can affect a number of organs and tissues with a profound impact on quality of life and often present with comorbidities. Psoriasis is a chronic IMID affecting the skin which presents with both local and systemic inflammation as part of its pathophysiology. An oil rich in polar lipids extracted from the roe sacks of herring has been shown to have immunomodulatory functions and to improve the clinical symptoms and impact inflammatory cytokine pathways in psoriasis in a clinical trial. The lipidic nature of herring roe oil and its high content of marine polyunsaturated fatty acids could suggest involvement of lipid mediator pathways for the observed alleviation of psoriatic inflammation. Of particular interest is the super-family of lipid mediators termed specialized pro-resolving mediators (SPMs), due to their known involvement in the resolution of inflammation and return to homeostasis. We have therefore explored the influence of herring roe oil and phospholipid esters from herring roe on lipid mediator and SPM biosynthesis in IFN-γ and LPS-stimulated human monocyte-derived macrophages and an IL-17A-stimulated skin cell co-culture with keratinocytes and fibroblasts. Lipid mediators including SPMs were quantified from resulting cell supernatants using a validated LC-MS/MS protocol. In these experiments we observed broad SPM biosynthesis with dominant upregulation of RvE2 and RvE3 in both cell systems and upregulation of DHA-derived SPMs such as RvD2 and PDX. Observations of PCTR2 in the macrophage cell supernatants also indicate activation of reparative pathways upon treatment with herring roe oil. In conclusion, we observed a promotion of SPM biosynthesis associated with a shift towards a protective and possibly

reparative macrophage phenotype as well as promotion of biosynthesis of pro-resolving lipid mediators in a skin cell co-culture, thus demonstrating a possible mechanism for resolution of inflammation in the skin niche using herring roe oil.

Introduction

Inflammation is a physiological process that has been described by scholars for centuries, even millennia, where inflammation was famously described two thousand years ago by Aulus Cornelius Celsus (25 BC – 50 AD) by the four cardinal signs of redness, swelling, heat, and pain (1–3). With the progress of science, including growing access to modern powerful analytical instruments, an enormous amount of work has since then been put into expanding the knowledge of initiation and perpetuation of inflammation by describing the actions of cytokines and chemokines, as well as the pro-inflammatory eicosanoids (e.g. prostaglandins and leukotrienes) and their importance in driving inflammatory processes (4–6). And it has not been until the most recent decades that scientists have been able to elucidate the active processes such as the lipid mediator class switch for containing inflammation and restoring tissue homeostasis (4,7). Where before it was thought that resolution of inflammation was a passive process that took place through gradual dilution of chemoattractants, it has now been shown that these are active processes regulated through the expression of a super-family of lipid mediators termed specialized pro-resolving mediators (SPMs) (2,5,6,8). SPMs are potent and transient small molecule signaling agents that are endogenously biosynthesized from polyunsaturated fatty acids (PUFAs) and that, as implied by their name, promote the shift towards resolution of inflammation (4,6). The biosynthesis of SPMs from their parent fatty acids (FAs) is regulated by cell type and phenotype, enzyme expression, and substrate availability (2,9–11). In regulating this vital response, SPMs bind to and activate G protein-coupled receptors (GPCRs) (6,12,13) that result in, amongst other, the reduction of polymorphonuclear leukocyte (PMN) infiltration (6), promotion of efferocytosis/phagocytosis (6), and promotion of tissue regeneration (14,15).

The lack of, or incomplete, resolution of inflammation perpetuates what should have been appropriate and self-limiting inflammatory processes and has been linked to chronic inflammation and multiple disease states (16). A group of chronic inflammatory disorders with unknown etiology that share common inflammatory pathways has been collectively termed as immune-mediated inflammatory diseases (IMIDs) (17). IMIDs are a major public health issue with a global incidence of over 67 million cases (18). IMIDs can affect a number of organs and tissues, including the skin, and can have a profound impact on quality of life, often present with comorbidities (19–21). In 2019, 6.84% of new IMID cases were psoriasis (18), a chronic inflammatory skin disease which presents with both local and systemic inflammation as part of its pathophysiology. Hippocrates (460 – 377 BC) described the symptoms of psoriasis as *psora* (itch), and *lopoi (*scaling) and recommended treatment with tar followed by sun exposure (22). It is now known that albeit IMIDs display various symptoms depending on the organ they are affecting, IMIDs share an underlying pathogenesis and, indeed, many of the same drugs are therefore used to treat several of these diseases (23,24). Insights in the molecular basis of inflammatory disease has led to therapies targeting specific IMID-associated immune and inflammatory pathways, with recent focus on developing drugs targeting the cytokines IL-17 and IL-23 (25,26). However, due to their intricate involvement in cessation of inflammation responses, imbalances in lipid-mediated regulation of resolution/inflammation due to dysregulated lipid mediator and SPM biosynthesis and signaling have also been proposed to play a role in the pathophysiology of IMIDs (27–29), and it has been shown that intervention with PUFAs (30) or

81 SPMs (31,32) may hold promise in correcting these imbalances. Lack of immunoresolution due to
82 dysregulated SPM biosynthesis may arise both from dysregulated enzyme expression as a
83 consequence of the disease state (33) or substrate competition from pro-inflammatory precursors
84 released from membrane phospholipids (PLs) (34).

85 Marine PUFA oils have traditionally been considered to have anti-inflammatory properties and have
86 been reported to have potential in treatment of selected IMIDs (35,36). Furthermore, supplementation
87 with marine oils has also been reported to promote SPM and SPM precursor biosynthesis (37–39),
88 where biosynthesis and downstream SPM profiles changes according to different marine oils (39).
89 The difference in conversion between oils implies that the pharmacology may be impacted by other
90 parameters, such as lipid composition and oil source, rather than just the PUFA content/ratios. One
91 marine oil of interest in this regard is herring (*Clupea harengus*) roe oil (HRO), where the nature of
92 the raw material used for extraction causes HRO to have a high content of membrane phospholipid
93 esters enriched with marine PUFAs (PEHeRo = phospholipid esters from herring roe). The benefits
94 of marine PLs have been described previously in literature as having anti-inflammatory effects (40–
95 42) and reports also imply that marine PLs could be superior to other marine lipid forms with regards
96 to bioavailability and active uptake across the blood-brain barrier (43–47), neuroprotective properties
97 (48), and ability to reduce lesion progress in atherosclerosis (49).

98 The long-chain PUFAs (LC-PUFAs) provided by PEHeRo in HRO are primarily docosahexaenoic
99 acid (DHA) and eicosapentaenoic acid (EPA) in a ratio of approximately 3:1. As reported for other
100 marine PLs, the fatty acids in PEHeRo have also been shown to be readily bioavailable and
101 supplementation has shown reduction of plasma and RBC PC n-6/n-3 ratios (50), as well as a higher
102 acute uptake of DHA and EPA when compared to a triacylglycerol source in a head to head study
103 (51). The bioavailability of lipids and their corresponding fatty acids upon administration is relevant
104 for lipid mediator biosynthesis, as LC-PUFA supplementation has been shown to give increased
105 blood and plasma levels of lipid mediators (52–55). Being able to increase blood levels of lipid
106 mediators through supplementation coincides with observations of positive effects on inflammation
107 through direct supplementation of lipid mediators (33,56,57).

108 In addition to the general reports on benefits of marine PLs pertaining to inflammation, HRO
109 specifically has been shown to display effect on peripheral inflammation (58) and has been shown to
110 dampen psoriasis *in vivo* in a clinical trial (59–61). The dampening effect of HRO on psoriasis has
111 been shown in human trials where oral daily treatment with HRO gave statistically significant
112 improvement of mean Psoriasis Area Severity Index (PASI) score (59,60). *Post hoc* analyses of
113 blood samples from the study by Tveit *et al.* (59) further supports the beneficial effects of HRO for
114 dampening of psoriasis by showing that HRO had a beneficial effect on immune cell activation and
115 influenced the immune cell profile by, amongst others, reducing CCL2 over time and leading to an
116 increase in IFN-γR1 (61). The *in vitro* experiments performed by Mildenberger *et al.* (62) further
117 corroborates the potential benefit of HRO in the treatment of psoriasis by showing that HRO limits
118 secretion of IL-23 in macrophages in a dose-dependent manner and limits secretion of IL-17 from
119 CD^{4+} T-cells. Thus, showing that HRO positively impacts immune cells and skin cells impacted by
120 psoriasis in multiple ways.

121 To understand how HRO exerts anti-inflammatory actions, we have studied its impact on lipid
122 mediator biosynthesis in several cell systems. We have studied the effects of HRO on SPM
123 biosynthesis in primary human monocyte-derived-macrophages (MDMs), as these cells have a role in
124 all inflammatory processes and are known to readily express SPMs as part of their signaling in
125 inflammation regulation. Macrophages are also known to change their phenotype towards more anti-

126 inflammatory phenotypes upon stimulation with lipid mediators (63), which is followed by a shift in
127 lipid mediator biosynthesis. In addition, the effects of HRO on SPM biosynthesis in a skin cell model
128 consisting of a co-culture of IL-17A-treated keratinocytes and fibroblasts, which mimics psoriasis,
129 are studied (64). Current knowledge about SPM biosynthesis in skin cells is limited. Therefore, the
130 study aims to elucidate the role of these cells in SPM and lipid mediator biosynthesis during
131 resolution of inflammation. Studying both the immune cells and skin cells simultaneously may give
132 an image of the cell-cell interplay between these cell types locally in the skin niche. Lastly, we have
133 also mapped the lipid mediator content of HRO and isolated PEHeRo in order to have a greater
134 understanding of which mediators are presented to the cells exogenously compared to the output after
135 treatment, and if any of the identified lipid mediators has described roles in specific interleukin
136 pathways relevant to the pathogenesis of inflammatory diseases including psoriasis.

137

138 **Materials and methods**

139 **Preparation of HRO and PEHeRo**

140 Immature herring roe (IHR) was dried under reduced pressure (until H_2O <20% w/w), then contacted
141 with EtOH/H_2O to extract the lipids. Centrifugation of the resulting suspension yielded a clarified
142 lipid extract which then was concentrated under reduced pressure, desalinated by crystallization in
143 EtOH (abs.), and filtered to give a desalinated phospholipid concentrate. The final HRO was prepared
144 by standardization and viscosity modification through addition of a reconstituted triglyceride oil and
145 evaporation under reduced pressure.

146 IHR was contacted with EtOH/H_2O to extract lipids before the resulting extract was clarified by
147 centrifugation and dried under reduced pressure. The dried crude extract was then desalinated by
148 crystallization in EtOH (abs.) and filtration. PEHeRo (>90% PLs w/w dry matter, 85% DM) was
149 purified from the desalinated extract using normal-phase flash column chromatography (EtOH/H_2O
150 88:12, 200 nm).

151 Phospholipid analyses were performed using quantitative ^{31}P NMR (Spectral Service AG) and oil
152 fatty acid analyses were performed using GC-FID according to Ph. Eur. 2.4.29 with modifications to
153 accommodate for PL-rich oils (Mylnefield Lipid Analysis).

154 **HRO and PEHeRo emulsions**

155 HRO and PEHeRo were prepared as 5% emulsions in dH_2O by stirring at 800 rpm under inert
156 atmosphere (N_2 flushing) at 37˚C for 1 hour. Emulsions were then pasteurized under inert
157 atmosphere by sonication at 70˚C for 2 min in an ultrasonic water bath (Branson 2200, 40 kHz) or by
158 an ultrasonic probe. Emulsions were stored at 4˚C for maximum 4 days.

159 **Macrophage cell culture**

160 Buffy coats were obtained from the blood bank Ålesund (Helse Møre og Romsdal, Norway) with
161 approval by the Regional Ethics Committee (REK, ref.: 230804). Peripheral blood mononuclear cells
162 (PBMC) were isolated from buffy coats by Lymphoprep™ (AXIS-SHIELD PoC AS, 1114547)
163 density gradient and monocytes were selected by plastic adherence and differentiated into monocyte-
164 derived macrophages (MDM) by culture in AIM V medium (Gibco, 12055083) with 5 % CTS™

165 Immune Cell Serum Replacement (ICSR) and 10 ng/ml M-Csf (Miltenyi Biotec, 130-093-963) for 5
166 d, followed by incubation without M-Csf.

167 MDMs from each donor were treated in 1.5 mL per well in a 6-well plate (3 wells per condition) with
168 indicated final percentages (w/w) of HRO or PEHeRo, by dilution of the prepared emulsions in
169 phenol- and serum-free CTS™ AIM-V™ Medium (Gibco, A3830801) supplemented with Pen-Strep.
170 MDMs were either pretreated with HRO for 16 h, followed by change of medium with addition of 1
171 µg/mL lipopolysaccharide (LPS, Sigma-Aldrich, L2630) and 50 ng/mL IFN-γ (Miltenyi Biotec, 130-
172 096-481) for further 24 h or co-treated with either HRO or PEHeRo and 1 ng/mL LPS for 24 h.
173 Supernatants were collected, pooled per condition, centrifuged and frozen at -80°C until shipping on
174 dry ice for analysis.

175 **Keratinocyte and fibroblast co-culture**

176 HaCaT keratinocytes (CLS Cell line service GmbH, cat no 300493) were co-cultured with normal
177 human primary dermal fibroblasts (ATCC PCS-201-012) in Dulbecco´s modified Eagle´s medium
178 (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL
179 streptomycin and 250 µg/mL fungizone (all purchased from Thermo Fisher Scientific) in T25 tissue
180 culture flasks (Nunc™ EasYFlask™, VWR) and stimulated with and without 100 ng/mL IL-17A
181 (Sigma-Aldrich, cat.no SRP0675) and 5 µg/mL HRO for 96 h, before media were collected and
182 stored at -80 ºC for further fatty acid and lipid mediator analysis. The co-culture was established by
183 first seeding fibroblasts at $1x10^3$ cells per cm^2 and pre-cultured in the media described above for 2-
184 days, before adding 4,3 $x10^3$ keratinocytes per cm^2 and co-culturing additional 5 days before
185 initiating stimulation with HRO with and without IL-17A for 96 h as described above.

186 **Fatty acid composition of cells**

187 Total lipids were extracted from the cells according to the protocol by Folch *et al*. (65). The $CHCl_3$
188 phase was dried under nitrogen gas, and thereafter the samples were methylated over night with
189 benzene, 2% H_2SO_4 in MeOH, and dimethoxypropanate at 50 °C. The methyl esters of the fatty acids
190 were separated using GC (Hewlett Packard 6890) with flame-ionizing detector (FID) using a BPX70
191 column (60 m, 0.25 mm i.d., 0.25 µm film) with a temperature program starting at 50 °C, and
192 increasing at a rate of 4 °C /min until 170 °C, then raised to 0.5 °C/min until 200°C, and finally to 10
193 °C/min until 240 °C. The peaks were integrated using GC ChemStation software (Agilent
194 Technologies, Rev. B.01.01 SR1, 2001-2005). The fatty acids were identified by an external standard
195 (GLC 463, Nu-Chek Prep), and the concentration of the individual fatty acids was calculated based
196 on an internal standard (C23:0, Larodan). For analysis of fatty acid composition in lipid classes, the
197 $CHCl_3$ lipid extracts were first evaporated under nitrogen gas and re-dissolved in hexane. The lipid
198 classes (phospholipids, triacylglycerides and free fatty acids) were separated by thin layer
199 chromatography using a mixture of petroleum ether, Et_2O and AcOH (113:20:2, by volume) as the
200 mobile phase. The lipids were visualized by spraying the TLC plates with 0.2% (w/v) 2′,7′-
201 dichlorofluorescein in MeOH, and then identified by comparing with known standards (Sigma
202 Chemical Co.) under UV light. The spots corresponding to the different fractions were scraped off
203 into glass tubes, trans-methylated following the aforementioned procedure and the fatty acid
204 composition determined by GC-FID.

205 **Lipid mediator analyses**

206 Lipid mediator analysis was performed using methodologies and approaches recently published by
207 Dooley *et al.* (66).

208 Snap frozen media was placed in 2 volumes of MeOH containing deuterated internal standards (IS)
209 (d_4-LTB$_4$, d_5-MaR1, d_5-MaR2, d_4-PGE$_2$, d_5-LXA$_4$, d_5-RvD$_3$, d_5-RvD2, d_4-RvE1, d_5-17R-RvD1, d_5-
210 LTE$_4$, d_5-LTD$_4$ and d_5-LTC$_4$), representing the chromatographic regions of interest, were added to
211 facilitate lipid mediator identification and quantification. This was placed at -20 ºC for at least 45
212 minutes then centrifuged and supernatants were subjected to solid-phase extraction (SPE) using the
213 ExtraHera system (Biotage) and Isolute C18 500 mg columns (Biotage).

214 Oils samples were placed in 2 volumes of MeOH containing 2.5 ng of deuterated IS (d_4-LTB$_4$, d_5-
215 MaR1, d_5-MaR2, d_4-PGE$_2$, d_5-LXA$_4$, d_5-RvD$_3$, d_5-RvD2, d_4-RvE1, d_5-17R-RvD1, d_5-LTE$_4$, d_5-LTD$_4$
216 and d_5-LTC$_4$) and incubated in 2 volumes of KOH (1N) for 45 minutes at 45 ºC. At the end of the
217 incubation the samples were immediately acidified and subjected to SPE using the ExtraHera system
218 (Biotage) and Isolute C18 500 mg columns (Biotage). Oils analysed for unesterified lipid mediators
219 were not saponified prior to being subjected to SPE.

220 Methyl formate and MeOH fractions from C18 SPE were collected, brought to dryness, and
221 suspended in phase (MeOH/H_2O, 1:1. v/v) for injection on a Shimadzu LC-20AD HPLC and a
222 Shimadzu SIL-20AC autoinjector, paired with a QTrap 6500+ (Sciex). Analysis of mediators isolated
223 in the methyl formate fraction was conducted as follows: an Agilent Poroshell 120 EC-C18 column
224 (100 mm × 4.6 mm × 2.7 µm) was kept at 50 °C and mediators eluted using a mobile phase consisting
225 of MeOH/H_2O/AcOH of 20:80:0.01 (v/v/v) that was ramped to 50:50:0.01 (v/v/v) over 0.5 min and
226 then to 80:20:0.01 (v/v/v) from 2 min to 11 min, maintained till 14.5 min and then rapidly ramped to
227 98:2:0.01 (v/v/v) for the next 0.1 min. This was subsequently maintained at 98:2:0.01 (v/v/v) for
228 5.4 min, and the flow rate was maintained at 0.5 mL/min. In the analysis of mediators isolated in the
229 MeOH fraction, the initial mobile phase was MeOH/H_2O/AcOH of 20:80:0.5 (v/v/v) which was
230 ramped to 55:45:0.5 (v/v/v) over 0.2 min and then to 70:30:0.5 (v/v/v) over 5 min and then ramped to
231 80:20:0.5 (v/v/v) for the next 2 min. The mobile phase was maintained for 3 min and ramped to
232 98:2:0.5 (v/v/v) for 2 min. QTrap 6500+ was operated using a multiple reaction monitoring (MRM)
233 method. Each lipid mediator was identified using the following criteria: (1) matching retention time
234 to synthetic or authentic standards (±0.05 min), (2) a signal-to-noise ratio ≥ 5 for a primary transition,
235 and (3) a signal-to-noise ratio of ≥3 for a secondary transition. Data was analyzed using validated
236 protocols (66) and Sciex OS v3.0. Chromatograms were reviewed using the AutoPeak algorithm,
237 using 'low' smoothing setting and signal to noise ratios were calculated using the relative noise
238 algorithm (66). External calibration curves were used to quantify identified mediators. Where
239 available calibration curves were obtained for each mediator using synthetic compound mixtures that
240 gave linear calibration curves with R^2 values of 0.98–0.99. These calibration curves were then used
241 to calculate the abundance of each mediator. Where synthetic standards were not available for the
242 construction of calibration curves, calibration curves for mediators with similar physical properties
243 (e.g. carbon chain length, number of double bonds, number of hydroxyl groups and similar elution
244 time) were used.

245 **Statistical analysis**

246 Statistical significances were determined using; the Mann-Whitney U test (one-tailed/two-tailed as
247 appropriate) for comparison of two groups, one-factor ANOVA and one-way/two-way Dunnett's
248 multiples comparison test or Tukey's HSD for comparison of >2 groups. Statistical significance
249 criteria for difference between groups was $P < 0.05$. All results are presented as mean values ± the
250 standard error of the mean (SEM). PLS-DA analyses were performed using MetaboAnalyst v6.0
251 (67), all other statistical analyses were performed using Microsoft Excel for Microsoft 365 MSO.

252

253 **Results**

254 **Oil composition and lipid mediator content in HRO and PEHeRo**

255 In order to understand the effects of phospholipid ester rich oils on cellular systems, it is important to
256 understand the composition of the oils both with regards to fatty acid profile and lipid class
257 distribution, but also with regards to lipid mediators potentially present in the oil. We therefore
258 started off with characterizing the composition of the oils used.

259 Herring roe oil (HRO) is a lipid extract from herring roe that has been standardized with regards to
260 content of *phospholipid esters from herring roe* (PEHeRo) using a marine triacylglyceride (TAG) oil.
261 The phospholipid esters in PEHeRo are predominantly phosphatidylcholine (>80%) that are enriched
262 with LC-PUFAs. The fatty acid profiles in both HRO and PEHeRo display a high content of DHA
263 with a DHA/EPA ratio of approximately 3:1, where DHA and EPA make up 50-60% of the total fatty
264 acid profile in area %. The HRO and PEHeRo investigated in this work had a phospholipid content of
265 34% and 91% (per anhydrous substance) and 425 mgTAG/g and 318 mgTAG/g DHA+EPA,
266 respectively. Lastly, HRO and PEHeRo were found to contain 6% area and 1% area of omega-3
267 docosapentaenoic acid (n-3 DPA), respectively.

268 After establishing the macroscopic lipid composition, the content of lipid mediators in HRO and
269 PEHeRo was determined, as it is possible that the lipid mediator levels in the oils could influence the
270 behavior and SPM biosynthesis in the cell systems. The oils were screened for SPMs and
271 monohydroxylated fatty acids (including SPM precursors) originating from DHA, n-3 DPA, EPA,
272 and arachidonic acid (AA). The qualitative lipid mediator profiles of the two oils were,
273 unsurprisingly, found to be similar, with the predominant SPMs originating mostly from the DHA
274 metabolome in form of RvD5, RvD6, PDX, 17R-PD1, MaR1, and MaR2 (Fig. 1A). The most similar
275 relative abundance between HRO and PEHeRo was found for 18-HEPE, RvD5, MaR2, and RvD5 $_{n\text{-}3}$
276 $_{DPA}$ where the groups rank sums indicated a $P > 0.1$ (two-way) as denoted with # in (Fig 1 A-C).
277 Notable differences between the oils included a relative lower abundance of PDX and higher relative
278 abundance of MaR1 and RvE4 in PEHeRo compared to HRO. Furthermore, there were also notable
279 contributions from SPMs originating from the n-3 DPA (RvT4 and RvD5 $_{n\text{-}3\ DPA}$) and EPA (RvE4)
280 metabolomes, whereas lipid mediators from the AA metabolome, as expected, only made up 1-2% of
281 the total SPM content in the oils.

282 The key difference between HRO and PEHeRo pertaining to SPM content, was the >10 times higher
283 concentration of overall SPMs in the less refined HRO compared to the concentrated phospholipid oil
284 PEHeRo. The same was also observed for the monohydroxylated fatty acid levels in the two oils.

285 Furthermore, the monohydroxylated fatty acid (HDHAs, HEPEs, HDPAs, and HETEs) content was
286 found to be surprisingly high in HEPEs given the overall fatty acid composition, with 5-HEPE and
287 18-HEPE being the two major contributors (Fig. 1B and Fig. 1C). The HEPE/HDHA ratio was found
288 to be 1.8 and 1.4 for HRO and PEHeRo respectively (Fig. 1B), where a significant portion (>50%) of
289 total HEPEs and HDHAs were found to be present as metabolically available free fatty acids not
290 bound in lipids. The sum of HEPEs and HDHAs made up >95% of the total amount of
291 monohydroxylated fatty acids in the two oils. Lastly, we found higher levels of unesterified EPA in
292 both HRO and PEHeRo compared to DHA free fatty acid.

293 **Supplemented HRO is taken up by macrophages and keratinocytes/fibroblasts**

294 The uptake of fatty acids from HRO was analyzed by GC-FID, showing a significant increase in
295 DHA, n-3 DPA, and EPA in total lipids in the MDMs (Fig. 2A and supplementary table S1). In the
296 direct keratinocyte/fibroblast co-culture, it was further shown that the changes introduced by HRO
297 could be observed in the phospholipid fractions of the cells with similarly significantly increased
298 EPA and DHA and decreased AA (Fig. 2B and supplementary table S2).

299 **HRO and PEHeRo promote SPM biosynthesis in monocyte-derived macrophages**

300 The macrophage experiments conducted by Mildenberger *et al.* demonstrated reduction in cytokine
301 secretion, and we therefore wanted to study SPM biosynthesis in the macrophages treated with HRO
302 in order to better understand how this oil and its phospholipids (PEHeRo) elicits their anti-
303 inflammatory and possibly proresolving activities (62).

304 We therefore quantified the SPMs present in the supernatants from likewise stimulated MDMs,
305 which showed a significant upregulation of SPM concentrations when macrophages were pre-treated
306 with HRO prior to stimulation with LPS+IFN-γ (Fig. 3A-D and supplementary table S3). The
307 biosynthesis was found to be significantly upregulated for SPMs from the DHA, EPA, and AA
308 metabolomes, including a non-significant trend for upregulation of n-3 DPA SPMs. The EPA SPM
309 RvE3 was found in highest concentration in the treated samples. The overall SPM profiles of the
310 treated macrophages showed activation of both ALOX5 and ALOX15 pathways.

311 Partial least squares discriminant analysis (PLS-DA) of the SPMs in the cell supernatants from the
312 treated and untreated macrophages (including a fresh media control sample) as shown in Fig 2C also
313 displayed a difference between the groups. This shift in SPM concentrations indicated by the
314 separation of the clusters in green (2, untreated) and blue (3, treated with HRO) in the PLS-DA
315 scores plot. The chart shown in Fig. 3D shows the variable importance in projection (VIP) scores of
316 individual SPMs that contribute to the separation of the clusters in the PLS-DA with a VIP score >1
317 identifying those mediators that contribute to the observed separation to the greatest extent. VIP
318 scores >1 were observed for 4 SPMs from the DHA metabolome (RvD1, RvD4, PD1, and PDX) and
319 2 SPMs from the n-3 DPA metabolome ($RvD5_{n\text{-}3\ DPA}$ and $PD1_{n\text{-}3\ DPA}$).

320 Considering the promotion of SPM biosynthesis, we observed in the macrophages when pretreated
321 with HRO, we wanted to further explore this and see if we were able to optimize the experiment
322 setup with regards to measured concentrations of SPMs in the supernatant after
323 treatment/stimulation. Also, considering that we this far have only looked at the complete HRO, we
324 also wanted to evaluate if there were any differences between HRO and a purified sample of PEHeRo
325 in terms of ability to promote SPM biosynthesis in primary immune cells. So, we tested two
326 concentrations of HRO (0.05% and 0.25%) and one concentration of PEHeRo (0.25%) in an
327 experiment where the macrophages were co-stimulated with the oils and 1.0 ng/mL LPS for 24 hours
328 before SPM analysis of the cell supernatants, similarly to the conditions employed by Sobrino *et al.*
329 (39).

330 Similarly to the initial experiment, we also observed a significant upregulation of SPM biosynthesis
331 in the macrophages treated with HRO and PEHeRo (Fig. 4A-D and supplementary table S4). The
332 results also showed, as expected, generally higher concentrations of SPMs in the cell supernatants,
333 thus offering a potential for greater understanding of the pathways activated upon treatment with
334 HRO and PEHeRo.

335 All three treatments conditions (0.05% HRO, 0.25% HRO, and 0.25% PEHeRo) gave significant
336 upregulation of the sum of SPMs from the DHA and EPA metabolomes (Fig. 4A-B) when compared

337 to media stimulated with LPS (control). We also observed a nonsignificant trend for upregulation of
338 SPMs from the DPA metabolome and, in contrast to the first experiment, no upregulation of AA
339 SPMs. Similarly to the first experiments, the most strongly expressed SPM was the EPA-derived
340 SPM RvE3.When comparing the two treatments concentrations of HRO (0.05% and 0.25%), we
341 observed a nonsignificant dose-dependent trend where the higher treatment concentration gave higher
342 mean values than the lower concentration. The macrophage SPM biosynthetic response to the
343 treatment did not reflect the 5-times increase in treatment concentration and could indicate saturation
344 of the system with regards to accessible fatty acids for SPM biosynthesis.

345 Looking at individual SPMs through the PLS-DA analysis from the experiments, we also see here
346 that PEHeRo elicits a different response from the treated macrophages compared to the two
347 concentrations of HRO. This is well visualized through the clear separation of the cluster for
348 PEHeRo and the two HRO clusters in Fig. 4C. The partial overlap of the two different doses of HRO
349 is in alignment with the observation made for the abundance of SPMs from the different
350 metabolomes, where a nonsignificant trend for dose-dependency was observed. The VIP scores
351 shown in Fig. 4D indicate which individual SPMs are drivers for the separation of the clusters
352 similarly to Fig. 3C. The VIP scoring shows also here that RvD1 is the biggest driver for separation
353 of the clusters, followed by RvE3 which was found to be the most abundant SPM present in both
354 experiments. Furthermore, we see that individual SPMs from all four metabolomes (DHA, n-3 DPA,
355 EPA, and AA) contribute to the separation of the different clusters. Despite not observing significant
356 upregulation of total n-3 DPA SPMs between the groups, the individual n-3 DPA SPMs with a VIP-
357 score >1 were found to be significantly upregulated: RvD1 $_{n\text{-}3\ DPA}$ for HRO and PEHeRo at 0.25%,
358 RvT2 for PEHeRo at 0.25%, and RvT1 for both concentrations of HRO, thus corroborating their
359 significance in the separation of the clusters in the PLS-DA analysis.

360 Interestingly, the VIP scoring in Fig. 4D also shows the cysteinyl DHA-derived SPM PCTR2 (PCTR
361 = protectin conjugates in tissue regeneration) from the DHA metabolome to be a significant
362 contributor to the separation of the different clusters, thus indicating activation of reparative
363 pathways and not only protective SPM pathways.

364 **HRO promotes SPM biosynthesis in IL-17A stimulated fibroblast/keratinocyte co-culture**

365 To study the potential effects of HRO on the IL-23/IL-17 cytokine axis in psoriasis, a co-culture of
366 keratinocytes and fibroblasts that mimics psoriasis upon stimulation with IL-17A was developed.
367 Mildenberger *et al.* (62) showed that HRO dampens psoriatic marker S100A7 (psoriasine) and the
368 IL-17 response gene NFKBIZ in this co-culture. Considering the dampening effects observed in these
369 co-culture experiments, we wanted to see if the SPM biosynthesis was upregulated from the co-
370 culture in this *in vitro* psoriasis-mimicking cell model.

371 Quantification of SPMs and monohydroxylated fatty acids in the co-culture cell media showed
372 significant upregulation of SPMs from the DHA and EPA metabolomes, as well as significant
373 upregulation of monohydroxylated DHA, EPA, and n-3 DPA (Fig. 5A-D and supplementary table
374 S5). It was encouraging to see a significant promotion of SPM biosynthesis taking place despite only
375 treating the cells with 1/100 (0.0005%) of the amount of HRO compared to the 0.05% HRO used in
376 the macrophages and reporting on 1/3 of the amount of media (3 mL vs 1 mL). The amounts
377 measured in the media were of course affected by the decreased concentration of HRO, and the
378 overall quantities are therefore lower compared to what was observed in the macrophage
379 experiments. It should be noted that a parallel co-culture experiment (n=6) showed similar results
380 albeit with somewhat more moderate levels of lipid mediators. This experiment did not show

381 significant upregulation of sum DHA-derived SPMs (only PD1 in the HRO group). Nor was a
382 significant increase in the sum of HDPAs observed, only a significant increase of 17-HDPA was
383 observed compared to the stimulated control (see supplementary table S6 for more information).

384 The DHA SPMs that contributed to the significant upregulation of sum of DHA SPMs (Fig. 5A) in
385 both the groups treated with HRO included RvD1, RvD2, RvD3, RvD5, RvD6, and PD1, of which
386 RvD5 and RvD6 were significant in HRO+IL-17A compared to the stimulated control. The
387 contributors to the overall activation of the DHA SPM metabolome coincide with observations made
388 in the macrophage experiments where RvD1 and PD1 had VIP scores >1 when HRO was used to
389 pretreat macrophages prior to stimulation with LPS+IFN-γ and RvD1 and RvD2 had VIP scores >1
390 when macrophages were costimulated with HRO/PEHeRo + LPS. Significant upregulation of
391 monohydroxylated fatty acids (HDHAs and HDPAs) also could indicate that the HRO indeed
392 promotes activation of the metabolic pathways for SPM biosynthesis from the DHA and DPA
393 metabolomes. The HDHA profile measured in the media supports the observations made of the
394 SPMs (Fig. 5C), as the main monohydroxylated fatty acid found was 17-HDHA where the 17S-
395 HDHA isomer is a precursor for the D-series resolvins formed through ALOX15. 17-HDHA was
396 significantly favored over 14-HDHA where the 14S isomer is a pathway marker for the biosynthesis
397 of MaR1 and MaR2 through ALOX12. The apparent preference for 17-HDHA thus correspond with
398 the observed levels of MaR1 and MaR2 where neither of which were measured above the LLOQ.
399 The same also being the case for the experiments with the macrophages where no consistent or
400 significant measurements of MaR1 and MaR2 were made. It should be noted that this was more
401 pronounced for the HDPAs, as only 17-HDPA was measured in the media samples.

402 Furthermore, SPMs from the EPA metabolome were also significantly increased in both treatment
403 groups compared to the stimulated control (Fig. 5B). This was primarily driven by RvE3 and to a
404 lesser extent RvE2 (approximately 1:40 RvE2/RvE3), both of which were also separately
405 significantly upregulated compared to the stimulated control. RvE2 and RvE3 being the main EPA
406 SPMs measured is not surprising when looking at the HEPE profile of both treatment groups (Fig.
407 5D), where 18-HEPE was found in the, by far, highest concentration in the media. The 18R isomer of
408 18-HEPE is a SPM precursor biosynthesized via CYP450 and GPX4 and is the main intermediate
409 before a one-step conversion via ALOX15 into RvE3 or the conversion to RvE2 via ALOX5. The
410 abundance of 15-HEPE was far lower than the abundance of 18-HEPE, which may explain why
411 RvE4 was not found above the LLOQ as 15S-HEPE is the main precursor for RvE4.

412 Including a group of "healthy" cells only treated with HRO shows, that there may be an impact to the
413 lipid mediator biosynthesis when the cells are stimulated with IL-17A. The greatest impact of IL-17A
414 stimulation was observed for the EPA metabolome, with a significant reduction of 18-HEPE, sum
415 HEPEs, and EPA SPMs when comparing the HRO group with the HRO+IL-17A group.
416 Interestingly, only RvE3 was significantly reduced, whereas only a nonsignificant trend was seen for
417 RvE2. An impact of IL-17A stimulation was also observed for SPMs in the DHA metabolome, albeit
418 to a lesser extent. Here we observed that both RvD2 and PD1 were significantly upregulated in the
419 HRO group, but not in the HRO+IL-17A group. The negating effect of IL-17A on lipid mediator
420 biosynthesis was only observed in one out of two data sets, as can be seen from Table S6 in the
421 supplementary data.

422

423 **Discussion**

424 An interesting finding from the macrophage experiments in the present study was the comparison of
425 total SPMs across different metabolomes. Where notably, treatment with PEHeRo significantly
426 increased the biosynthesis of SPMs from the DHA metabolome compared to HRO. In addition to the
427 significant difference between PEHeRo and HRO in the DHA metabolome, a similar observation was
428 made for SPMs from the EPA metabolome albeit as a nonsignificant trend. The observed increase in
429 SPM biosynthesis in macrophages treated with PEHeRo cannot be explained by availability of
430 metabolically available free fatty acid precursors or availability of mono-hydroxylated fatty acids,
431 including lipid mediator precursors, as PEHeRo was shown to contain far less of these compounds
432 compared to the HRO used in these experiments. Thus, any normalization based on availability of
433 free EPA/DHA, bound EPA/DHA, or monohydroxylated fatty acids (18-HEPE and 17-HDHA) only
434 increases the difference observed when comparing HRO and PEHeRo based on overall
435 concentration. This indicates that the more purified PEHeRo more efficiently promotes upregulation
436 of biosynthesis and that the oils' ability to upregulate these pathways depends on other factors such
437 as matrix complexity or lipid class composition and not just the precursor availability. For instance,
438 phospholipids have been indicated to give increased *in vivo* bioavailability of PUFAs compared to
439 corresponding triacylglycerides (51,68), and their lysoforms may also themselves act as signaling
440 molecules (69). The observations correspond with existing data that indicates that different oils elicit
441 different ability to promote conversion of PUFAs and SPM precursors into SPMs (39). Pinpointing
442 the impacting factors for these observations needs further studies to be verified.

443 Consistent dominant upregulation of the EPA-derived SPMs RvE2 and RvE3 was observed in all
444 experiments, with RvE3 being the most abundant of the two. The higher relative concentration of
445 RvE3 compared to other SPMs can possibly be attributed to RvE3 being available in one enzymatic
446 transformation from 18R-HEPE by ALOX15, which was likely abundant in both HRO and PEHeRo
447 from the measured levels of 18-HEPE (achiral analysis). RvE2 is also biosynthesized from 18R-
448 HEPE but through two steps by ALOX5 and a dehydrogenase. The availability of 18-HEPE in the
449 oils does not fully explain the strong upregulation of RvE3, as more RvE3 was found in cell media
450 when the macrophages were stimulated with PEHeRo which contained >10x less 18-HEPE than
451 HRO. The consistent upregulation of RvE2 and RvE3 across both the macrophages and the skin cell
452 co-culture experiments is particularly interesting given that RvE2 has been reported to be a regulator
453 of neutrophil infiltration in addition to enhancing phagocytosis (70), and more importantly, RvE3 has
454 been reported to attenuate inflammation via the IL-23/IL-17A-axis (71) which is highly relevant for
455 psoriatic inflammation.

456 The most recurring DHA-derived SPM was found to be RvD2, which was observed consistently in
457 the macrophage experiments and for the HRO treated group in the keratinocyte/fibroblast co-culture
458 experiments. RvD2 takes part in regulating inflammation by increasing phagocytosis and reducing
459 neutrophil infiltration (72), promoting macrophage polarization (73), and reducing expression of
460 proinflammatory cytokines (72). The protectin PDX has been reported to be implicated in regulation
461 of psoriasis, as it has been found to be elevated in psoriasis lesional skin (74), and was measured in
462 all the macrophage experiments. By being upregulated in the immune cells treated with HRO and
463 PEHeRo and involved in regulation of inflammation in psoriasis, PDX could be a promising
464 biomarker for future studies in psoriasis using marine phospholipids.

465 Showing that we were able to upregulate SPM biosynthesis in the keratinocyte/fibroblast co-culture
466 was intriguing, considering that there, to our knowledge, are few studies detailing the ability of lipid
467 supplementation to regulate SPM production in these cells. The monohydroxylated fatty acid profiles
468 could imply that a key pathway for synthesis of lipid mediators from DHA and DPA goes through
469 17-HDHA, as there were found significantly higher concentrations of 17-HDHA compared to 14-

470 HDHA. This was even more pronounced for n-3 DPA, where only 17-HDPA was found in the media
471 upon analysis. Also, it was interesting to see activation of the DPA metabolome considering the
472 much lower content of this PUFA in HRO, thus implicating the importance of this pathway in
473 regulation of inflammation. The apparent preference for the 17-hydroxylated DHA and n-3 DPA are
474 aligned with literature showing 17-HDHA biosynthesis through ALOX15 in normal human
475 keratinocytes when treated with DHA (75). Morin *et al.* have shown that supplementation of a
476 psoriatic skin cell model with pure EPA also led to increased epidermal production of 17-HDHA,
477 besides PGE3 and 12-HEPE, but not in the presence of polarized T-cells (76). Future experiments in
478 the keratinocyte/fibroblast co-cultures should include chiral analyses of the monohydroxylated fatty
479 acids in order to further elucidate which enzymes that are involved. Our findings furthermore
480 indicates that the SPM biosynthesis from DHA and DPA is maintained in an inflamed state, as the
481 precursor levels were found to be very similar between the HRO and the HRO + IL-17A groups.
482 However, where ALOX15 seems to be important for conversion of DHA and DPA, this does not
483 seem to be the case for EPA. Biosynthesis via 18-HEPE seems to be the main pathway for EPA
484 derived lipid mediators with 15-HEPE levels being comparatively lower. This could be due to
485 substrate vs enzyme availability in the system, but the finding is also consistent with the SPMs found
486 in both the skin cells and the macrophages, where the downstream product of 15S-HEPE RvE4 was
487 not measured in any of the experiments.

488 The observed biosynthesis of the cysteinyl SPM PCTR2 in the macrophages may also indicate a shift
489 towards a reparative phenotype as well as a protective one, as the PCTRs have been reported to be
490 biosynthesized by macrophages and to be involved in macrophage phenotype regulation and tissue
491 regeneration (14,77,78). The PCTRs are biosynthesized similarly to PD1 through ALOX15
492 conversion of 17S-HpDHA into 16S,17S-epoxy-DHA, but with a last step through a
493 glutamyltransferase instead of an epoxide hydrolase to give PCTR1 instead of PD1. PCTR1 is then
494 hydrolyzed to PCTR2 which may be taken through a final hydrolysis to PCTR3. The linear nature of
495 their biosynthesis implies that PCTR1 also was produced by these cells prior to being hydrolyzed to
496 PCTR2, which is interesting given that PCTR1 has been shown to stimulate keratinocyte migration
497 and facilitate host defense (14).

498 In addition to promoting lipid mediator biosynthesis, we also observed that HRO significantly
499 increased the levels of EPA/DHA in the total fatty acids in the MDMs and significantly increased the
500 levels of EPA/DHA and reduced the levels of AA in the PL-fraction of the fibroblast/keratinocyte co-
501 culture. Allowing for potential replacement of AA in cell membranes could positively impact
502 psoriasis, as overexpressed PLA2 enzymes liberating AA for conversion into pro-inflammatory lipid
503 mediators is a driver for psoriatic inflammation (79).

504 The results presented in this work also shows that both HRO and PEHeRo contain SPMs, including:
505 1) PDX that has been shown to ameliorate inflammation and promote macrophage polarization in
506 experimental models of infection (80). 2) MaR1 that has been reported to display immunoresolving
507 effects in models of skin inflammation through regulation of IL-23 (81), and 3) RvE4 that increases
508 efferocytosis of apoptotic cells (82). The impact of these existing SPMs in the oils was not elucidated
509 in this work, but considering their reported effects, they may provide anti-inflammatory effects and
510 could potentially impact SPM biosynthesis. The effect of exogenous administration of lipid
511 mediators has been shown in humans, where supplementation has been shown to increase blood lipid
512 mediator levels (83). However, as stated above, the treatment of MDMs indicated that the existing
513 lipid mediator levels in HRO and PEHeRo were not the primary drivers for biosynthesis, as the
514 PEHeRo contained far less SPMs and monohydroxylated fatty acids than HRO but resulted in
515 significantly increased levels of DHA-derived SPMs in the cell media.

516 The results shown in this work indicates relevance for understanding more of the underlying
517 mechanisms that allows for regulation of cell processes involved in the pathophysiology of psoriasis
518 through lipid mediators and SPMs. The key limitation to the data, however, is this being *in vitro*
519 experiments, where all fatty acids and lipid mediators are made very accessible in concentrations that
520 may not reflect realistic *in vivo* concentrations. Thus, the effects of directly metabolically available
521 PUFAs and, particularly, lipid mediators present in the oils may therefore be over-emphasized. It is
522 therefore important to take this into further *in vivo* trials for confirming the relevance of the findings.

523 The data on skin cells mimicking psoriasis indicates a possible mechanism of resolution of
524 inflammation in the local skin niche which could provide a treatment modality for psoriasis, and it is
525 plausible that these mechanisms also could contribute to resolution of inflammation in other organs
526 and IMIDs. The findings will also contribute to the broader understanding of the pharmacological
527 factors that are in play when administering exogenous sources of SPMs and SPM precursors into
528 biological systems.

529 In summary, we observed that HRO promotes broad SPM and lipid mediator biosynthesis in
530 macrophages and keratinocytes/fibroblasts, with a subset of SPMs that are consistently upregulated
531 throughout the stimulation/treatment conditions and cell types. Thus, indicating that these pathways
532 may carry significance for signaling and inflammation resolution across several cell types. When
533 treating macrophages with HRO and PEHeRo we found that the macrophages exhibited a dose-
534 dependent trend for biosynthesis of SPMs associated with resolution of inflammation and a shift
535 towards a more protective and possibly reparative phenotype. Given the human interventions and
536 preclinical data that are available pertaining to the use of HRO in the management of psoriasis, the
537 presented results suggest as a model of inflammatory disease for studies of local cellular resolution of
538 inflammation processes.

539

540 **Conflict of Interest**

541 TRB, MB, and RG are employed by Arctic Bioscience AS.

542 The authors declare that all experiments performed were partially funded by Arctic Bioscience AS.

543 JD is an inventor on patents related to the composition of matter and/or use of pro-resolving
544 mediators some of which are licensed by Brigham and Women's Hospital or Queen Mary University
545 of London for clinical development.

546

547 **Author Contributions**

548 TRB performed all statistical tests aside from the PLS-DA and the fatty acid uptake experiments,
549 evaluated the data, and wrote the original draft of the paper. JD, MD and AS performed all lipid
550 mediator/SPM analyses on both oils and cell media and performed the PLS-DA analyses. TKØ
551 performed fatty acid uptake experiments including statistical analysis of these experiments and was
552 part of performing all co-culture experiments. JM performed all macrophage experiments. MB
553 provided HRO and PEHeRo. MP and NS performed the co-culture experiments. RG contributed to
554 the writing of the paper. RG, TRB, TKØ, JM, and MP also contributed to describing the scope of the
555 project.

556 All authors contributed to review and finalization of the final article for publication.

557

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567

568 **References**

569 1. Cavaillon JM. Once upon a time, inflammation. J Venom Anim Toxins Trop Dis. 2021 Apr
570 9;27:e20200147.

571 2. Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. Annu
572 Rev Pathol. 2008;3:279–312.

573 3. Recchiuti A, Serhan CN. Pro-Resolving Lipid Mediators (SPMs) and Their Actions in
574 Regulating miRNA in Novel Resolution Circuits in Inflammation. Front Immunol. 2012 Oct
575 22;3:298.

576 4. Serhan CN. Novel Pro-Resolving Lipid Mediators in Inflammation Are Leads for Resolution
577 Physiology. Nature. 2014 Jun 5;510(7503):92.

578 5. Serhan CN, Chiang N, Dalli J. New pro-resolving n-3 mediators bridge resolution of infectious
579 inflammation to tissue regeneration. Mol Aspects Med. 2018 Dec;64:1–17.

580 6. Serhan CN, Chiang N, Dalli J, Levy BD. Lipid Mediators in the Resolution of Inflammation.
581 Cold Spring Harb Perspect Biol. 2015 Feb 1;7(2):a016311.

582 7. Serhan CN. The resolution of inflammation: the devil in the flask and in the details. FASEB J.
583 2011 May;25(5):1441.

584 8. Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of
585 mediators. J Clin Invest. 2018 Jul 2;128(7):2657–69.

586 9. Basil MC, Levy BD. Specialized pro-resolving mediators: endogenous regulators of infection and
587 inflammation. Nat Rev Immunol. 2016 Jan;16(1):51–67.

588 10. Jordan PM, Werz O. Specialized pro-resolving mediators: biosynthesis and biological role in
589 bacterial infections. FEBS J. 2022;289(14):4212–27.

590 11. Bannenberg G, Serhan CN. Specialized Pro-Resolving Lipid Mediators in the Inflammatory
591 Response: An Update. Biochim Biophys Acta. 2010 Aug 10;1801(12):1260.

592 12. Murakami M. Lipid Mediators in Life Science. Exp Anim. 2011;60(1):7–20.

593 13. Lim JY, Park CK, Hwang SW. Biological Roles of Resolvins and Related Substances in the
594 Resolution of Pain. BioMed Res Int. 2015;2015:830930.

595 14. Sansbury BE, Li X, Wong B, Riley CO, Shay AE, Nshimiyimana R, et al. PCTR1 Enhances
596 Repair and Bacterial Clearance in Skin Wounds. Am J Pathol. 2021 Jun 1;191(6):1049–63.

597 15. Chiang N, de la Rosa X, Libreros S, Pan H, Dreyfuss JM, Serhan CN. Cysteinyl-specialized
598 proresolving mediators link resolution of infectious inflammation and tissue regeneration via
599 TRAF3 activation. Proc Natl Acad Sci U S A. 2021 Mar 9;118(10):e2013374118.

600 16. Dalli J, Gomez EA, Jouvene CC. Utility of the Specialized Pro-Resolving Mediators as
601 Diagnostic and Prognostic Biomarkers in Disease. Biomolecules. 2022 Mar;12(3):353.

602 17. Kuek A, Hazleman BL, Östör AJK. Immune-mediated inflammatory diseases (IMIDs) and
603 biologic therapy: a medical revolution. Postgrad Med J. 2007 Apr 1;83(978):251–60.

604 18. Wu D, Jin Y, Xing Y, Abate MD, Abbasian M, Abbasi-Kangevari M, et al. Global, regional, and
605 national incidence of six major immune-mediated inflammatory diseases: findings from the
606 global burden of disease study 2019. eClinicalMedicine [Internet]. 2023 Oct 1 [cited 2025 Jan
607 6];64. Available from: https://www.thelancet.com/journals/eclinm/article/PIIS2589-
608 5370(23)00370-X/fulltext

609 19. Conrad N, Verbeke G, Molenberghs G, Goetschalckx L, Callender T, Cambridge G, et al.
610 Autoimmune diseases and cardiovascular risk: a population-based study on 19 autoimmune
611 diseases and 12 cardiovascular diseases in 22 million individuals in the UK. The Lancet. 2022
612 Sep 3;400(10354):733–43.

613 20. El-Gabalawy H, Guenther LC, Bernstein CN. Epidemiology of Immune-Mediated Inflammatory
614 Diseases: Incidence, Prevalence, Natural History, and Comorbidities. J Rheumatol Suppl. 2010
615 May 1;85:2–10.

616 21. Takeshita J, Grewal S, Langan SM, Mehta NN, Ogdie A, Van Voorhees AS, et al. Psoriasis and
617 Comorbid Diseases Part I. Epidemiology. J Am Acad Dermatol. 2017 Mar;76(3):377–90.

618 22. Olejnik M, Adamski Z, Dorocka-Bobkowska B. Psoriasis: From antiquity to the present. Indian J
619 Dermatol Venereol Leprol. 2023;89(1):149–53.

620 23. Patel DD, Lee DM, Kolbinger F, Antoni C. Effect of IL-17A blockade with secukinumab in
621 autoimmune diseases. Ann Rheum Dis. 2013 Apr 1;72(suppl 2):iii116–23.

622 24. Curtis JR, Mariette X, Gaujoux-Viala C, Blauvelt A, Kvien TK, Sandborn WJ, et al. Long-term
623 safety of certolizumab pegol in rheumatoid arthritis, axial spondyloarthritis, psoriatic arthritis,

624 psoriasis and Crohn's disease: a pooled analysis of 11 317 patients across clinical trials. RMD
625 Open. 2019 May 1;5(1):e000942.

626 25. Navarro-Compán V, Puig L, Vidal S, Ramírez J, Llamas-Velasco M, Fernández-Carballido C, et
627 al. The paradigm of IL-23-independent production of IL-17F and IL-17A and their role in
628 chronic inflammatory diseases. Front Immunol. 2023;14:1191782.

629 26. Krueger JG, Eyerich K, Kuchroo VK, Ritchlin CT, Abreu MT, Elloso MM, et al. IL-23 past,
630 present, and future: a roadmap to advancing IL-23 science and therapy. Front Immunol. 2024 Apr
631 15;15:1331217.

632 27. Simard M, Morin S, Ridha Z, Pouliot R. Current knowledge of the implication of lipid mediators
633 in psoriasis. Front Immunol. 2022 Aug 26;13:961107.

634 28. Coras R, Kavanaugh A, Kluzniak A, Holt D, Weilgosz A, Aaron A, et al. Differences in oxylipin
635 profile in psoriasis versus psoriatic arthritis. Arthritis Res Ther. 2021 Jul 24;23(1):200.

636 29. Sorokin AV, Domenichiello AF, Dey AK, Yuan ZX, Goyal A, Rose SM, et al. Bioactive Lipid
637 Mediator Profiles in Human Psoriasis Skin and Blood. J Invest Dermatol. 2018 Jul;138(7):1518–
638 28.

639 30. Sorokin AV, Arnardottir H, Svirydava M, Ng Q, Baumer Y, Berg A, et al. Comparison of the
640 dietary omega-3 fatty acids impact on murine psoriasis-like skin inflammation and associated
641 lipid dysfunction. J Nutr Biochem. 2023 Jul 1;117:109348.

642 31. Sawada Y, Honda T, Nakamizo S, Otsuka A, Ogawa N, Kobayashi Y, et al. Resolvin E1
643 attenuates murine psoriatic dermatitis. Sci Rep. 2018 Aug 8;8(1):11873.

644 32. Xu J, Duan X, Hu F, Poorun D, Liu X, Wang X, et al. Resolvin D1 attenuates imiquimod-
645 induced mice psoriasiform dermatitis through MAPKs and NF-κB pathways. J Dermatol Sci.
646 2018 Feb 1;89(2):127–35.

647 33. Neuhofer A, Zeyda M, Mascher D, Itariu BK, Murano I, Leitner L, et al. Impaired Local
648 Production of Proresolving Lipid Mediators in Obesity and 17-HDHA as a Potential Treatment
649 for Obesity-Associated Inflammation. Diabetes. 2013 May 20;62(6):1945–56.

650 34. Calder PC. Eicosapentaenoic and docosahexaenoic acid derived specialised pro-resolving
651 mediators: Concentrations in humans and the effects of age, sex, disease and increased omega-3
652 fatty acid intake. Biochimie. 2020 Nov 1;178:105–23.

653 35. Simopoulos AP. Omega-3 Fatty Acids in Inflammation and Autoimmune Diseases. J Am Coll
654 Nutr. 2002 Dec 1;21(6):495–505.

655 36. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and
656 clinical relevance. Biochim Biophys Acta BBA - Mol Cell Biol Lipids. 2015 Apr 1;1851(4):469–
657 84.

658 37. Nordgren TM, Anderson Berry A, Van Ormer M, Zoucha S, Elliott E, Johnson R, et al. Omega-3
659 Fatty Acid Supplementation, Pro-Resolving Mediators, and Clinical Outcomes in Maternal-Infant
660 Pairs. Nutrients. 2019 Jan;11(1):98.

661 38. Grenon SM, Owens CD, Nosova EV, Hughes-Fulford M, Alley HF, Chong K, et al. Short-Term,
662 High-Dose Fish Oil Supplementation Increases the Production of Omega-3 Fatty Acid–Derived
663 Mediators in Patients With Peripheral Artery Disease (the OMEGA-PAD I Trial). J Am Heart
664 Assoc. 2015 Aug 25;4(8):e002034.

665 39. Sobrino A, Walker ME, Colas RA, Dalli J. Protective activities of distinct omega-3 enriched oils
666 are linked to their ability to upregulate specialized pro-resolving mediators. PLoS ONE. 2020
667 Dec 16;15(12):e0242543.

668 40. Ahmmed MK, Ahmmed F, Tian H (Sabrina), Carne A, Bekhit AED. Marine omega-3 (n-3)
669 phospholipids: A comprehensive review of their properties, sources, bioavailability, and relation
670 to brain health. Compr Rev Food Sci Food Saf. 2020;19(1):64–123.

671 41. Lordan R, Tsoupras A, Zabetakis I. Phospholipids of Animal and Marine Origin: Structure,
672 Function, and Anti-Inflammatory Properties. Molecules. 2017 Nov;22(11):1964.

673 42. Shabana S, Hamouda HI, Hamadou AH, Ahmed B, Chi Z, Liu C. Marine phospholipid
674 nanoliposomes: A promising therapeutic approach for inflammatory bowel disease: Preparation,
675 safety, and efficacy evaluation. Colloids Surf B Biointerfaces. 2024 Feb 1;234:113702.

676 43. Liu L, Bartke N, Daele HV, Lawrence P, Qin X, Park HG, et al. Higher efficacy of dietary DHA
677 provided as a phospholipid than as a triglyceride for brain DHA accretion in neonatal piglets. J
678 Lipid Res. 2014 Mar 1;55(3):531–9.

679 44. Sugasini D, Yalagala PCR, Goggin A, Tai LM, Subbaiah PV. Enrichment of brain
680 docosahexaenoic acid (DHA) is highly dependent upon the molecular carrier of dietary DHA:
681 lysophosphatidylcholine is more efficient than either phosphatidylcholine or triacylglycerol. J
682 Nutr Biochem. 2019 Dec 1;74:108231.

683 45. Patrick RP. Role of phosphatidylcholine-DHA in preventing APOE4-associated Alzheimer's
684 disease. FASEB J. 2019 Feb;33(2):1554–64.

685 46. Sugasini D, Thomas R, Yalagala PCR, Tai LM, Subbaiah PV. Dietary docosahexaenoic acid
686 (DHA) as lysophosphatidylcholine, but not as free acid, enriches brain DHA and improves
687 memory in adult mice. Sci Rep. 2017 Sep 12;7(1):11263.

688 47. Hachem M, Géloën A, Van AL, Foumaux B, Fenart L, Gosselet F, et al. Efficient
689 Docosahexaenoic Acid Uptake by the Brain from a Structured Phospholipid. Mol Neurobiol.
690 2016 Jul;53(5):3205–15.

691 48. Wang D, Zhang L, Wen M, Du L, Gao X, Xue C, et al. Enhanced neuroprotective effect of DHA
692 and EPA-enriched phospholipids against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)
693 induced oxidative stress in mice brain. J Funct Foods. 2016 Aug 1;25:385–96.

694 49. Zhang LY, Ding L, Shi HH, Xu J, Xue CH, Zhang TT, et al. Eicosapentaenoic acid in the form of
695 phospholipids exerts superior anti-atherosclerosis effects to its triglyceride form in ApoE-/- mice.
696 Food Funct. 2019 Jul 17;10(7):4177–88.

697 50. Bjørndal B, Strand E, Gjerde J, Bohov P, Svardal A, Diehl BW, et al. Phospholipids from herring
698 roe improve plasma lipids and glucose tolerance in healthy, young adults. Lipids Health Dis.
699 2014 May 17;13:82.

700 51. Cook CM, Hallaråker H, Sæbø PC, Innis SM, Kelley KM, Sanoshy KD, et al. Bioavailability of
701 long chain omega-3 polyunsaturated fatty acids from phospholipid-rich herring roe oil in men
702 and women with mildly elevated triacylglycerols. Prostaglandins Leukot Essent Fatty Acids.
703 2016 Aug;111:17–24.

704 52. Ramirez JL, Gasper WJ, Khetani SA, Zahner GJ, Hills NK, Mitchell PT, et al. Fish Oil Increases
705 Specialized Pro-resolving Lipid Mediators in PAD (The OMEGA-PAD II Trial). J Surg Res.
706 2019 Jun 1;238:164–74.

707 53. Marchand NE, Choi MY, Oakes EG, Cook NR, Stevens E, Gomelskaya N, et al. Over-the-
708 counter fish oil supplementation and pro-resolving and pro-inflammatory lipid mediators in
709 rheumatoid arthritis. Prostaglandins Leukot Essent Fatty Acids. 2023 Mar 1;190:102542.

710 54. Mas E, Croft KD, Zahra P, Barden A, Mori TA. Resolvins D1, D2, and Other Mediators of Self-
711 Limited Resolution of Inflammation in Human Blood following n-3 Fatty Acid Supplementation.
712 Clin Chem. 2012 Oct 1;58(10):1476–84.

713 55. Norris PC, Skulas-Ray AC, Riley I, Richter CK, Kris-Etherton PM, Jensen GL, et al.
714 Identification of specialized pro-resolving mediator clusters from healthy adults after intravenous
715 low-dose endotoxin and omega-3 supplementation: a methodological validation. Sci Rep. 2018
716 Dec 21;8(1):18050.

717 56. Mottola G, Werlin E, Wu B, Chen M, Chatterjee A, Schaller M, et al. Oral Resolvin D1
718 attenuates early inflammation but not intimal hyperplasia in a rat carotid angioplasty model.
719 Prostaglandins Other Lipid Mediat. 2019 Dec 10;146:106401.

720 57. Callan N, Hanes D, Bradley R. Early evidence of efficacy for orally administered SPM-enriched
721 marine lipid fraction on quality of life and pain in a sample of adults with chronic pain. J Transl
722 Med. 2020 Oct 21;18(1):401.

723 58. Caputo MP, Radlowski EC, Lawson MA, Antonson AM, Watson JE, Matt SM, et al. Herring roe
724 oil supplementation alters microglial cell gene expression and reduces peripheral inflammation
725 after immune activation in a neonatal piglet model. Brain Behav Immun. 2019 Oct 1;81:455–69.

726 59. Tveit KS, Brokstad KA, Berge RK, Sæbø PC, Hallaråker H, Brekke S, et al. A Randomized,
727 Double-blind, Placebo-controlled Clinical Study to Investigate the efficacy of Herring Roe Oil
728 for treatment of Psoriasis. Acta Derm Venereol. 2020 May 28;100(10):adv00154.

729 60. Tveit KS, Brokstad KA, Berge RK, Saeboe PC, Hallaraaker H, Brekke S, et al. Long Term
730 Efficacy and Safety of Herring Roe Oil in the Treatment of Psoriasis, a 39-week Open-label
731 Extension Study. Int J Clin Exp Med Sci. 2021 Jan;7(1):13–20.

732 61. Petrovic A, Bueide I, Tveit KS, Hallaråker H, Bjørndal B, Holmes TD, et al. Herring roe oil in
733 treatment of psoriasis – influence on immune cells and cytokine network. Front Immunol. 2023
734 Sep 8;14:1128986.

735 62. Mildenberger J, Solberg N, Østbye TKK, Høst V, Federico P, Gammelsæter R, et al.
736 Phospholipid Esters from Herring Roe have immunomodulatory anti-psoriatic effects by
737 affecting signaling on the IL-17/23 axis in immune cells and psoriatic skin cell models in vitro.
738 In: EADV Congress, Conference poster 3243. Amsterdam; 2024.

739 63. Titos E, Rius B, González-Périz A, López-Vicario C, Morán-Salvador E, Martínez-Clemente M,
740 et al. Resolvin D1 and its precursor docosahexaenoic acid promote resolution of adipose tissue
741 inflammation by eliciting macrophage polarization toward an M2-like phenotype. J Immunol
742 Baltim Md 1950. 2011 Nov 15;187(10):5408–18.

743 64. Furue M, Furue K, Tsuji G, Nakahara T. Interleukin-17A and Keratinocytes in Psoriasis. Int J
744 Mol Sci. 2020 Feb 13;21(4):1275.

745 65. Folch J, Lees M, Stanley GHS. A SIMPLE METHOD FOR THE ISOLATION AND
746 PURIFICATION OF TOTAL LIPIDES FROM ANIMAL TISSUES. J Biol Chem. 1957 May
747 1;226(1):497–509.

748 66. Dooley M, Saliani A, Dalli J. Development and Validation of Methodologies for the
749 Identification of Specialized Pro-Resolving Lipid Mediators and Classic Eicosanoids in
750 Biological Matrices. J Am Soc Mass Spectrom. 2024 Oct 2;35(10):2331–43.

751 67. Pang Z, Lu Y, Zhou G, Hui F, Xu L, Viau C, et al. MetaboAnalyst 6.0: towards a unified
752 platform for metabolomics data processing, analysis and interpretation. Nucleic Acids Res. 2024
753 Jul 5;52(W1):W398–406.

754 68. Wang DD, Wu F, Wen M, Ding L, Du L, Xue CH, et al. Replenishment of Docosahexaenoic
755 Acid (DHA) in Dietary n-3-Deficient Mice Fed DHA in Triglycerides or Phosphatidylcholines
756 After Weaning. J Food Sci. 2018 Feb;83(2):481–8.

757 69. Meyer zu Heringdorf D, Jakobs KH. Lysophospholipid receptors: Signalling, pharmacology and
758 regulation by lysophospholipid metabolism. Biochim Biophys Acta BBA - Biomembr. 2007 Apr
759 1;1768(4):923–40.

760 70. Oh SF, Dona M, Fredman G, Krishnamoorthy S, Irimia D, Serhan CN. Resolvin E2 Formation
761 and Impact in Inflammation Resolution. J Immunol. 2012 May 1;188(9):4527–34.

762 71. Sato M, Aoki-Saito H, Fukuda H, Ikeda H, Koga Y, Yatomi M, et al. Resolvin E3 attenuates
763 allergic airway inflammation via the interleukin-23-interleukin-17A pathway. FASEB J Off Publ
764 Fed Am Soc Exp Biol. 2019 Nov;33(11):12750–9.

765 72. Spite M, Norling LV, Summers L, Yang R, Cooper D, Petasis NA, et al. Resolvin D2 is a potent
766 regulator of leukocytes and controls microbial sepsis. Nature. 2009 Oct;461(7268):1287–91.

767 73. Shan K, Feng N, Cui J, Wang S, Qu H, Fu G, et al. Resolvin D1 and D2 inhibit tumour growth
768 and inflammation via modulating macrophage polarization. J Cell Mol Med. 2020
769 Jul;24(14):8045–56.

770 74. Sorokin AV, Norris P, English J, Dey AK, Chaturvedi A, Baumer Y, et al. Identification of pro-
771 resolving and inflammatory lipid mediators in human psoriasis. J Clin Lipidol. 2018 Apr
772 6;12(4):1047.

773 75. Hellmann J, Sansbury BE, Wong B, Li X, Singh M, Nuutila K, et al. Biosynthesis of D-Series
774 Resolvins in Skin Provides Insights into their Role in Tissue Repair. J Invest Dermatol. 2018 Sep
775 1;138(9):2051–60.

776 76. Morin S, Tremblay A, Dumais E, Julien P, Flamand N, Pouliot R. Eicosapentaenoic Acid
777 Influences the Lipid Profile of an In Vitro Psoriatic Skin Model Produced with T Cells.
778 Biomolecules. 2023 Sep;13(9):1413.

779 77. Dalli J, Ramon S, Norris PC, Colas RA, Serhan CN. Novel proresolving and tissue-regenerative
780 resolvin and protectin sulfido-conjugated pathways. FASEB J. 2015 May;29(5):2120–36.

781 78. Dalli J, Serhan CN. Identification and structure elucidation of the pro-resolving mediators
782 provides novel leads for resolution pharmacology. Br J Pharmacol. 2019 Apr;176(8):1024–37.

783 79. Shao S, Chen J, Swindell WR, Tsoi LC, Xing X, Ma F, et al. Phospholipase A2 enzymes
784 represent a shared pathogenic pathway in psoriasis and pityriasis rubra pilaris. JCI Insight.
785 6(20):e151911.

786 80. Xia H, Chen L, Liu H, Sun Z, Yang W, Yang Y, et al. Protectin DX increases survival in a mouse
787 model of sepsis by ameliorating inflammation and modulating macrophage phenotype. Sci Rep.
788 2017 Mar 7;7(1):99.

789 81. Saito-Sasaki N, Sawada Y, Mashima E, Yamaguchi T, Ohmori S, Yoshioka H, et al. Maresin-1
790 suppresses imiquimod-induced skin inflammation by regulating IL-23 receptor expression. Sci
791 Rep. 2018 Apr 3;8(1):5522.

792 82. Libreros S, Shay AE, Nshimiyimana R, Fichtner D, Martin MJ, Wourms N, et al. A New E-
793 Series Resolvin: RvE4 Stereochemistry and Function in Efferocytosis of Inflammation-
794 Resolution. Front Immunol. 2021 Feb 10;11:631319.

795 83. Souza PR, Marques RM, Gomez EA, Colas RA, De Matteis R, Zak A, et al. Enriched Marine Oil
796 Supplements Increase Peripheral Blood Specialized Pro-Resolving Mediators Concentrations and
797 Reprogram Host Immune Responses: A Randomized Double-Blind Placebo-Controlled Study.
798 Circ Res. 2020 Jan 3;126(1):75–90.

799

800

801 **Figure legends**

802 **Fig. 1** A) Relative concentrations of select SPMs in HRO and PEHeRo (mean of 3 replicates ±SEM)
803 as a percentage of total SPMs found in the oils. B) Relative concentrations of monohydroxylated
804 fatty acids in HRO and PEHeRo (mean of 3 replicates ±SEM) as a percentage of the total amount of
805 monohydroxylated fatty acids found in the oils. C) Relative distribution of monohydroxylated fatty
806 acids in HRO and PEHeRo (mean of 3 replicates ±SEM) as a percentage of the sum
807 monohydroxylated fatty caids from DHA, EPA, and n-3 DPA. SPMs were identified and quantified
808 using LC-MS/MS. # $P > 0.1$ (Mann-Whitney U test, two-tailed) and indicating similarity between
809 HRO and PEHeRo for the analyte, remaining parameters were $P < 0.1$.

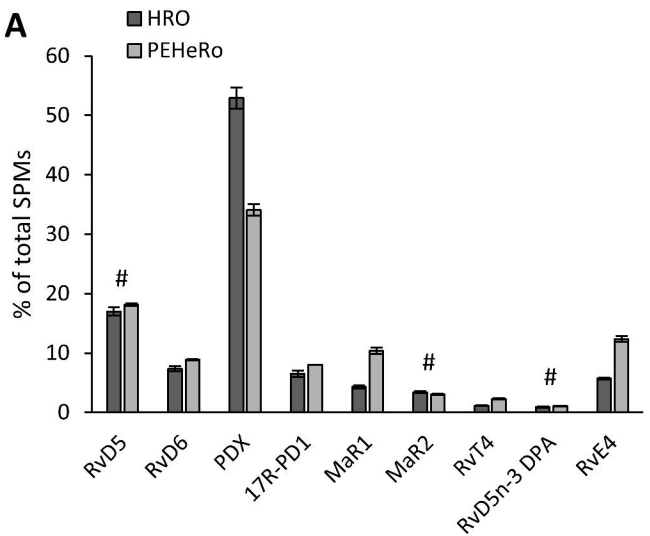
810 **Fig. 2** A) Relative concentrations from GC-FID of fatty acids as % of total fatty acids in MDMs
811 (mean of 3 replicates ±SEM) when treated with HRO versus control. B) Relative concentrations from
812 GC-FID of fatty acids as % of total fatty acids in the phospholipid fraction of the
813 keratinocyte/fibroblast co-culture (mean of 9 replicates ±SEM) when treated with HRO versus
814 control. *$P < 0.05$ (One-factor ANOVA with Tukey's HSD).

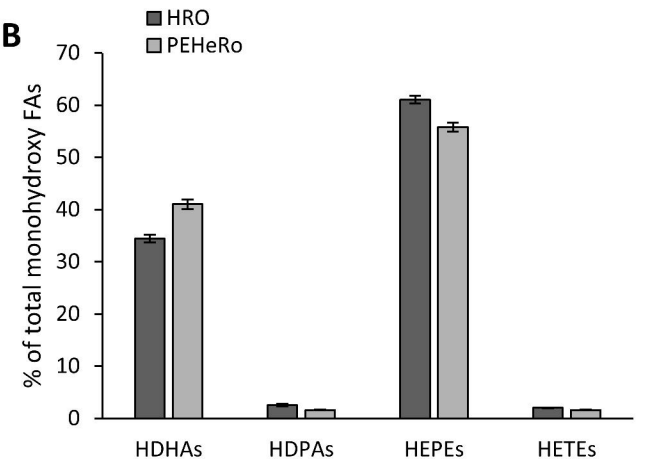
815 **Fig. 3** A) SPM levels (mean of 4 donors/experiments ± SEM) in supernatants from macrophages
816 incubated with HRO prior to stimulation with LPS+IFN-γ versus only stimulated with LPS+IFN-γ
817 (control). B) Partial least squares discriminant analysis (PLS-DA) of supernatants from macrophages
818 incubated with HRO prior to stimulation with LPS+IFN-γ versus only stimulated with LPS+IFN-γ
819 (control). Results are compared to lipid mediator concentrations identified in fresh media. C)
820 Variable importance in projection (VIP) scores of SPMs that contribute to the difference between the
821 tested conditions. D) Figure showing the treatment conditions and the highlighted measured
822 outcomes. SPMs were identified and quantified using LC-MS/MS. *$P < 0.05$ (Mann-Whitney U test,
823 two-tailed).

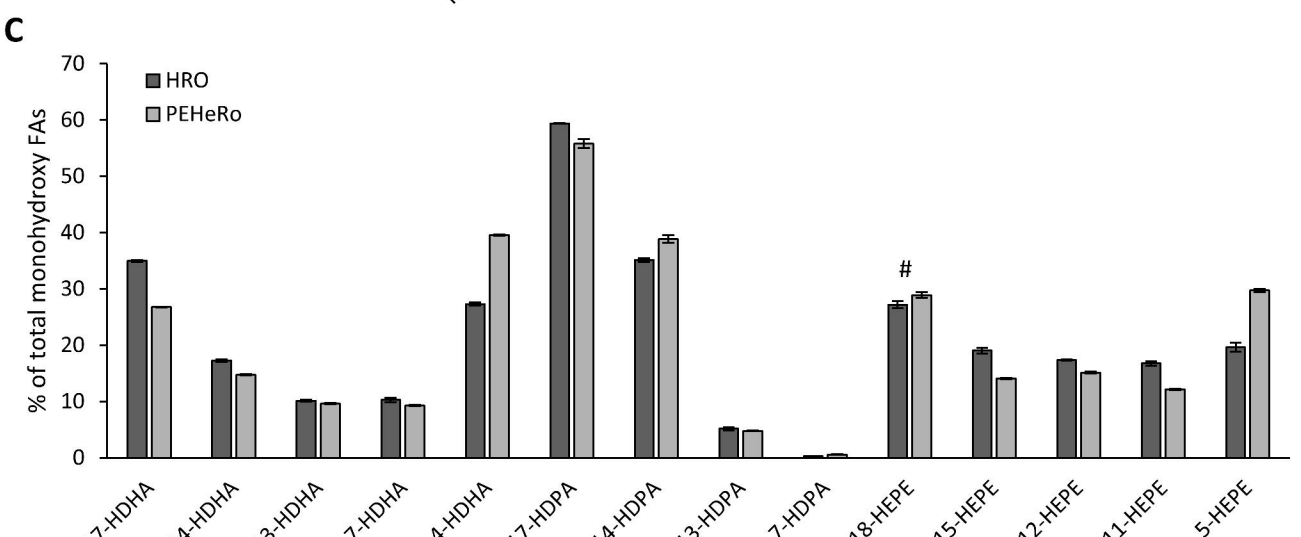
824 **Fig. 4** SPM levels (mean of 4 donors/experiments ± SEM) from DHA (A) and EPA (B) metabolomes
825 in macrophage cell supernatants (pg/3 mL) when incubated with HRO+LPS, PEHeRo+LPS, LPS
826 (control), or untreated. C) Partial least squares discriminant analysis (PLS-DA) of supernatants from
827 macrophages incubated with HRO+LPS, PEHeRo+LPS, LPS (control), or untreated. D) Variable
828 importance in projection (VIP) scores of SPMs that contribute to the difference between the tested
829 conditions. SPMs were identified and quantified using LC-MS/MS. *$P < 0.05$ and **$P < 0.01$ (One-
830 factor ANOVA with one-way Dunnett's multiple comparisons test for comparison to control and
831 Tukey's HSD for comparison between treatment groups).

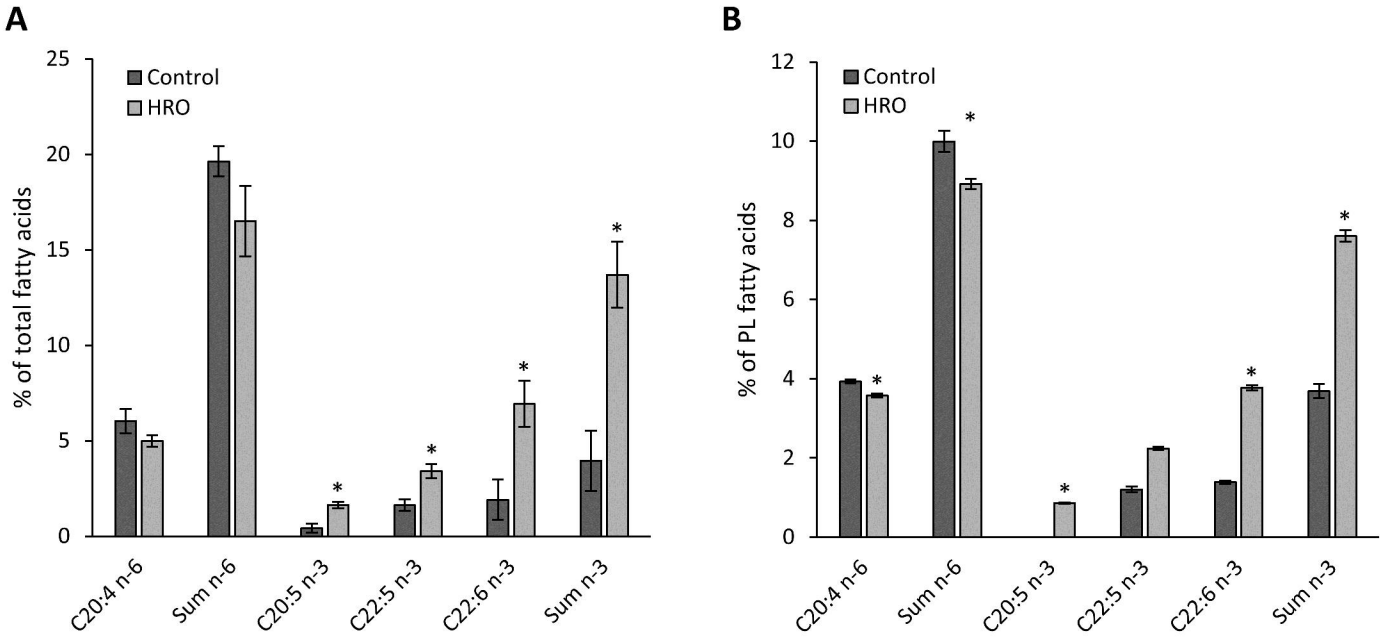
832 **Fig. 5** SPM and SPM precursor levels (mean of 3 biological replicates ± SEM) in
833 fibroblast/keratinocyte co-culture cell supernatants (pg/mL) incubated with IL-17A (control), HRO,
834 HRO+IL-17A, or untreated. SPMs were identified and quantified using LC-MS/MS. A) SPMs and
835 monohydroxylated fatty acids from DHA and monohydroxylated fatty acids from DPA. B) SPMs and
836 monohydroxylated fatty acids from EPA. C) Monohydroxylated fatty acids from DHA. D)
837 Monohydroxylated fatty acids from EPA. *$P < 0.05$ and **$P < 0.01$ (One-factor ANOVA with one-
838 way Dunnett's multiple comparisons test for comparison to control and Tukey's HSD for comparison
839 between treatment groups and between different monohydroxylated fatty acids).

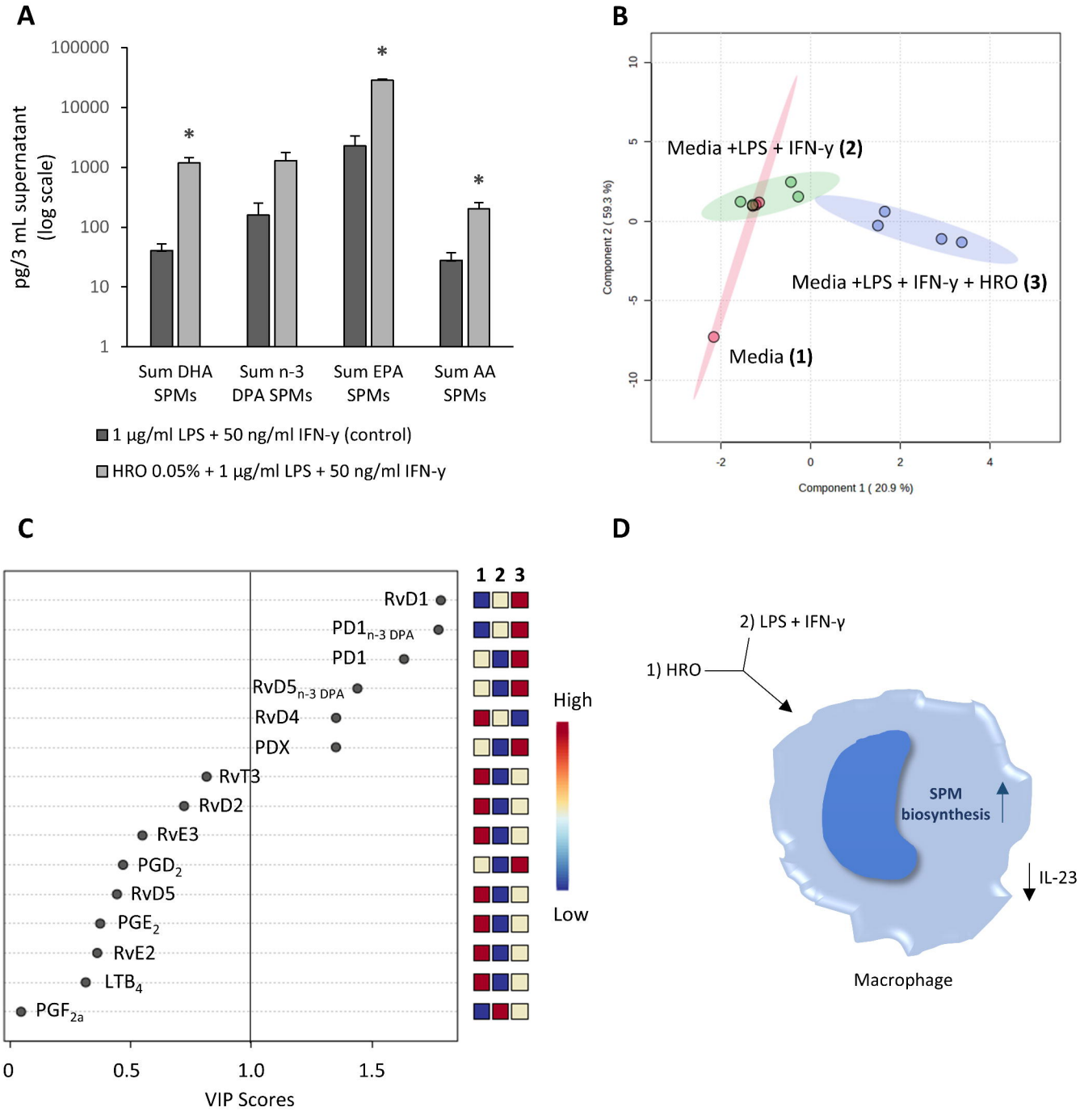
840

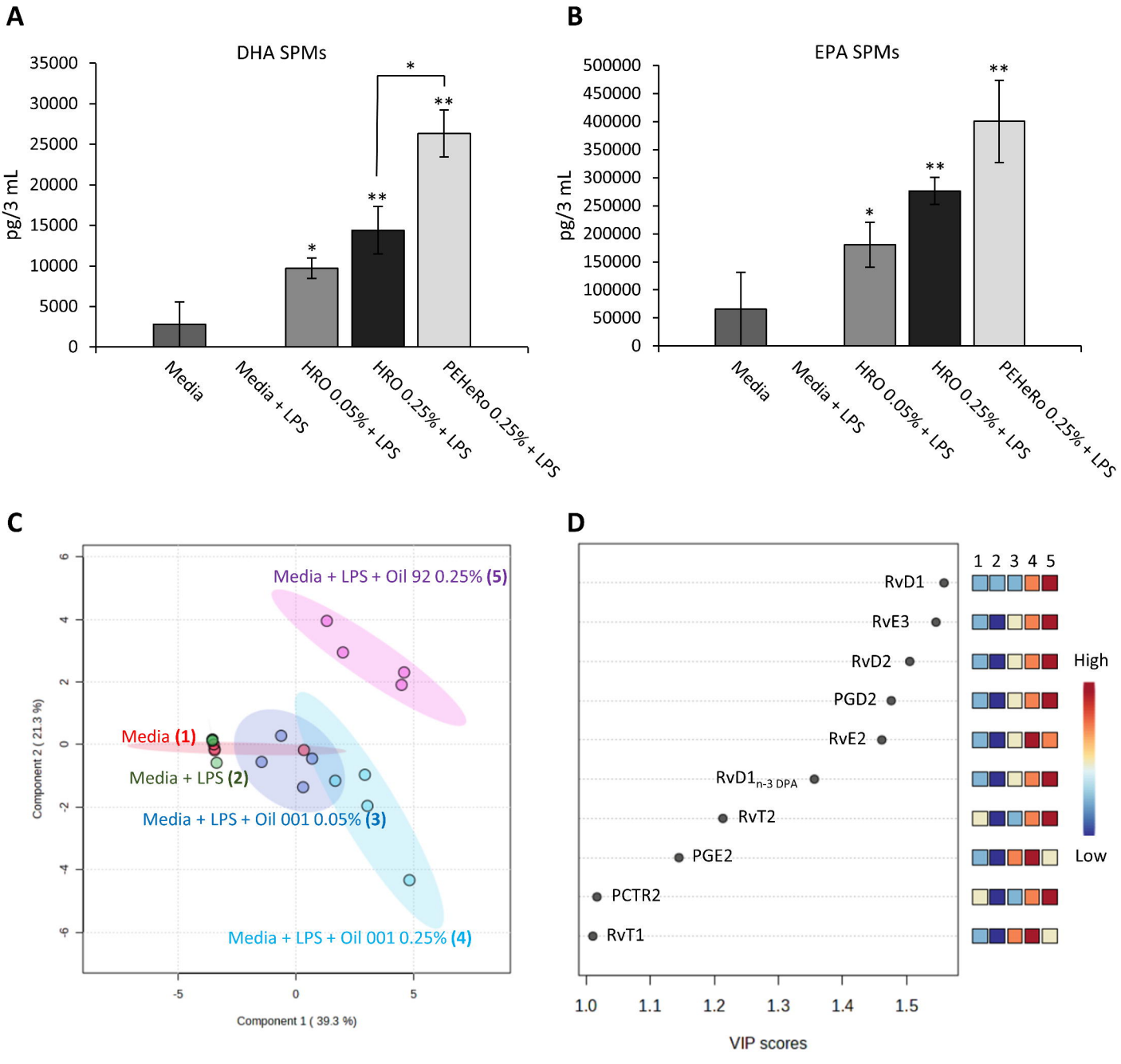

A
HRO
PEHeRo
% of total SPMs
60
50
40
30
20
10
0
#
#
#
RvD5
RvD6
PDX
17R-PD1
MaR1
MaR2
RvT4
RvD5n-3 DPA
RvE4

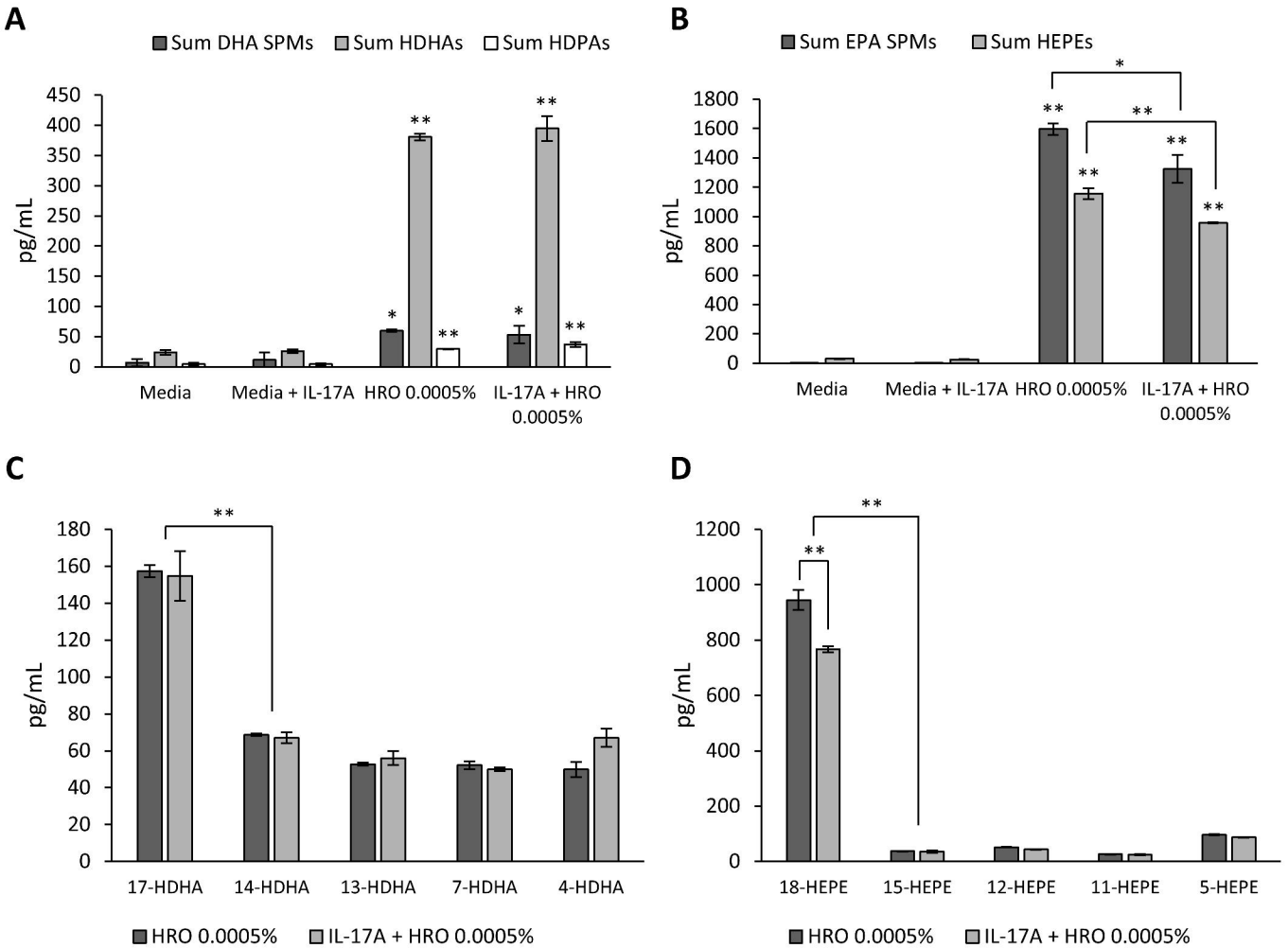

B
HRO
PEHeRo
% of total monohydroxy FAs
70
60
50
40
30
20
10
0
HDHAs
HDPAs
HEPEs
HETEs


C
HRO
PEHeRo
% of total monohydroxy FAs
70
60
50
40
30
20
10
0
#
17-HDHA
14-HDHA
13-HDHA
7-HDHA
4-HDHA
17-HDPA
14-HDPA
13-HDPA
7-HDPA
18-HEPE
15-HEPE
12-HEPE
11-HEPE
5-HEPE


A
Control
HRO
% of total fatty acids
25
20
15
10
5
0
*
C20:4 n-6
Sum n-6
C20:5 n-3
C22:5 n-3
C22:6 n-3
Sum n-3
B
Control
HRO
% of PL fatty acids
12
10
8
6
4
2
0
*
C20:4 n-6
Sum n-6
C20:5 n-3
C22:5 n-3
C22:6 n-3
Sum n-3


A
pg/3 mL supernatant (log scale)
100000
10000
1000
100
10
1
*
*
*
Sum DHA SPMs
Sum n-3 DPA SPMs
Sum EPA SPMs
Sum AA SPMs
1 µg/ml LPS + 50 ng/ml IFN-γ (control)
HRO 0.05% + 1 µg/ml LPS + 50 ng/ml IFN-γ
B
Media +LPS + IFN-γ (2)
Media +LPS + IFN-γ + HRO (3)
Media (1)
Component 2 (59.3 %)
Component 1 (20.9 %)
C
1 2 3
RvD1
PD1n-3 DPA
PD1
RvD5n-3 DPA
RvD4
PDX
RvT3
RvD2
RvE3
PGD2
RvD5
PGE2
RvE2
LTB4
PGF2a
High
Low
VIP Scores
D
1) HRO
2) LPS + IFN-γ
SPM biosynthesis
IL-23
Macrophage


A
DHA SPMs
pg/3 mL
35000
30000
25000
20000
15000
10000
5000
0
*
**
*
**
Media
Media + LPS
HRO 0.05% + LPS
HRO 0.25% + LPS
PEHeRo 0.25% + LPS
B
EPA SPMs
pg/3 mL
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
*
**
**
Media
Media + LPS
HRO 0.05% + LPS
HRO 0.25% + LPS
PEHeRo 0.25% + LPS
C
Media + LPS + Oil 92 0.25% (5)
Media (1)
Media + LPS (2)
Media + LPS + Oil 001 0.05% (3)
Media + LPS + Oil 001 0.25% (4)
Component 2 (21.3 %)
Component 1 (39.3 %)
D
RvD1
RvE3
RvD2
PGD2
RvE2
RvD1n-3 DPA
RvT2
PGE2
PCTR2
RvT1
1 2 3 4 5
High
Low
VIP scores


A
Sum DHA SPMs
Sum HDHAs
Sum HDPAs
pg/mL
450
400
350
300
250
200
150
100
50
0
Media
Media + IL-17A
HRO 0.0005%
IL-17A + HRO 0.0005%
B
Sum EPA SPMs
Sum HEPEs
pg/mL
1800
1600
1400
1200
1000
800
600
400
200
0
Media
Media + IL-17A
HRO 0.0005%
IL-17A + HRO 0.0005%
C
pg/mL
180
160
140
120
100
80
60
40
20
0
17-HDHA
14-HDHA
13-HDHA
7-HDHA
4-HDHA
HRO 0.0005%
IL-17A + HRO 0.0005%
D
pg/mL
1200
1000
800
600
400
200
0
18-HEPE
15-HEPE
12-HEPE
11-HEPE
5-HEPE
HRO 0.0005%
IL-17A + HRO 0.0005%