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The effects of triglycerides and fatty acids on T cells: role in atherosclerosis

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CHAPTER 4

EPA induces anti-inflammatory transcriptomics in T cells, implicating a triglyceride-independent pathway for cardiovascular risk reduction

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Abstract

Background: A twice-daily dose of highly purified eicosapentaenoic acid (EPA) reduces the risk of atherosclerotic cardiovascular disease among patients with high triglycerides and either known cardiovascular disease or those at high risk for developing it. However, the process by which EPA exerts its beneficial effects remains poorly understood.

Objectives: We show that EPA can induce an anti-inflammatory transcriptional profile in non-activated CD4⁺ T cells.

Methods: Non-activated CD4⁺ T cells from 8 different donors were exposed to 100μM EPA, oleic acid, palmitic acid, or control. RNA and ATAC-sequencing were performed after 48h of exposure to determine changes in the transcriptomic and epigenetic landscape of the exposed cells as compared to control.

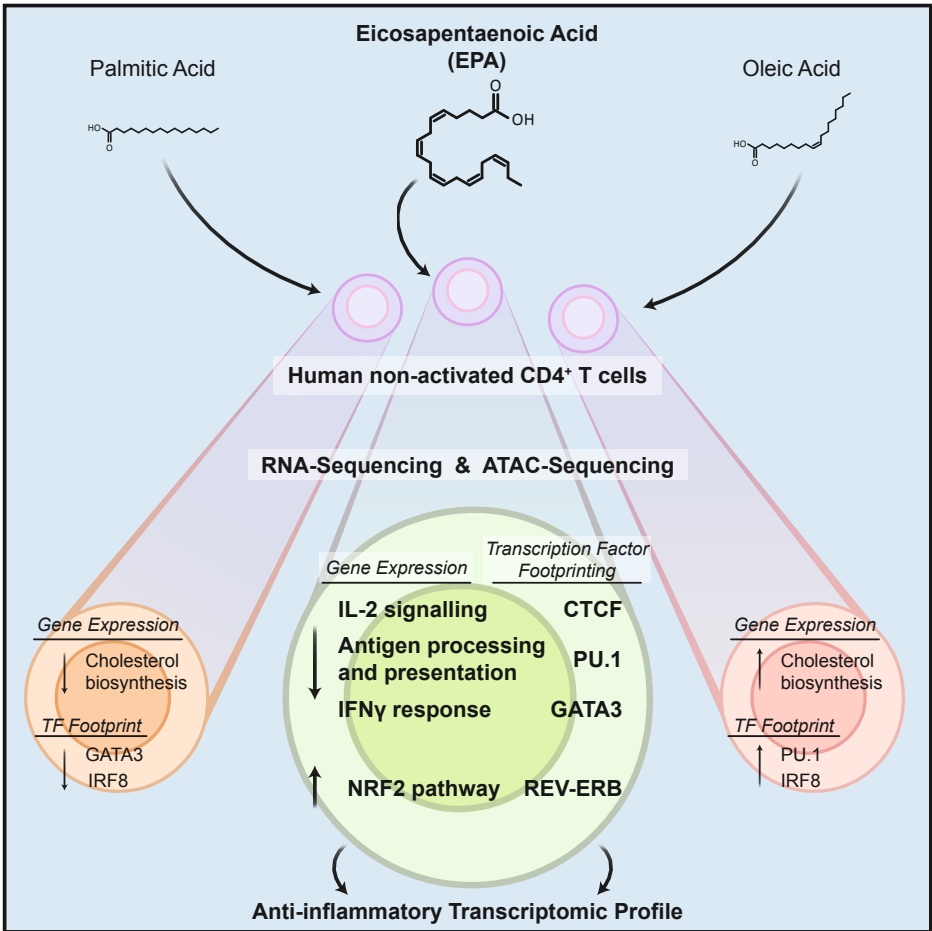
Results: We find that EPA-exposed CD4⁺ T cells downregulate immune response-related genes, such as *HLA-DRA*, *CD69*, and *IL2RA*, while upregulating genes involved in oxidative stress prevention, such as *NQO1*. Furthermore, transcription footprint analysis based on ATAC-sequencing reveals downregulation of GATA3 and PU.1, key transcription factors in T_H2 and T_H9 differentiation, and upregulation of REV-ERB, an antagonist of T_H17 differentiation. By in parallel examining T cell responses to oleic acid, a monounsaturated fatty acid, and palmitic acid, a saturated fatty acid, we find that both the intensity of the transcriptomic response and the involvement of anti-inflammatory pathways is highly specific for EPA.

Conclusions: Thus, EPA can induce an anti-inflammatory transcriptomic landscape in CD4⁺ T cells, a process that may contribute to the unexpectedly strong beneficial effects of EPA on the risk of atherosclerotic cardiovascular disease in clinical trials.

Highlights

- The mechanism by which EPA reduces the risk of ASCVD in clinical trials remains unclear.
- EPA can induce an anti-inflammatory transcriptomic landscape in non-activated CD4⁺ T cells *in vitro*.
- T cell reactions to palmitic and oleic acid reveal EPA's unique anti-inflammatory transcriptomic response
- Examining T-cells during IPE interventions may reveal insights into EPA's benefits independent of triglyceride reduction.

Graphical abstract



Introduction

The risk of atherosclerotic cardiovascular disease (ASCVD) persists despite therapies that effectively control blood cholesterol levels including statins and PCSK9 inhibitors¹⁻³. This residual risk has been attributed, in part, to elevated triglyceride levels in blood⁴. Nevertheless, most triglyceride influencing therapies, such as fibrates or niacin, have little cardiovascular benefit⁵⁻⁹. However, there is one triglyceride lowering drug that was found to strongly reduce ASCVD risk, namely, icosapent ethyl (IPE), which in the body is metabolized to eicosapentaenoic acid (EPA), a polyunsaturated fatty acid. The REDUCE-IT trial showed that patients who received 4g of IPE administered as 2g twice daily was superior to placebo in reducing triglycerides, cardiovascular events, and cardiovascular death among patients with high triglycerides and either known cardiovascular disease or those at high risk for developing it, and who were already on statin therapy with relatively well-controlled low density lipoprotein (LDL) levels¹⁰. The results of the trial and its interpretation has been much debated in literature^{11, 12}. In particular, it remains largely unknown how EPA exerts its beneficial effects, and only limited studies have been carried out in model membranes or by examining whole blood¹³⁻¹⁵.

Atherosclerosis is regarded as a lipid-driven immune disease¹⁶. As such, the majority of immune cells in the atherosclerotic plaque are T cells, of which half are CD4⁺^{17, 18}. Furthermore, CD4⁺ T cells aggravate atherosclerosis in established mouse models^{19, 20}. Therefore, the study of CD4⁺ T cells is a promising route to further understanding ASCVD and investigating how EPA can influence these cells can indicate a potential mechanism underlying the beneficial effects of EPA on atherosclerosis. Interestingly, EPA was suggested to have anti-inflammatory properties as indicated by a reduction in CD4⁺ T cell proliferation, decreased differentiation towards T helper 1 (T_H1) and T helper 17 (T_H17), and increased or no effect on differentiation towards T helper 2 (T_H2) and T regulatory (T_{reg}) cells²¹. However, these studies were largely carried out in mouse models, or investigated *in vitro* during T cell activation, under polarizing conditions, or by measuring general T cell markers²²⁻²⁸. Thus, the effects of EPA on T cells remain incompletely understood and, in particular, it is unknown whether EPA can affect human CD4⁺ T cells in a non-activated state, as they occur in the circulation and where the primary interaction with EPA takes place.

We aimed to further elucidate the effects of EPA on CD4⁺ T cells by performing transcriptomic analysis on non-activated exposed cells. Furthermore, we assessed the specificity of the effects of EPA by exposing cells to two other fatty acids of different saturation, oleic acid (OA), a monounsaturated fatty acid, and palmitic acid (PA), a saturated fatty acid. To do so, we performed RNA and ATAC-sequencing on non-activated CD4⁺ T cells exposed to EPA, OA, PA, or control after 48h exposure. We show that EPA leads to a marked downregulation of many anti-inflammatory genes in non-activated CD4⁺ T cells as compared to control. The pronounced and specific effects on the transcriptomics landscape contrasted with the relatively modest effects of OA and PA.

Methods

Peripheral blood CD4⁺ T cell isolation and culture conditions

CD4⁺ T cell isolation and fatty acid exposure model were based on our previously described *in vitro* model with minor changes²⁹. To obtain non-activated CD4⁺ T cells, peripheral blood mononuclear cells (PBMCs) were isolated from 8 different buffy coats of anonymous blood bank donors (Sanquin, Amsterdam, The Netherlands) by Ficoll paque (Apotheek LUMC, 97902861) gradient centrifugation. All donors provided written informed consent in accordance with the protocol of the local institutional review board, the Medical Ethics committee of Sanquin blood supply in accordance with the Declaration of Helsinki. The sex of the cells could not be determined due to the anonymity of the donors. However, RNA sequencing showed that, of 8 donors sequenced, 6 were female and 2 were male, which was accounted for during the statistical analysis by correcting for donor effect. Next, CD4⁺ T cells were purified from the PBMCs using lyophilized human anti-CD4⁺ magnetically labeled microbeads (Miltenyi, 130-097-048) scaling the manufacturer's instructions to 1/3 of the recommended volumes. CD4⁺ T cell purity was assessed on an LSR-II instrument at the Leiden University Medical Center Flow Cytometry Core Facility (<https://www.lumc.nl/research/facilities/fcf/>) with the BD FACSDiva™ v9.0 software (BD Biosciences). Cells were stained with anti-CD3-PE (BD Biosciences, 345765), anti-CD4-APC (BD Biosciences, 345771), anti-CD8-FITC (BD Biosciences, 555634), and anti-CD14-PEcy7 (BD Biosciences, 560919) and resuspended in 1% paraformaldehyde (Apotheek LUMC, 120810-001) to fix the cells prior to acquisition. Purity was >98% for all donors.

Prior to fatty acid exposure, $\sim 1 \times 10^8$ isolated cells were cultured overnight to allow the cells to return to a resting state after the stress of the isolation procedure. This was done in T75 flasks (Greiner Bio-One, 658-175) at a density of $\sim 2.5 \times 10^6$ cells/mL in 5% fetal calf serum (FCS) (Bodinco BDC, 16941) DMEM (Dulbecco's Modified Eagle's Serum (Sigma, 05796), 1% Pen-Strep (Lonza, DE17-602E), 1% GlutaMAX-1 (100x) (Gibco, 35050-038)) medium supplemented with 50 IU/mL IL-2 (Peprotech, 200-02) and incubated at 37°C under 5% CO₂. To keep the cells in a non-activated state, no additional stimulus was added. Any CD4⁺ T cells not used directly after the isolation were kept in DMEM supplemented with 30% FCS, 1% Pen-Strep, 1% GlutaMAX-1, and 20% Dimethyl Sulfoxide (DMSO) (WAK-Chemie Medical GmbH, WAK-DMSO-10) medium at a density of $\sim 25 \times 10^6$ cells/mL, and stored in liquid nitrogen.

Next, non-activated CD4⁺ T cells were cultured with either EPA (Cayman, 90110), OA (Sigma, O1383), PA (Cayman, 10006627), or control for 48 hours at 37°C under 5% CO₂. The cells were exposed for 48h based off previous findings when establishing our previously described *in vitro* model²⁹. To this end, CD4⁺ T cells from each donor were plated in a 24 wells plate (density of $\sim 3.5 \times 10^6$ cells/well) in 2mL 5% FCS DMEM for each condition (Fig. 1a). Cells were cultured in medium containing FCS to ensure cell viability during culture and to be more comparable to physiological conditions of the circulation where other lipids are also present. To assess the additional EPA, OA, or PA stimulus to the non-activated CD4⁺ T cells due to FCS in the culture

medium, an FCS sample was measured via the Shotgun Lipidomics Assistant (SLA) method³⁰ to estimate the fraction of fatty acids in the sample. The sample was prepped as previously described³¹ but with two modifications, a starting volume of 25 μ L FCS and 600 μ L MTBE was added instead of 575 μ L during the first extraction. Free EPA was 0.02 μ g/mL and EPA as components of larger molecules including cholesterol esters and sphingolipids was 0.13 μ g/mL. Free OA was 0.29 μ g/mL and OA as components of larger molecules including cholesterol esters and sphingolipids was 4.93 μ g/mL. Free PA was 0.23 μ g/mL and PA as components of larger molecules including cholesterol esters and sphingolipids was 3.45 μ g/mL.

PA was dissolved in HPLC grade ethanol (Fisher Scientific, 64-17-5) to a final concentration of 5mg/mL to create a stock solution. The stock solution was vortexed briefly, sonicated in a sonicator (Branson, 2800) for 15 min, and heated for 15 min at 45°C. A small portion of the stock was extracted into a glass HPLC vial (Agilent Technologies, 5182-0714) to a final concentration of 5,000 μ g/mL. EPA and OA were dissolved from their stock in HPLC grade ethanol to a final concentration of 25,000 and 30,000 μ g/mL, respectively. The HPLC grade EtOH was then evaporated before the fatty acids were complexed to fatty acid-free (FAF) bovine serum albumin (BSA) (Sigma, A7030) in a 2% FAF BSA DMEM mixture (Dulbecco's Modified Eagle's Serum, 2% FAF BSA, 1% Pen-Strep, 1% GlutaMAX-1 (100x)) to a concentration of 151.25 μ g/mL for EPA, 141.25 μ g/mL for OA, and 128.2 μ g/mL for PA. Complexing fatty acids to BSA mimics physiological conditions as fatty acids are also bound to albumin in the human circulations³². Each fatty acid was further diluted to the final concentrations of 100 μ M (30.25 μ g/mL for EPA, 28.25 μ g/mL for OA, and 25.64 μ g/mL for PA) upon addition to the cells. The concentration tested was kept equal to ensure the cells were exposed the same amount of fatty acid particles and not influenced by concentration differences. Fatty acid stocks were stored under argon gas at -20°C to avoid oxidation.

As a control, HPLC grade EtOH was evaporated in a glass HPLC vial before adding 2% FAF BSA DMEM medium and added to the cells. The amount of 2% FAF BSA DMEM added to the wells was equal for each condition to keep the volumes equivalent. The CD4⁺ T cells were cultured for 48h at 37°C under 5% CO₂. After exposure, the cells were washed and 1*10⁵ cells were used directly for ATAC sequencing preparation. Cells from the same donors for which ATAC sequencing was performed were later thawed from liquid nitrogen and exposed to the fatty acids as described previously. After 48h exposure, the cells were washed and 3*10⁶ cells were flash frozen in liquid nitrogen and stored at -80°C for RNA isolations. Cell viability and diameter were measured by Via1-Cassette™ (Chemometec, 941-0012) on a NucleoCounter® NC-200™ (Chemometec, 900-0200) and found to be > 95% and on average 9 μ m for each condition.

RNA isolation

To isolate total RNA for RNA sequencing and RT-qPCR, RNA was extracted from the cell samples using the Quick-RNA Microprep Kit (Zymo, R1050) according to manufacturer's instructions. The RNA was quantified using a Qubit® 2.0 Fluorometer (Q32866) with the Qubit® RNA BR Assay Kit (ThermoFisher, Q10211) according to manufacturer's instructions. The RNA was placed over a second Zymo-Spin IC Column, washed, and a second DNase treatment performed to

remove any residual DNA contamination from the samples. RNA integrity (RIN) values of the samples were on average 7.8 SE 0.1 as determined using an Agilent 2100 Bioanalyzer Instrument (G2939BA) with the Agilent RNA 6000 Nano Reagents (Agilent, 5067-1511). RNA was divided into two samples and stored at -80°C, 1µg for RNA sequencing and the rest for cDNA synthesis and RT-qPCR measurements.

Real time-quantitative PCR

To measure the expression of *CPT1A* in all the cell samples, cDNA was synthesized with 200ng of the stored RNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche, 04897030001) according to the manufacturer's instructions. Quantitative real time PCR's for *CPT1A* (Thermofisher, Hs00912671_m1, 4331182) were performed using the TaqMan™ Fast Advanced Master Mix (Thermofisher, 4444557) with 10ng cDNA per reaction on a QuantStudio 6 Real-Time PCR system (Applied Biosystems). All RT-qPCR reactions were performed in triplicate and outliers were removed if the Ct value measured differed more than 0.5% from the mean. Relative gene expression levels ($-\Delta\text{Ct}$) were calculated using the average of Ct values of *RPL13A* (Thermofisher, Hs03043887_gH, 4448892) and *SDHA* (Thermofisher, Hs00188166_m1, 4453320) as internal controls³³. The fold change was determined using the $2^{-\Delta\Delta\text{Ct}}$ method, using the control as the reference. All statistical analyses were performed in R. Data are expressed as mean of the relative fold change and standard error. The reported P values were determined by applying a paired two-tailed student's T test. P values < 0.05 were considered to be statistically significant.

RNA sequencing analysis

RNA sequencing (RNA-seq) was performed to determine the differences in the transcriptome of control versus fatty acid exposed non-activated CD4⁺ T cells across time. The RNA from each of the samples was sent for sequencing (Macrogen, Amsterdam, NL). RNA-sequencing libraries were prepared from 200ng RNA using the Illumina Truseq stranded mRNA library prep (Illumina, 20020594) with a poly A selection. Both whole-transcriptome amplification and sequencing library preparations were performed in two 96-well plates with 26 samples in one plate and 6 in another. Quality control steps were included to determine total RNA quality and quantity, the optimal number of PCR preamplification cycles, and fragment size selection. No samples were eliminated from further downstream steps. Barcoded libraries were divided across two plates with 26 samples in one and 6 in the other and sequenced separately. Barcoded libraries were sequenced to a read depth of 20 million reads using the Novaseq 6000 (Illumina) to generate 100 base pair paired-end reads.

FastQ files are analyzed using the RNAseq pipeline (v5.0.0) from BioWDL (<https://zenodo.org/record/5109461>), developed by SASC (LUMC). The pipeline performed preprocessing on the FastQ files (including quality control, quality trimming, and adapter clipping), read mapping, and expression quantification. *FastQC* (v0.11.9) is used to check raw reads and *Cutadapt* (v2.10) to perform adapter clipping. Reads are mapped to a reference genome (Ensembl v105) using *STAR aligner* (v2.7.5a), and with *HTSeq Count* (v0.12.4) the number of assigned reads to genes per sample is determined.

Based on Ensembl gene biotype annotation, we included only protein coding genes for further downstream analysis (19,991 genes in total). We used the Bioconductor package *DESeq2*³⁴ (v1.40.2) to test whether EPA, OA, or PA had an effect on gene expression as compared to the control. *DESeq2* fits a generalized linear model (GLM) assuming the negative binomial distribution for the counts. The model expresses the logarithm of the average of the counts in terms of one or more predictors. In this case, we used three models that had one of the fatty acids, subject identifier, and batch as predictors each. By including the subject identifier and batch in the models, we account for the dependence between measurements within the same subject and between different batches of sequencing³⁴. Lowly expressed genes, i.e. that did not have at least a count of 1 in half of the samples per fatty acid and control, were removed, resulting in 12,938 genes for EPA, 12,949 genes for OA, and 12,971 genes for PA. The Benjamini-Hochberg procedure was used to correct for multiple testing at a false discovery rate (FDR) of 5%.

Differentially expressed genes per fatty acid were divided into upregulated or downregulated based on the log₂ fold change values. 10 human pathway databases (BioPlanet 2019, WikiPathways 2019 Human, KEGG 2019 Human, Elsevier Pathway Collection, BioCarta 2015, Reactome 2016, HumanCyc 2016, NCI-Nature 2016, Panther 2016 and MSigDB Hallmark 2020) were queried using gene symbols, with 904 of 1170 queried genes for EPA, 51 of 60 queried genes for OA, and 26 of 33 queried genes for PA, present in at least 1 database. The identified clusters were then mapped for pathway enrichment using *clusterProfiler*³⁵ (v4.8.3) with the background set to the 12,938 expressed genes for EPA, 12,949 expressed genes for OA, and 12,971 expressed genes for PA as determined above. Multiple testing correction using the Benjamini-Hochberg method at 5% FDR was performed over the combined results from the 10 databases. Pathways that included highly similar gene sets were grouped (Jaccard index > 0.7) and only the most significantly enriched pathway per group was retained.

ATAC sequencing analysis

Post-exposure, the 1×10^5 cells were taken off for ATAC sequencing and placed into DNA LoBind 1.5mL tubes (Eppendorf, 2231000945). The cells were washed 3x in ice cold buffered sodium chloride (PBS; pH 7.4; Fresen, 15360679). The samples were then handed off to the Leiden Genome Technology Center for library generation. The ATAC-sequencing libraries were generated using the Omni-ATAC protocol³⁶. Briefly, the nuclei were isolated by lysing the cells in ATAC-Resuspension Buffer (RSB) (0.1% NP40 (ThermoFisher, 85124), 0.1% Tween-20 (ThermoFisher, 28320), and 0.01% digitonin (Promega, G9441)) for 3 min on ice. After washing the nuclei with 1mL wash buffer (RSB and 0.1% Tween) the nuclei were centrifuged for 10min at 4°C. After removing the supernatant, carefully avoiding the pelleted nuclei, the nuclei were resuspended in PBS. The nuclei were counted and normalized to 25,000 cells using the TC20 cell counter (BioRad, 1450102). The nuclei were combined with 25µL 2x TD buffer (TrisHCl pH 7.5 (ThermoFisher, 15567027), NaCl (ThermoFisher, A57006) and MgCl₂ (ThermoFisher, AM9530G)), 2µL Tn5 enzyme (Tn5 enzyme (Illumina, 15027865) and TD Tagment DNA Buffer (Illumina, 15027866)), 0.5µL 1% digitonin, 0.5µL 10% Tween-20 up to a volume of 50µL. The reaction was incubated at 37°C for 30min and then purified using AMPure Beads (Beckman Coulter, A63881) with a ratio of 1.8x and

eluted in 10 μ L of EB (10mM Tris-HCl). The PCR was done using 2x Kapa HiFi Master mix (Roche, 09420398001) with the barcoded primers described in the Omni-ATAC protocol. After the PCR, the products were dual size selected using AMPure beads, first using 0.4x, followed directly by 1.2x. The ATAC-sequencing libraries were checked on the Femto Pulse (Agilent, M5330AA) and pooled equimolar for sequencing. No samples were eliminated from further downstream steps.

Barcoded libraries were sent for sequencing (Macrogen, Amsterdam, NL). An additional round of quality control was performed and the samples were then pooled and divided across one lane. Barcoded libraries were sequenced to a read depth of 30 million 150 base pair paired-end reads using the Novaseq 6000 (Illumina).

FastQ files were analyzed using the ChIP-seq pipeline from BioWDL (<https://github.com/biowdl/ChIP-seq>), developed by SASC (LUMC). The pipeline performed preprocessing on the FastQ files (including quality control, quality trimming, and adapter clipping), read mapping, and peak calling. *FastQC* (v0.11.9) is used to check raw reads and *Cutadapt* (v2.10) to perform adapter clipping. Reads are mapped to a reference genome (Encode GRCh38) using *BWA aligner* (v0.7.17), and *MACS2* (v2.1.2) was used to perform the peakcalling. These peak files were then processed using R (v4.3.0). Using *DiffBind* (v3.10.0), reads in the BAM files were counted for each peak. Next, the read counts per peak for each sample were merged to create one table containing all peaks and read counts of all the samples combined. *De novo* motif analysis was then performed using HOMER³⁷.

Data availability

The data supporting the findings of this study are available within the article and its Supplementary information files. All other data including the raw files are available at the Gene Expression Omnibus repository, accession GEO (main combined submission: GSE254749, RNA sequencing submission: GSE254695, and ATAC sequencing submission: GSE254468).

Results

Transcriptomic analysis of EPA exposed non-activated CD4⁺ T cells

Non-activated CD4⁺ T cells from 8 different donors were each exposed to 100 μ M EPA, OA, PA, or control for 48h (Fig. 1a). Exposure did not affect cell viability or diameter (Supp. Fig. 1a and b). To confirm a response by the cells due to the fatty acid exposure, the expression of *CPT1A*, the rate limiting enzyme in β -fatty acid oxidation, was measured. *CPT1A* expression increased as compared to control (EPA: 12.4-fold, SE 1.9 ($p < 0.001$); OA: 19.5-fold, SE 3.0 ($p < 0.001$); PA: 11.3-fold, SE 2.2 ($p < 0.003$); Fig. 1b). This signifies a consistent response to EPA, OA, and PA exposure.

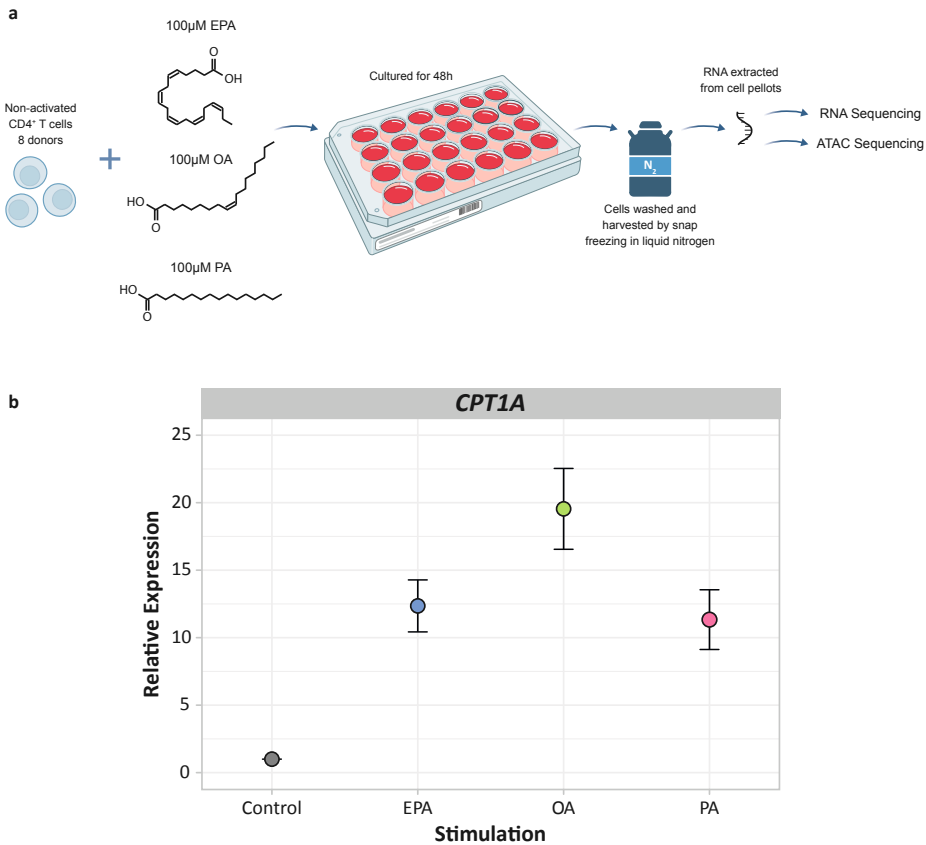


Fig. 1 | Experimental set up and *in vitro* model verification. (a) Experimental set up for RNA and ATAC sequencing of EPA, OA, and PA exposed non-activated CD4⁺ T cells, n = 8. (b) Dot plot showing the relative expression of *CPT1A* after 48h of fatty acid exposure as a confirmation of the *in vitro* model by RT-qPCR. Values are colored by fatty acid. On average *CPT1A* was upregulated 12.4 SE 1.9 fold for EPA ($p < 0.001$), 19.5 SE 3.0 fold for OA ($p < 0.001$), and 11.3 SE 2.2 fold for PA ($p < 0.003$), n = 8. Abbreviations, EPA = eicosapentaenoic acid, h = hours, OA = oleic acid, PA = palmitic acid.

Next, we studied the transcriptomic response of CD4⁺ T cells to EPA, OA, and PA as compared to control using RNA-seq. The transcriptional response was compared to the control condition for each fatty acid. The number of differentially expressed genes (DEGs) and effect sizes were markedly larger for EPA, than for OA and PA (Fig. 2a) and there was limited overlap between the DEGs of each fatty acid (Fig. 2b). EPA induced 1170 DEGs ($P_{\text{FDR}} < 0.05$), 723 of which were downregulated and 447 of which were upregulated (Supp. Table 1a and b). In contrast, OA induced 60 DEGs ($P_{\text{FDR}} < 0.05$; 13 downregulated and 47 upregulated; Supp. Table 1c and d). PA induced 33 DEGs ($P_{\text{FDR}} < 0.05$; 15 downregulated and 18 upregulated; Supp. Table 1e and f). Despite the high specificity of the transcriptional response of each fatty acid, 4 genes were upregulated upon exposure of all three fatty acids. These genes were involved in β -fatty acid oxidation (*CPT1A*, *SLC25A20*, *ACADVL*, and *ACAA2*) in line with a generic cellular response to fatty acid exposure regardless of the fatty acid type (Supp. Table 1g).

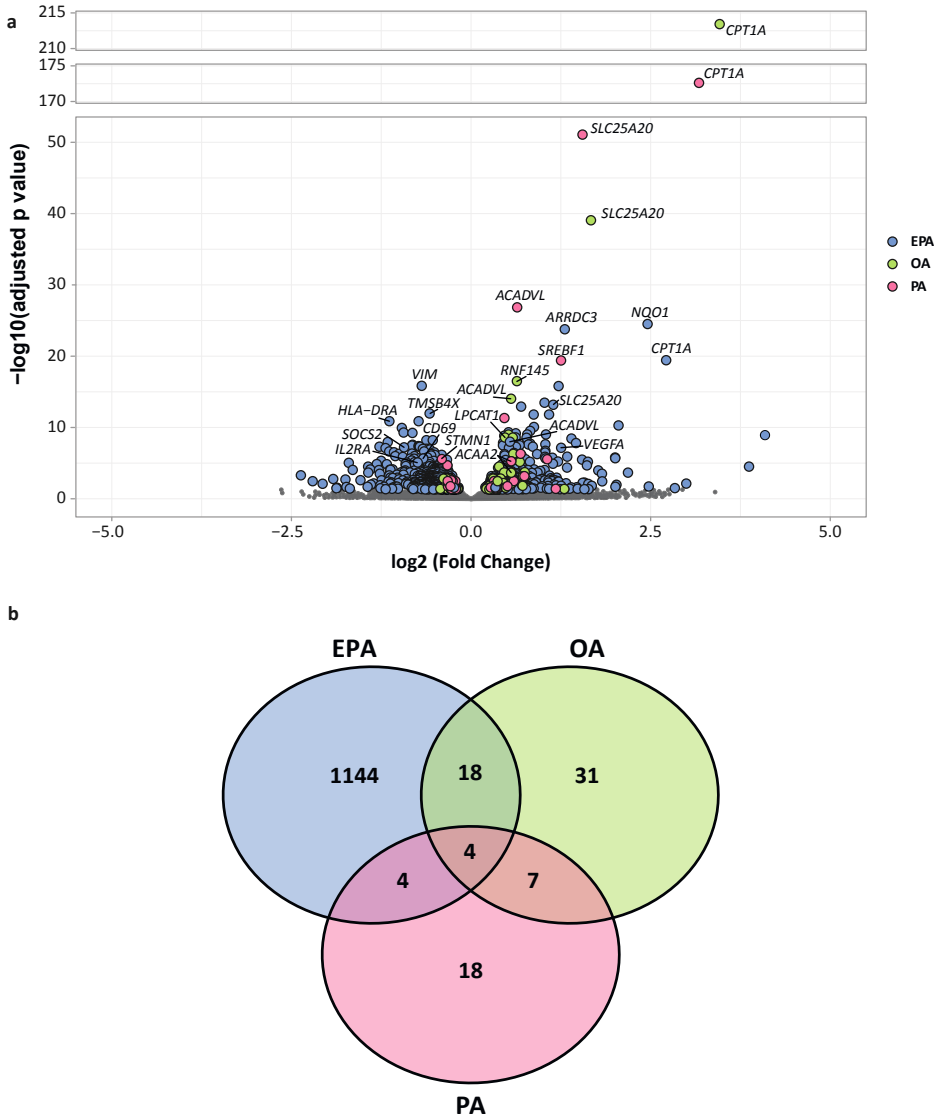


Fig. 2 | EPA, OA, and PA exposure in non-activated CD4⁺ T cells induces changes in transcriptomics. (a) Volcano plot showing the gene expression of non-activated CD4⁺ T cells exposed to either EPA, OA, or PA. All 19,991 protein coding genes are shown for each fatty acid. DEGs are colored by fatty acid and denoted by a larger size. Non-significant genes are shown in grey and denoted by a smaller size. Log₂ fold change is used to show the direction of gene expression. **(b)** Venn diagram showing the unique response of non-activated CD4⁺ T cells to each fatty acid. Values are colored by fatty acid. There are 6 DEGs overlapping between all three fatty acids, 18 DEGs overlapping between EPA and OA, 4 DEGs overlapping between EPA and PA, and 7 DEGs overlapping between OA and PA. Abbreviations, EPA = eicosapentaenoic acid, OA = oleic acid, PA = palmitic acid.

We focused on the marked transcriptomic response of CD4⁺ T cells to EPA. Firstly, we analyzed the 723 downregulated genes in EPA exposed non-activated CD4⁺ T cells. The top three DEGs were *VIM* (vimentin), *TMSB4X* (thymosin beta 4 X-linked) and *HLA-DRA* (major histocompatibility complex, class II, DR alpha). *VIM* and *TMSB4X* both encode structures involved in the makeup of the cytoskeleton. *HLA-DRA* plays a central role in the immune response by presenting peptides to T cells. Remarkably, many other immune response genes were also downregulated, including *SOCS2* (suppressor of cytokine signaling 2), *CD69* (CD69 molecule), and *IL2RA* (interleukin 2 receptor subunit alpha). *SOCS2* is a negative regulator of cytokine receptor signaling, particularly of IGF1R, an Insulin-Like Growth Factor whose expression is associated with the development of T_H17 over T_{reg} subsets. *CD69* plays an integral part in T cell activation, and *IL2RA* is an important regulator of T cell differentiation. A strong downregulation of immune-related processes was confirmed by a formal analysis of enriched biological processes. In particular, interleukin (IL)-2 signaling pathway ($P_{\text{FDR}} < 0.001$; 110 DEGs), antigen processing and presentation ($P_{\text{FDR}} < 0.001$; 27 DEGs), and interferon gamma response ($P_{\text{FDR}} < 0.001$; 47 DEGs) were enriched (Fig. 3a; Supp. Table 1h). This indicates that EPA reduces immune related gene expression in non-activated CD4⁺ T cells.

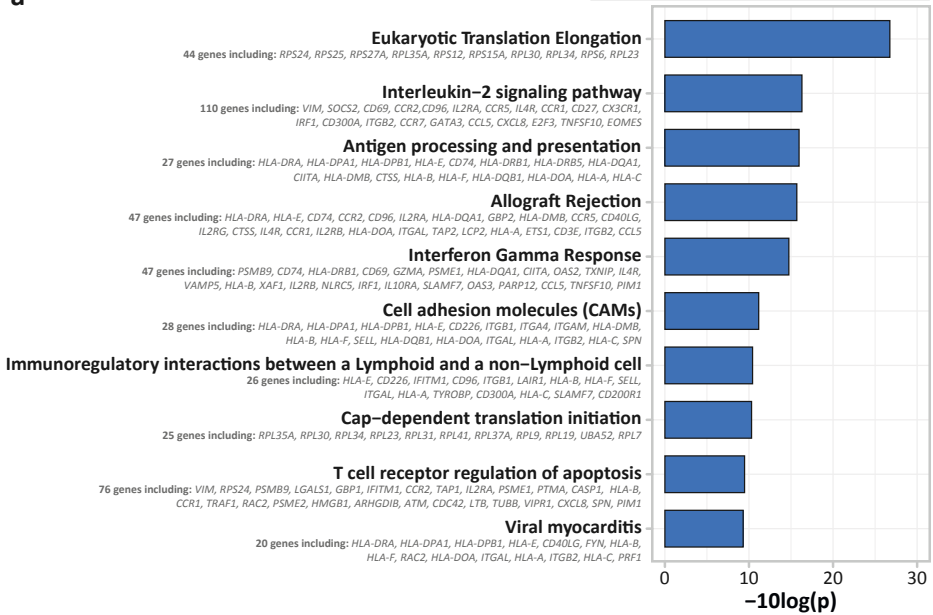
Secondly, we analyzed the 447 upregulated genes in EPA exposed non-activated CD4⁺ T cells. The top three DEGs were *NQO1* (NAD(P)H quinone dehydrogenase 1), *ARRDC3* (arrestin domain containing 3) and *CPT1A*. *NQO1* is involved in protecting cells against oxidative stress, which can be caused by lipid peroxidation and *ARRDC3* encodes a regulator of G protein-mediated signaling. Another gene of interest that was upregulated was *VEGFA* (vascular endothelial growth factor A). The enzyme encoded by this gene is a proangiogenic molecule known to be involved in creating immunosuppressive environments. This immunosuppressive profile was further supported by a formal analysis of enriched biological processes, which showed upregulation of the NRF2 pathway ($P_{\text{FDR}} < 0.001$; 20 DEGs; Fig. 3b; Supp. Table 1i). This pathway is the most important pathway for protecting cells against oxidative stress and has been shown to be involved in anti-inflammatory responses. Overall, these results suggest that EPA exposure can alter gene expression in non-activated T cells towards an anti-inflammatory profile by decreasing immune response related genes and increasing protective genes such as those in the NRF2 pathway.

Next, we investigated whether specific transcription factors may underlie the differential gene expression. To do this, we examined the enrichment of transcription factor binding motifs in loci that were more closed (down) versus more open (up) as determined by ATAC-seq. The top EPA downregulated motifs included CTCF, GATA3, RUNX1, and PU.1 (Fig. 3c; Supp. Table 1j). CTCF is a master regulator of chromatin looping and moreover, involved in effector cell differentiation^{38,39}. GATA3 and PU.1 are the key transcription factors for the development of T_H2 and T_H9 cells, respectively^{40,41}. RUNX1 is necessary for T cell maturation, knock outs of this transcription factor results in phenotypically and functionally immature T cells⁴². We next examined the enrichment of transcription factor binding motifs in upregulated versus downregulated genes. EPA upregulated motifs included, REV-ERB, TCF7, and FOXA1 (Fig. 3d; Supp. Table 1k). REV-ERB is an antagonist of ROR γ t, the key transcription factor for the development of T_H17 cells⁴³. TCF7 plays a role in the regulation of autoinflammatory T cell responses⁴⁴. FOXA1

is involved in giving T_{reg} cells their suppressive properties⁴⁵. These results further suggest that non-activated CD4⁺ T cells may decrease their ability to induce an immune response or effector T cell profile after EPA exposure.

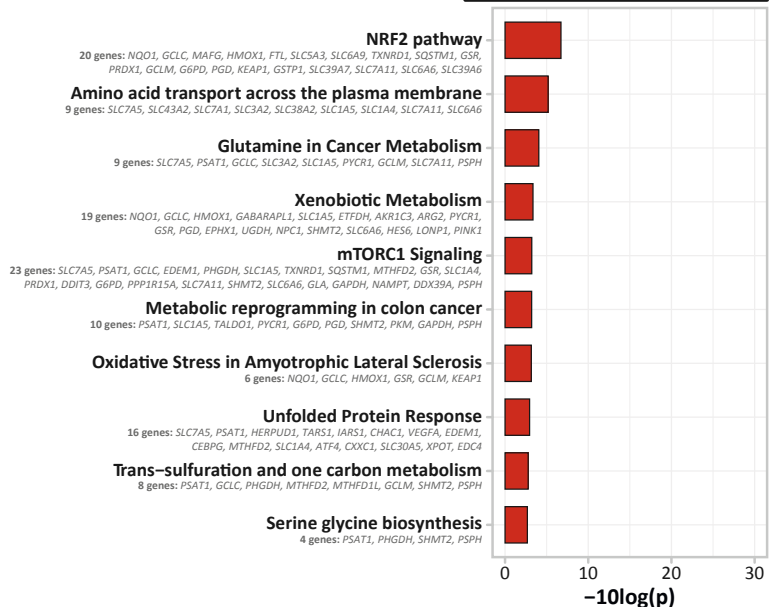
a

Downregulated Pathways EPA



b

Upregulated Pathways EPA



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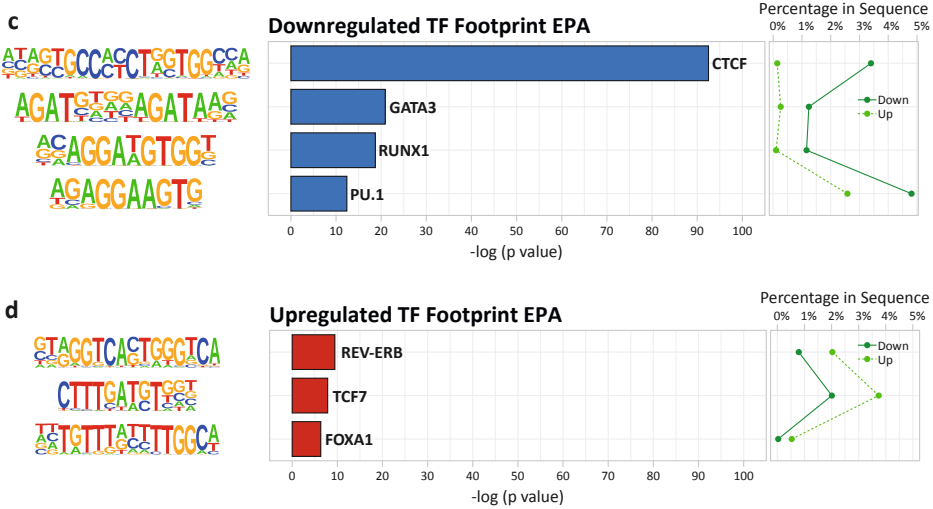


Fig. 3 | Up- and downregulated pathways and transcription factors in EPA-exposed non-activated CD4⁺ T cells. (a) Pathway enrichment analysis of all downregulated EPA DEGs generated using *clusterProfiler* using 10 human pathway databases. Top 10 enrichments are shown. (b) Pathway enrichment analysis of all upregulated EPA DEGs generated using *clusterProfiler* using 10 human pathway databases. Top 10 enrichments are shown. (c) Known motif analysis on promoters of down versus upregulated EPA ATAC peaks. Enrichment of transcription factor binding motifs was performed using HOMER. 4 motifs are shown with supplementing information on p-value, percentage of genes in upregulated gene set and percentage of genes in downregulated gene set, transcription factor name, -log(p-value), and percentage in sequence. (d) Known motif analysis on promoters of up versus downregulated EPA ATAC peaks. Enrichment of transcription factor binding motifs was performed using HOMER. 4 motifs are shown with supplementing information on p-value, percentage of genes in upregulated gene set and percentage of genes in downregulated gene set, transcription factor name, -log(p-value), and percentage in sequence. Abbreviations, EPA = eicosapentaenoic acid, TF = transcription factor.

Transcriptomic analysis of OA and PA exposed non-activated CD4⁺ T cells

Non-activated CD4⁺ T cells were also exposed to either OA or PA and differential gene expression was measured. In line with our previous experiments, we found that OA exposure lead to the downregulation of endogenous peptide antigen presentation (*HSPA5* and *PDIA3*; $P_{FDR} < 0.001$; 2 DEGs), electron transport chain and oxidative phosphorylation activity (*NDUFA12*, *NDUFB4*, and *ATP5F1C*; $P_{FDR} < 0.001$; 3 DEGs) and upregulation of cholesterol biosynthesis (*HMGR*, *HMGS1* and *DHCR24*; $P_{FDR} < 0.001$; 4 DEGs; Supp. Fig. 2a and b; Supp. Table 1l and m) related genes. Exposure to PA induced an opposite response, with the downregulation of cholesterol biosynthesis pathway (*HMGR* and *SQLE*; $P_{FDR} < 0.05$; 2 DEGs), and upregulated beta fatty acid oxidation (*CPT1A*, *SLC25A20*, and *ACADVL*; $P_{FDR} < 0.001$; 3 DEGs; Supp. Fig. 2c and d; Supp. Table 1n and o) related genes. Thus, the changes in the transcriptome of OA and PA exposed cells seem to have a greater effect on genes involved in cellular metabolism, particularly cholesterol metabolism, as compared to EPA.

The transcriptional responses observed were in line with the results of ATAC-sequencing based transcription factor footprint analysis. For OA only three motifs were downregulated including RAR:RXR, a motif known to play a part in the development of T_{reg} over T_H17 cells (Supp. Fig. 3a;

Supp. Table 1p). OA upregulated motifs included PU.1, as was found previously²⁹ as well as IRF8, which is also involved in T_H9 differentiation (Supp. Fig. 3b; Supp. Table 1q). PA downregulated motifs included IRF8 and GATA3 (Supp. Fig. 3c; Supp. Table 1r) and upregulated motifs included REV-ERB (Supp. Fig. 3d; Supp. Table 1s). OA and PA showed reversed effects on cholesterol metabolism processes which were mirrored in opposite associations with transcription factor binding motifs, indicating fatty-acid specific responses in non-activated CD4⁺ T cells.

Discussion

IPE, the highly purified form of EPA, has been associated with reduced triglycerides, cardiovascular events, and cardiovascular death in individuals with relatively well controlled LDL levels, even when corrected for placebo response in the mineral oil control group, LDL, and C-reactive protein (CRP) in the REDUCE-IT trial^{10, 46-49}. The trials outcomes and interpretation have been widely debated and the mechanisms by which EPA exerts its beneficial effects remains incompletely understood^{11, 12}. We show that EPA exposure can already produce distinct changes in T cells prior to activation by decreasing the expression of immune response genes and increasing the expression of genes involved in oxidative stress protection. This is further supported by changes in transcription factor binding sites in our ATAC-sequencing motif analysis, indicating a change in the epigenetic landscape of EPA exposed T cells. Furthermore, we show that EPA induces a unique response in non-activated CD4⁺ T cells as two other fatty acids of varying degrees of saturation, OA and PA, generated a smaller yet distinct effect on gene expression profiles in T cells as compared to control. Our findings imply that different fatty acids in the circulation can induce diverse effects on T cell transcriptomics, and that specifically EPA exposure may poise T cells to have clearer anti-inflammatory responses. These results underscore a potential mechanism by which EPA may mitigate ASCVD risk, suggesting its anti-inflammatory impact on T cells as a contributing factor. This is particularly noteworthy as T cells comprise over half of the immune cell population within atherosclerotic plaques^{17, 18}.

Our results show that EPA exposure, but not OA nor PA, leads to a strong downregulation of immune response related genes. Particularly, genes involved in antigen processing and presentation were downregulated in EPA exposed cells, denoted by, amongst others, the decreased expression of 14 different HLA genes. This gene group is crucial in inducing immune responses⁵⁰ and has also been found to be associated with T cell activation and effector memory phenotype in CD4⁺ T cells⁵¹. In addition, genes involved in IL-2 signaling were also downregulated, which is required for T cell activation⁵². Downregulation of genes in these pathways suggests that EPA exposed T cells may have a reduced ability to initiate an immune response, a key component of inflammatory responses in atherosclerotic plaques⁵³. This result can support the finding that higher plasma EPA levels are associated with lower CVD risk in humans⁵⁴. Furthermore, genes involved in pro-inflammatory pathways, such as interferon gamma response were downregulated in EPA exposed cells. IFN γ is primarily produced by pro-inflammatory T cell subset, T_H1 cells, which have also been found to decrease upon EPA exposure^{27, 55-57}. Moreover, the key transcription

factors in T_H2 and T_H9 differentiation, GATA3 and PU.1, were also found to be decreased in our motif analysis. While T_H2 cells have inconclusive effects on ASCVD, T_H9 cells have been shown to aggravate it⁵⁸⁻⁶⁰. Thus, EPA exposure decreased genes involved in immune response and pro-inflammatory pathways as well as suggests a reduced ability for key T cell differentiation transcription factors to bind.

In further support of EPA's anti-inflammatory properties on non-activated CD4⁺ T cells, we found that genes involved in the NRF2 pathway were upregulated upon EPA exposure. This pathway mainly functions in preventing oxidative stress in cells by activating genes involved in detoxification and removal of reactive oxygen species⁶¹. However, the NRF2 pathway has also been shown to aid in the anti-inflammatory responses of macrophages⁶² and has been suggested as a beneficial pleiotropic effect of statins⁶³, as oxidative stress has been found to be a risk factor for ASCVD⁶⁴. We also found an increased footprint for the transcription factors REV-ERB, TCF7, and FOXA1. These transcription factors are each involved in regulating T cell responses and generating a more anti-inflammatory T cell profile⁴³⁻⁴⁵. Overall, these data indicate that non-activated CD4⁺ T cells can already acquire an anti-inflammatory transcriptomic profile, which may play a role in the anti-inflammatory properties observed of EPA in clinical trials.

EPA has a distinct effect on CD4⁺ T cells. This is observed by our analysis of the effects of OA and PA on non-activated CD4⁺ T cells. The number of DEGs and effect sizes were smaller upon OA and PA exposure and distinctly different. Interestingly, OA and PA each had opposed effects on cholesterol biosynthesis related genes, with OA upregulating and PA downregulating genes in this pathway. Upregulation of cholesterol biosynthesis has been related to the development of T_H17 cells by controlling ROR γ t activity, the key transcription factor in T_H17 differentiation^{65, 66}. This observation can be further supported by OA downregulating the RAR:RXR motif, which is involved in generating T_{reg} cells over T_H17 and PA upregulating REV-ERB^{43, 67}. The results of OA exposure also show the robustness of our approach as our findings here match what was found previously by our group²⁹. These data suggest that our model is robust and each fatty acid induces its own unique response in non-activated CD4⁺ T cells.

Conclusion

In conclusion, our data points to the fact that EPA produces a strong and specific anti-inflammatory transcriptional profile in non-activated CD4⁺ T cells comprised of both the downregulation of immune related genes and the upregulation of antioxidant genes. This profile is supported by transcription factor motif analysis and by the analysis of two other fatty acids of varying degrees of saturation. Our results contribute to the debate of how EPA exerts beneficial effects in human ASCVD. Our study gives an indication that the beneficial effects observed of EPA, as asserted in clinical trials, can already start in the circulation by inducing an anti-inflammatory transcriptional profile in non-activated T cells with potentially anti-atherosclerotic properties.

Limitations of the study

We show that EPA exposure has beneficial anti-inflammatory effects on non-activated CD4⁺ T cells. This is relevant because T cells are largely non-activated in the circulation and it is in the circulation where T cells will encounter EPA when individuals are treated with IPE to reduce ASCVD risk. Nevertheless, our results are in line with experiments on activated T cells, which showed that EPA exposure decreased proliferation²²⁻²⁶, decreased T_H1 and T_H17 populations^{27, 28}, and had no effect on or increased T_H2 and T_{reg} populations²⁶⁻²⁸. Therefore, the encounter with EPA in a non-activated state may induce transcriptomic changes that influence the functional changes post-activation. Furthermore, our use of non-activated CD4⁺ T cells with no additional selection towards naïve, effector, memory, or specific T helper subsets, as well as in culture medium containing other lipids more closely represents the diversity of T cells and environment of the circulation in which EPA exposure takes place. Additionally, we utilized OA and PA, two fatty acids of varying degrees of saturation to establish the distinct effects of EPA. Nevertheless, this does not rule out that other fatty acids may have marked effects on non-activated CD4⁺ T cells as well²¹. A final limitation of our study is that we have employed an *in vitro* model and the effects of EPA on T cells *in vivo* should be studied in the context of trials of IPE. However, in mouse models, EPA supplementation has also been shown to reduce cholesterol levels⁶⁸, whereas, in humans, the effects of EPA on ASCVD risk were independent of LDL lowering⁴⁷. Therefore, using a validated *in vitro* model provides valuable insights to study the effects of EPA on human CD4⁺ T cells.

Translational perspectives

We report that EPA induces an anti-inflammatory transcriptomic profile in non-activated CD4⁺ T cells. This observation is important to better understand the mechanism through which EPA reduces cardiovascular disease risk in studies such as the REDUCE-IT trial. The REDUCE-IT trial showed that intervention with IPE, which the body metabolizes to EPA, reduces the risk of cardiovascular events and death in patients with high triglycerides. The findings of this trial have sparked considerable debate in the literature as the results appeared to occur irrespective of the attained triglyceride level after one year¹⁰⁻¹². Testing T cells from individuals undergoing IPE interventions can provide additional insights into how EPA exerts its beneficial effects independent of triglyceride reduction.

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Author contributions

B.T.H and J.W.J conceived the project. N.A.R. designed and conducted the experiments, analyzed the results, and drafted the manuscript. K.F.D. designed the analysis model and analyzed the RNA sequencing data. J.M. designed and performed *in vitro* model and prepped samples for ATAC sequencing. S. A. designed and performed *in vitro* model and prepped samples for RNA sequencing. T.K. aligned the RNA and ATAC sequencing data. Y.A. performed the ATAC sequencing library preparation. M.A.H. performed and analyzed the transcription factor footprint analysis. All authors contributed to the writing of the manuscript.

Competing interests

The authors declare no competing interests.

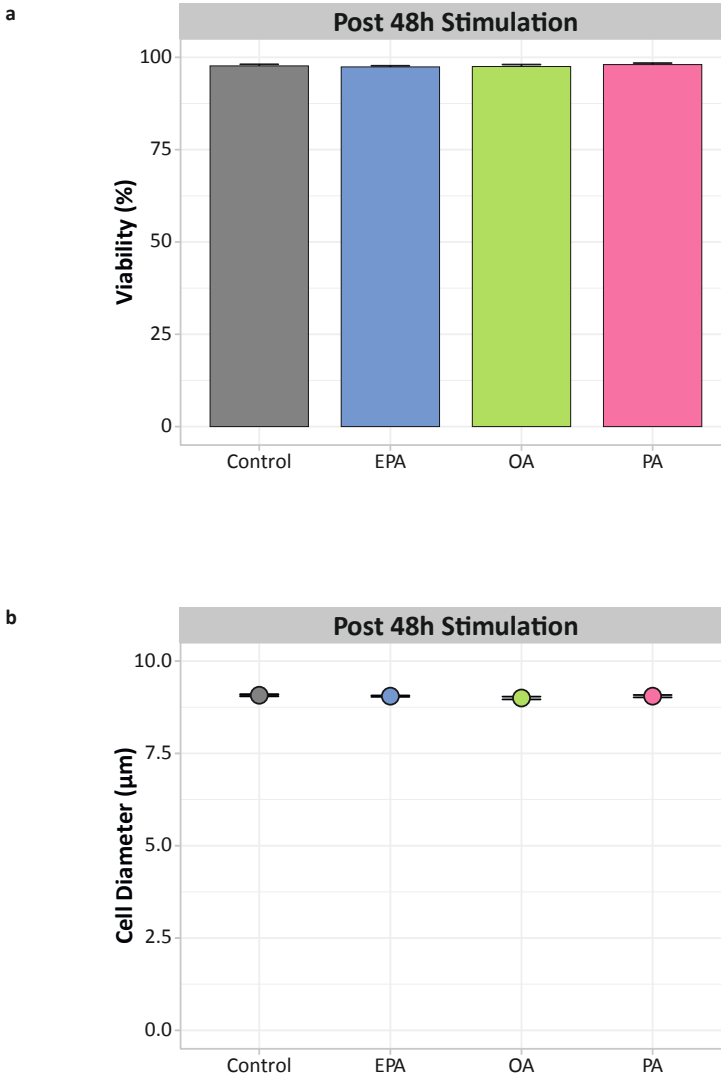
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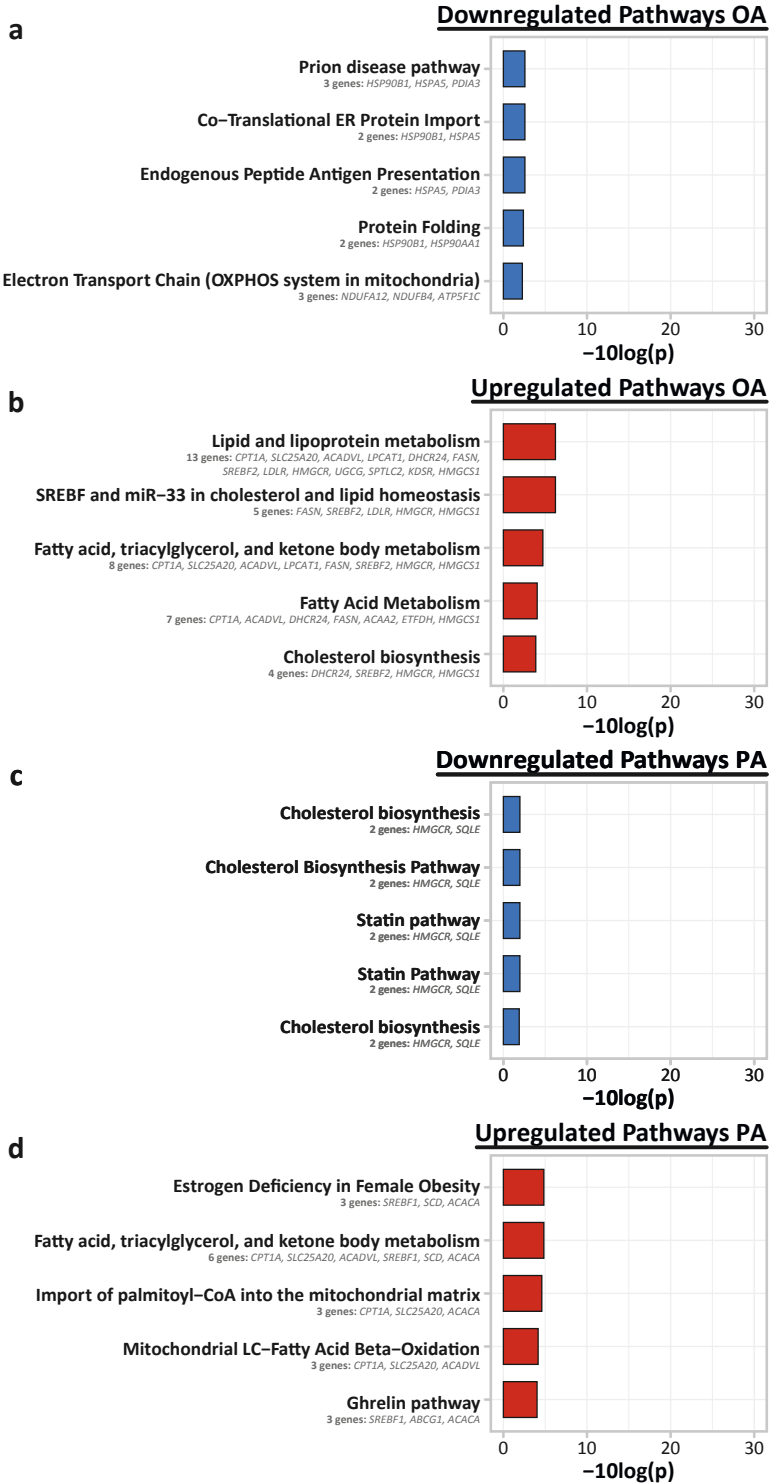
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Supplemental information

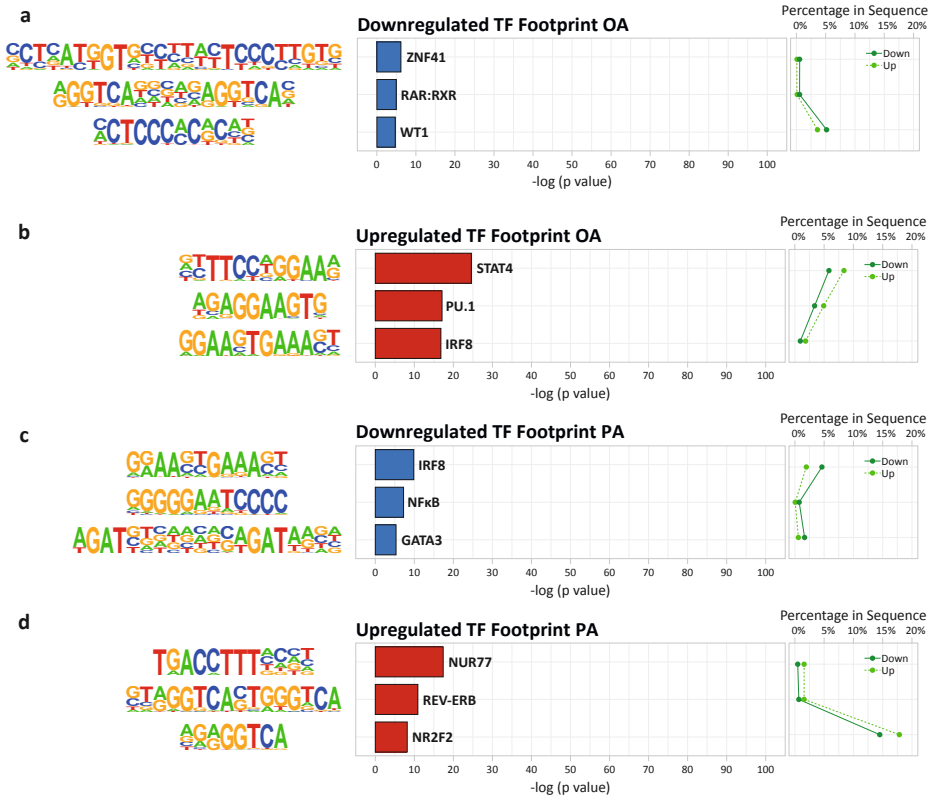


Supplemental Fig. 1 | Verification of viability and cell diameter post-exposure for RNA sequencing. (a) Bar plot showing the average cell viability and standard error in percent, as determined by Via1-Cassette™ on a NucleoCounter® NC-200™. On average the cell viability of control exposed cells was 97.7 SE 0.4%, EPA exposed cells was 97.4 SE 0.3% ($p > 0.05$), OA exposed cells was 97.5 SE 0.5% ($p > 0.05$), and PA exposed cells was 98.0 SE 0.4% ($p > 0.05$) at 48h. Thus, there was no effect on CD4⁺ T cell viability after 48h exposure, $n = 8$. **(b)** Dot plot showing the average cell diameter and standard error in μm , as determined by Via1-Cassette™ on a NucleoCounter® NC-200™. On average the cell diameter of control exposed cells was 9.1 SE 0.03 μm , EPA exposed cells was 9.1 SE 0.02 μm ($p > 0.05$), OA exposed cells was 9.0 SE 0.04 μm ($p > 0.05$), and PA exposed cells was 9.1 SE 0.03 μm ($p > 0.05$) at 48h. Thus, there was no effect on CD4⁺ T cell diameter after 48h exposure, $n = 8$. Abbreviations, EPA = eicosapentaenoic acid, h = hours, OA = oleic acid, PA = palmitic acid.



Supplemental Fig. 2 | Up- and downregulated pathways in PA and OA-exposed non-activated CD4⁺ T cells.

(a) Pathway enrichment analysis of all downregulated OA DEGs generated using *clusterProfiler* using 10 human pathway databases. Top 5 enrichments are shown. **(b)** Pathway enrichment analysis of all upregulated OA DEGs generated using *clusterProfiler* using 10 human pathway databases. Top 5 enrichments are shown. **(c)** Pathway enrichment analysis of all downregulated PA DEGs generated using *clusterProfiler* using 10 human pathway databases. Top 5 enrichments are shown. **(d)** Pathway enrichment analysis of all upregulated PA DEGs generated using *clusterProfiler* using 10 human pathway databases. Top 5 enrichments are shown. Abbreviations, OA = oleic acid, PA = palmitic acid.



Supplemental Fig. 3 | Up- and downregulated transcription factors in PA and OA-exposed non-activated CD4⁺ T cells. (a) Known motif analysis on promoters of down versus upregulated OA ATAC peaks. Enrichment of transcription factor binding motifs was performed using HOMER. 3 motifs are shown with supplementing information on p-value, percentage of genes in upregulated gene set and percentage of genes in downregulated gene set, transcription factor name, $-\log(p\text{-value})$, and percentage in sequence. (b) Known motif analysis on promoters of up versus downregulated OA ATAC peaks. Enrichment of transcription factor binding motifs was performed using HOMER. 4 motifs are shown with supplementing information on p-value, percentage of genes in upregulated gene set and percentage of genes in downregulated gene set, transcription factor name, $-\log(p\text{-value})$, and percentage in sequence. (c) Known motif analysis on promoters of down versus upregulated PA ATAC peaks. Enrichment of transcription factor binding motifs was performed using HOMER. 3 motifs are shown with supplementing information on p-value, percentage of genes in upregulated gene set and percentage of genes in downregulated gene set, transcription factor name, $-\log(p\text{-value})$, and percentage in sequence. (d) Known motif analysis on promoters of up versus downregulated PA ATAC peaks. Enrichment of transcription factor binding motifs was performed using HOMER. 4 motifs are shown with supplementing information on p-value, percentage of genes in upregulated gene set and percentage of genes in downregulated gene set, transcription factor name, $-\log(p\text{-value})$, and percentage in sequence. Abbreviations, OA = oleic acid, PA = palmitic acid, TF = transcription factor.

Supplemental Table 1 | Changes in gene expression and CD4⁺ T cell markers due to EPA, PA, or OA exposure.

Supplemental Table 1 | (a) (partial) Downregulated DEGs for EPA exposed non-activated CD4⁺ T cells in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log₂ fold change, log fold change standard error, p value, and adjusted p value (FDR). Top 30 most significantly expressed genes are shown here.

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC		
						SE	P value	P _{FDR}
1	ENSG00000026025	VIM	P08670	10461.483	-0.686	0.075	4.44E-20	1.44E-16
2	ENSG00000205542	TMSB4X	P62328	48176.611	-0.577	0.072	7.52E-16	1.08E-12
3	ENSG00000204287	HLA-DRA	P01903	633.049	-1.137	0.147	1.27E-14	1.32E-11
4	ENSG00000138326	RPS24	P62847	18476.569	-0.730	0.095	1.33E-14	1.32E-11
5	ENSG00000231389	HLA-DPA1	P20036	417.419	-0.963	0.130	1.43E-13	1.16E-10
6	ENSG00000026950	BTN3A1	O00481	2825.734	-0.939	0.131	7.52E-13	5.12E-10
7	ENSG00000106952	TNFSF8	P32971	1146.126	-0.816	0.114	9.00E-13	5.83E-10
8	ENSG00000034510	TMSB10	P63313	16350.584	-0.622	0.092	1.37E-11	6.08E-09
9	ENSG00000118181	RPS25	P62851	30745.712	-0.533	0.079	1.41E-11	6.08E-09
10	ENSG00000223865	HLA-DPB1	P04440	318.848	-1.159	0.174	2.74E-11	1.11E-08
11	ENSG00000186470	BTN3A2	P78410	3097.909	-0.814	0.125	7.80E-11	2.65E-08
12	ENSG00000240065	PSMB9	P28065	851.254	-0.822	0.127	1.09E-10	3.44E-08
13	ENSG00000111801	BTN3A3	O00478	2129.494	-0.732	0.114	1.32E-10	4.08E-08
14	ENSG00000100097	LGALS1	P09382	476.452	-0.723	0.113	1.68E-10	4.93E-08
15	ENSG00000204592	HLA-E	P13747	21754.754	-0.713	0.112	1.76E-10	4.96E-08
16	ENSG00000255150	EID3	Q8N140	109.010	-1.274	0.200	1.76E-10	4.96E-08
17	ENSG00000120833	SOCS2	O14508	272.816	-0.932	0.147	2.27E-10	6.12E-08
18	ENSG00000160856	FCRL3	Q96P31	201.018	-1.190	0.189	3.06E-10	7.76E-08
19	ENSG00000019582	CD74	P04233	2645.553	-0.622	0.099	3.92E-10	9.74E-08
20	ENSG00000008517	IL32	P24001	4786.915	-0.671	0.107	4.11E-10	1.00E-07
21	ENSG00000185880	TRIM69	Q86WT6	867.798	-0.572	0.092	5.10E-10	1.22E-07
22	ENSG00000131981	LGALS3	P17931	568.618	-0.653	0.107	9.88E-10	2.28E-07
23	ENSG00000113088	GZMK	P49863	365.982	-1.139	0.187	1.02E-09	2.31E-07

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Supplemental Table 1 | (a) (partial) [continued]

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC SE	P value	P _{FDR}
24	ENSG00000107736	<i>CDH23</i>	Q9H251	268.652	-1.072	0.177	1.35E-09	3.02E-07
25	ENSG00000243335	<i>KCTD7</i>	Q96MP8	1002.502	-0.512	0.085	1.40E-09	3.07E-07
26	ENSG00000114737	<i>CISH</i>	Q9NSE2	599.263	-0.822	0.137	2.18E-09	4.55E-07
27	ENSG00000196126	<i>HLA-DRB1</i>	P01911	300.924	-1.002	0.168	2.43E-09	5.00E-07
28	ENSG00000130755	<i>GMFG</i>	O60234	3215.210	-0.548	0.092	2.54E-09	5.14E-07
29	ENSG00000143947	<i>RPS27A</i>	P62979	45091.864	-0.408	0.069	3.60E-09	6.96E-07
30	ENSG00000101608	<i>MYL12A</i>	P19105	7915.620	-0.504	0.086	3.78E-09	7.19E-07

Supplemental Table 1 | (b) (partial) Upregulated DEGs for EPA exposed non-activated CD4⁺ T cells in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log₂ fold change, log fold change standard error, p value, and adjusted p value (FDR). Top 30 most significantly expressed genes are shown here.

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC SE	P value	P _{FDR}
1	ENSG00000181019	<i>NQO1</i>	P15559	346.693	2.457	0.218	2.37E-29	3.07E-25
2	ENSG00000113369	<i>ARRDC3</i>	Q96B67	3175.559	1.305	0.118	2.59E-28	1.68E-24
3	ENSG00000110090	<i>CPT1A</i>	P50416	603.707	2.716	0.270	8.52E-24	3.68E-20
4	ENSG00000103257	<i>SLC7A5</i>	Q01650	1346.098	1.219	0.133	5.98E-20	1.55E-16
5	ENSG00000115758	<i>ODC1</i>	P11926	1660.221	1.023	0.120	1.56E-17	3.37E-14
6	ENSG00000178537	<i>SLC25A20</i>	O43772	267.897	1.144	0.136	3.55E-17	6.57E-14
7	ENSG00000090861	<i>AARS1</i>	P49588	4381.327	0.697	0.084	7.34E-17	1.19E-13
8	ENSG00000135069	<i>PSAT1</i>	Q9Y617	1488.575	1.086	0.136	1.18E-15	1.53E-12
9	ENSG00000001084	<i>GCLC</i>	P48506	1258.380	0.872	0.109	1.31E-15	1.54E-12
10	ENSG00000167703	<i>SLC43A2</i>	Q8N370	169.252	2.053	0.273	5.60E-14	5.17E-11
11	ENSG00000125534	<i>PPDPF</i>	Q9H3Y8	1332.610	0.876	0.118	9.86E-14	8.50E-11
12	ENSG00000172613	<i>RAD9A</i>	Q99638	820.146	0.823	0.113	3.95E-13	3.01E-10
13	ENSG00000139514	<i>SLC7A1</i>	P30825	1604.807	0.522	0.073	6.51E-13	4.68E-10

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Supplemental Table 1 | (b) (partial) *[continued]*

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC		
						SE	P value	P _{FDR}
14	ENSG00000051108	<i>HERPUD1</i>	Q15011	1950.929	0.623	0.087	1.07E-12	6.59E-10
15	ENSG00000197063	<i>MAFG</i>	O15525	646.794	1.042	0.148	1.68E-12	9.53E-10
16	ENSG00000116852	<i>KIF21B</i>	O75037	5536.202	0.782	0.111	1.69E-12	9.53E-10
17	ENSG00000100292	<i>HMOX1</i>	P09601	118.300	4.091	0.583	2.20E-12	1.19E-09
18	ENSG00000198830	<i>HMGN2</i>	P05204	4201.608	0.462	0.066	2.80E-12	1.45E-09
19	ENSG00000113407	<i>TARS1</i>	P26639	3883.418	0.621	0.090	4.95E-12	2.46E-09
20	ENSG00000162413	<i>KLHL21</i>	Q9UJP4	1585.720	0.772	0.112	5.78E-12	2.77E-09
21	ENSG00000168003	<i>SLC3A2</i>	P08195	5743.672	1.397	0.204	7.76E-12	3.58E-09
22	ENSG00000072778	<i>ACADVL</i>	P49748	3071.367	0.541	0.081	2.37E-11	9.91E-09
23	ENSG00000196305	<i>IARS1</i>	P41252	3525.848	0.446	0.067	3.64E-11	1.43E-08
24	ENSG00000128965	<i>CHAC1</i>	Q9BUX1	266.899	1.461	0.221	4.02E-11	1.53E-08
25	ENSG00000082898	<i>XPO1</i>	O14980	4845.018	0.824	0.126	5.09E-11	1.88E-08
26	ENSG00000139112	<i>GABARAPL1</i>	Q9HOR8	821.575	1.034	0.158	6.32E-11	2.27E-08
27	ENSG00000087086	<i>FTL</i>	P02792	23835.068	0.800	0.123	6.97E-11	2.44E-08
28	ENSG00000134294	<i>SLC38A2</i>	Q96QD8	5627.146	0.676	0.104	9.33E-11	3.10E-08
29	ENSG00000166986	<i>MARS1</i>	P56192	5359.986	0.444	0.069	1.00E-10	3.24E-08
30	ENSG00000198743	<i>SLC5A3</i>	P53794	1939.433	0.860	0.135	1.66E-10	4.93E-08

Supplemental Table 1 | (c) Downregulated DEGs for OA exposed non-activated CD4⁺ T cells in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log2 fold change, log fold change standard error, p value, and adjusted p value (FDR).

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC		
						SE	P value	P _{FDR}
1	ENSG00000205542	<i>TMSB4X</i>	P62328	53784.953	-0.300	0.063	1.93E-06	0.00146
2	ENSG00000166598	<i>HSP90B1</i>	P14625	9262.938	-0.336	0.071	2.02E-06	0.00146
3	ENSG00000044574	<i>HSPA5</i>	P11021	5535.011	-0.382	0.081	2.19E-06	0.00149
4	ENSG00000184752	<i>NDUFA12</i>	Q9UI09	1467.880	-0.347	0.082	2.55E-05	0.01178
5	ENSG00000111348	<i>ARHGDI3</i>	P52566	18341.064	-0.224	0.057	9.10E-05	0.03021

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Supplemental Table 1 | (c) *[continued]*

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC SE	P value	P _{FDR}
6	ENSG00000065518	<i>NDUFB4</i>	O95168	923.826	-0.345	0.090	0.000119	0.03589
7	ENSG00000167004	<i>PDIA3</i>	P30101	5053.715	-0.275	0.072	0.000135	0.03820
8	ENSG00000034510	<i>TMSB10</i>	P63313	18121.778	-0.287	0.076	0.000146	0.03951
9	ENSG00000265972	<i>TXNIP</i>	Q9H3M7	19375.847	-0.185	0.049	0.000150	0.03973
10	ENSG00000145050	<i>MANF</i>	P55145	661.685	-0.427	0.113	0.000159	0.04114
11	ENSG00000111481	<i>COPZ1</i>	P61923	1920.473	-0.221	0.060	0.000207	0.04787
12	ENSG00000165629	<i>ATP5F1C</i>	P36542	1803.183	-0.277	0.075	0.000211	0.04787
13	ENSG00000080824	<i>HSP90AA1</i>	P07900	12647.574	-0.264	0.072	0.000222	0.04787

Supplemental Table 1 | (d) (partial) Upregulated DEGs for OA exposed non-activated CD4⁺ T cells in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log₂ fold change, log fold change standard error, p value, and adjusted p value (FDR). Top 30 most significantly expressed genes are shown here.

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC SE	P value	P _{FDR}
1	ENSG00000110090	<i>CPT1A</i>	P50416	893.439	3.460	0.110	2.87E-218	3.71E-214
2	ENSG00000178537	<i>SLC25A20</i>	O43772	352.353	1.669	0.121	1.34E-43	8.65E-40
3	ENSG00000145860	<i>RNF145</i>	Q96MT1	2335.306	0.638	0.068	7.35E-21	3.17E-17
4	ENSG00000072778	<i>ACADVL</i>	P49748	3128.902	0.557	0.064	2.87E-18	9.28E-15
5	ENSG00000143110	<i>C1orf162</i>	Q8NEQ5	1332.128	0.522	0.072	3.78E-13	9.78E-10
6	ENSG00000153395	<i>LPCAT1</i>	Q8NF37	1527.598	0.473	0.067	1.35E-12	2.59E-09
7	ENSG00000184602	<i>SNN</i>	O75324	905.754	0.581	0.082	1.40E-12	2.59E-09
8	ENSG00000116133	<i>DHCR24</i>	Q15392	474.003	0.588	0.093	3.08E-10	4.98E-07
9	ENSG00000169710	<i>FASN</i>	P49327	866.664	0.680	0.116	4.68E-09	6.74E-06
10	ENSG00000198911	<i>SREBF2</i>	Q12772	3184.981	0.390	0.070	2.37E-08	3.07E-05
11	ENSG00000102032	<i>RENBP</i>	P51606	250.558	0.747	0.142	1.57E-07	0.000184
12	ENSG00000130164	<i>LDLR</i>	P01130	1013.144	0.461	0.088	1.87E-07	0.000202
13	ENSG00000167315	<i>ACAA2</i>	P42765	483.439	0.558	0.108	2.23E-07	0.000222

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Supplemental Table 1 | (d) (partial) *[continued]*

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC		P _{FDR}
						SE	P value	
14	ENSG00000166575	TMEM135	Q86UB9	161.166	0.722	0.146	7.39E-07	0.000683
15	ENSG00000167106	FAM102A	Q5T9C2	7915.593	0.290	0.060	1.43E-06	0.001236
16	ENSG00000103249	CLCN7	P51798	1764.038	0.411	0.086	1.88E-06	0.001456
17	ENSG00000069424	KCNAB2	Q13303	4855.716	0.308	0.065	2.30E-06	0.001489
18	ENSG00000113161	HMGR	P04035	913.374	0.369	0.081	5.52E-06	0.003402
19	ENSG00000198355	PIM3	Q86V86	716.562	0.450	0.102	1.05E-05	0.006197
20	ENSG00000171503	ETFDH	Q16134	301.259	0.553	0.127	1.41E-05	0.007922
21	ENSG00000140526	ABHD2	P08910	1283.746	0.291	0.068	1.70E-05	0.009194
22	ENSG00000174903	RAB1B	Q9H0U4	2107.877	0.334	0.078	1.95E-05	0.010094
23	ENSG00000106266	SNX8	Q9Y5X2	144.357	0.710	0.168	2.40E-05	0.011780
24	ENSG00000079432	CIC	Q96RKO	2107.033	0.347	0.082	2.53E-05	0.011780
25	ENSG00000163162	RNF149	Q8NC42	1907.621	0.340	0.081	3.02E-05	0.013471
26	ENSG00000161011	SQSTM1	Q13501	3912.137	0.262	0.063	3.26E-05	0.014059
27	ENSG00000172059	KLF11	O14901	161.305	0.718	0.174	3.65E-05	0.015237
28	ENSG00000158470	B4GALT5	O43286	294.347	0.478	0.120	6.72E-05	0.026870
29	ENSG00000011021	CLCN6	P51797	556.903	0.388	0.098	6.85E-05	0.026870
30	ENSG00000113163	CERT1	Q9Y5P4	911.346	0.347	0.088	7.53E-05	0.028490

Supplemental Table 1 | (e) Downregulated DEGs for PA exposed non-activated CD4⁺ T cells in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log2 fold change, log fold change standard error, p value, and adjusted p value (FDR).

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC		P _{FDR}
						SE	P value	
1	ENSG00000117632	STMN1	P16949	1324.856	-0.407	0.067	1.44E-09	2.68E-06
2	ENSG00000163659	TIPARP	Q7Z3E1	2383.237	-0.329	0.058	1.57E-08	2.04E-05
3	ENSG00000153395	LPCAT1	Q8NF37	1166.324	-0.276	0.058	2.38E-06	0.00257
4	ENSG00000113161	HMGR	P04035	726.112	-0.325	0.070	3.58E-06	0.00328
5	ENSG00000118816	CCNI	Q14094	4732.443	-0.218	0.047	3.99E-06	0.00328

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Supplemental Table 1 | (e) [continued]

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC SE	P value	P _{FDR}
6	ENSG00000145741	<i>BTF3</i>	P20290	10617.724	-0.255	0.056	4.30E-06	0.00328
7	ENSG00000006451	<i>RALA</i>	P11233	1137.560	-0.283	0.067	2.66E-05	0.01724
8	ENSG00000145860	<i>RNF145</i>	Q96MT1	1666.498	-0.287	0.069	2.81E-05	0.01733
9	ENSG00000187109	<i>NAP1L1</i>	P55209	12784.530	-0.322	0.077	3.00E-05	0.01768
10	ENSG00000128989	<i>ARPP19</i>	P56211	1868.746	-0.278	0.067	3.43E-05	0.01932
11	ENSG00000104549	<i>SQLE</i>	Q14534	334.452	-0.364	0.090	4.68E-05	0.02427
12	ENSG00000117592	<i>PRDX6</i>	P30041	1454.171	-0.211	0.053	7.87E-05	0.03518
13	ENSG00000153283	<i>CD96</i>	P40200	6532.967	-0.210	0.054	0.000104	0.04298
14	ENSG00000130741	<i>EIF2S3</i>	P41091	5440.858	-0.211	0.055	0.000107	0.04298
15	ENSG00000125868	<i>DSTN</i>	P60981	923.114	-0.270	0.070	0.000109	0.04298

Supplemental Table 1 | (f) Upregulated DEGs for PA exposed non-activated CD4⁺ T cells in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log2 fold change, log fold change standard error, p value, and adjusted p value (FDR).

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC SE	P value	P _{FDR}
1	ENSG00000110090	<i>CPT1A</i>	P50416	709.522	3.173	0.112	1.88E-177	2.44E-173
2	ENSG00000178537	<i>SLC25A20</i>	O43772	328.362	1.552	0.099	1.28E-55	8.30E-52
3	ENSG00000072778	<i>ACADVL</i>	P49748	3226.461	0.642	0.055	3.26E-31	1.41E-27
4	ENSG00000072310	<i>SREBF1</i>	P36956	748.306	1.252	0.125	1.23E-23	3.99E-20
5	ENSG00000143110	<i>C1orf162</i>	Q8NEQ5	1302.359	0.464	0.058	1.83E-15	4.76E-12
6	ENSG00000111684	<i>LPCAT3</i>	Q6P1A2	415.027	0.690	0.109	2.26E-10	4.89E-07
7	ENSG00000160179	<i>ABCG1</i>	P45844	158.548	1.060	0.176	1.75E-09	2.84E-06
8	ENSG00000167315	<i>ACAA2</i>	P42765	486.997	0.560	0.095	3.79E-09	5.47E-06
9	ENSG00000166575	<i>TMEM135</i>	Q86UB9	164.063	0.743	0.148	5.64E-07	0.000665
10	ENSG00000149428	<i>HYOU1</i>	Q9Y4L1	2099.100	0.321	0.069	3.85E-06	0.003283
11	ENSG00000099194	<i>SCD</i>	O00767	183.550	0.595	0.129	4.11E-06	0.003283

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Supplemental Table 1 | (f) *[continued]*

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	log2FC	log2FC		
						SE	P value	P _{FDR}
12	ENSG00000196155	<i>PLEKHG4</i>	Q58EX7	674.104	0.305	0.072	2.22E-05	0.015991
13	ENSG00000182871	<i>COL18A1</i>	P39060	687.338	0.507	0.120	2.49E-05	0.016984
14	ENSG00000278540	<i>ACACA</i>	Q13085	410.104	0.359	0.088	4.54E-05	0.024270
15	ENSG00000141524	<i>TMC6</i>	Q7Z403	9025.505	0.274	0.068	5.20E-05	0.025954
16	ENSG00000079432	<i>CIC</i>	Q96RK0	2111.939	0.362	0.091	7.28E-05	0.034997
17	ENSG00000182095	<i>TNRC18</i>	O15417	1055.115	0.343	0.087	7.56E-05	0.035038
18	ENSG00000155090	<i>KLFI0</i>	Q13118	39.296	1.179	0.302	9.28E-05	0.040127

Supplemental Table 1 | (g) Table showing additional information on the genes that overlap between the different fatty acids. Information on Gene Symbol, Ensembl ID, chromosome location, log₂ fold change of that gene in each specific fatty acid, p value of that gene in each specific fatty acid, adjusted p value of that gene in each specific fatty acid, and whether the direction of gene expression is the same in each fatty acid is included.

Overlapping Genes	Ensembl ID	log₂FC EPA	log₂FC OA	log₂FC PA	P value EPA	P value OA	P value PA	P_{FDR} EPA	P_{FDR} OA	P_{FDR} PA	Direction Same
Overlapping Genes EPA vs OA vs PA											
CPT1A	ENSG00000110090	2.716	3.460	3.173	8.52E-24	2.87E-218	1.88E-177	3.68E-20	3.71E-214	2.44E-173	Yes Up
SLC25A20	ENSG00000178537	1.144	1.669	1.552	3.55E-17	1.34E-43	1.28E-55	6.57E-14	8.65E-40	8.30E-52	Yes Up
ACADVL	ENSG00000072778	0.541	0.557	0.642	2.37E-11	2.87E-18	3.26E-31	9.91E-09	9.28E-15	1.41E-27	Yes Up
ACAA2	ENSG00000167315	0.340	0.558	0.560	0.00177	2.23E-07	3.79E-09	0.02553	0.00022	5.47E-06	Yes Up
Overlapping Genes EPA vs OA											
TMSB4X	ENSG00000205542	-0.577	-0.300		7.52E-16	1.93E-06		1.08E-12	0.00146		Yes Down
PPDPF	ENSG00000125534	0.876	0.298		9.86E-14	0.00017		8.50E-11	0.04178		Yes Up
TMSB10	ENSG00000034510	-0.622	-0.287		1.37E-11	0.00015		6.08E-09	0.03951		Yes Down
CLCN7	ENSG00000103249	0.599	0.411		1.76E-09	1.88E-06		3.74E-07	0.00146		Yes Up
ETFDH	ENSG00000171503	0.758	0.553		1.98E-08	1.41E-05		2.84E-06	0.00792		Yes Up
SQSTM1	ENSG00000161011	0.618	0.262		4.20E-08	3.26E-05		5.49E-06	0.01406		Yes Up
RENBP	ENSG00000102032	0.716	0.747		8.37E-06	1.57E-07		0.00042	0.00018		Yes Up
PIM3	ENSG00000198355	0.479	0.450		2.24E-05	1.05E-05		0.00091	0.00620		Yes Up

(continued on next page)

Supplemental Table 1 | (g) [continued]

Overlapping Genes	Ensembl ID	log2FC EPA	log2FC OA	log2FC PA	P value EPA	P value OA	P value PA	P _{FDR} EPA	P _{FDR} OA	P _{FDR} PA	Direction Same
<i>TXNIP</i>	ENSG00000265972	-0.278	-0.185		2.93E-05	0.00015		0.00113	0.03973		Yes Down
<i>CLCN6</i>	ENSG0000011021	0.522	0.388		4.06E-05	6.85E-05		0.00147	0.02687		Yes Up
<i>FLCN</i>	ENSG00000154803	0.348	0.260		4.13E-05	9.78E-05		0.00149	0.03165		Yes Up
<i>AMDHD2</i>	ENSG00000162066	0.537	0.482		0.00012	8.14E-05		0.00352	0.02849		Yes Up
<i>PPP1R15A</i>	ENSG00000087074	0.459	0.344		0.00023	0.00022		0.00547	0.04787		Yes Up
<i>HSP90B1</i>	ENSG00000166598	-0.305	-0.336		0.00035	2.02E-06		0.00764	0.00146		Yes Down
<i>ARHGDI3</i>	ENSG00000111348	-0.247	-0.224		0.00067	9.10E-05		0.01230	0.03021		Yes Down
<i>SNX8</i>	ENSG00000106266	0.576	0.710		0.00124	2.40E-05		0.01963	0.01178		Yes Up
<i>ATXN2L</i>	ENSG00000168488	0.264	0.254		0.00133	0.00022		0.02050	0.04787		Yes Up
<i>HSPA5</i>	ENSG00000044574	-0.237	-0.382		0.00197	2.19E-06		0.02764	0.00149		Yes Down
Overlapping Genes EPA vs PA											
<i>CD96</i>	ENSG00000153283	-0.419		-0.210	6.55E-08		0.00010	7.70E-06		0.04298	Yes Down
<i>TNRC18</i>	ENSG00000182095	0.355		0.343	0.00012		7.56E-05	0.00353		0.03504	Yes Up
<i>PLEKHG4</i>	ENSG00000196155	-0.417		0.305	0.00128		2.22E-05	0.02012		0.01599	No EPA Down PA Up

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Supplemental Table 1 | (g) [continued]

Overlapping Genes	Ensembl ID	log ₂ FC EPA	log ₂ FC OA	log ₂ FC PA	P value EPA	P value OA	P value PA	P _{FDR} EPA	P _{FDR} OA	P _{FDR} PA	Direction Same
<i>COL18A1</i>	ENSG00000182871	-0.565		0.507	0.00196		2.49E-05	0.02757		0.01698	Yes Up
Overlapping Genes OA vs PA											
<i>RNF145</i>	ENSG00000145860		0.638	-0.287		7.35E-21	2.81E-05		3.17E-17	0.01733	No OA Up PA Down
<i>C1orf162</i>	ENSG00000143110		0.522	0.464		3.78E-13	1.83E-15		9.78E-10	4.76E-12	Yes Up
<i>LPCAT1</i>	ENSG00000111684		0.473	0.690		1.35E-12	2.26E-10		2.59E-09	4.89E-07	Yes Up
<i>TMEM135</i>	ENSG00000166575		0.722	0.743		7.39E-07	5.64E-07		0.00068	0.00067	Yes Up
<i>HMGCR</i>	ENSG00000113161		0.369	-0.325		5.52E-06	3.58E-06		0.00340	0.00328	No OA Up PA Down
<i>C1C</i>	ENSG00000079432		0.347	0.362		2.53E-05	7.28E-05		0.01178	0.03500	Yes Up
<i>KLFI10</i>	ENSG00000155090		1.297	1.179		0.00017	9.28E-05		0.04178	0.04013	Yes Up

Supplemental Table 1 (h) (partial) Pathway enrichment analysis of all downregulated EPA DEGs generated using *clusterProfiler* using 10 human pathway databases. Enriched pathways are shown with information on which group that term clusters into based on the Jaccard index > 0.70, pathway term, number of DEGs that overlap with that database gene set for that pathway, p value, adjusted p value, number of DEGs in that pathway that were upregulated, number of DEGs in that pathway that were downregulated, and gene names of all DEGs included in that pathway. Only the top term of the first 20 groups are shown here.

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated
1	Eukaryotic translation elongation	44/85	7.47E-31	1.75E-27	0	44
						RPS24, RPS25, RPS27A, RPL35A, RPS12, RPS15A, RPL30, RPL34, RPS6, RPL23, RPS29, RPL31, RPS7, RPS21, RPS20, RPS23, RPS8, RPS13, RPL21, RPL37, RPL23A, RPL11, RPS27, RPL32, RPL27, RPS3A, EEF1B2, RPL39, RPL38, RPL12, RPL4, RPL10A, RPL41, RPL37A, RPL9, RPL5, RPL24, RPL6, RPL19, UBA52, RPS11, RPL7, RPS18, RPS14
2	Interleukin-2 signaling pathway	110/728	3.38E-19	5.02E-17	0	110
						VIM, BTN3A1, BTN3A2, BTN3A3, SOCS2, GZMK, CISH, CD69, GBP1, CCR2, DPP4, TAP1, TRIB2, GZMA, CD96, IL2RA, RPS6, S100A11, ITGAM, AIF1, CD52, DENND2D, PTGER2, CASP1, RNF144A, NELL2, FCER1G, NCR3, RPL21, S100A4, MEOX1, CCR5, CD40LG, CTSO, ADTRP, GZMM, MAL, VNN2, CD2, FYN, IL2RG, CTSS, APOBEC3G, IL4R, MT2A, CYP11A1, LITAF, VAMP5, RGS10, SELL, RBM3, CCR1, TNFRSF1B, IL2RB, NMT2, KCNN4, BTG1, LAT, HEMGN, PDCD4, MLLT3, CD27, LCP2, NKG7, AHNAK, GNLY, ETS1, ACP5, CX3CR1, RAB33A, TBCE, FLOT1, PDE7A, TNFRSF25, IRF1, IFIT1, CD300A, ITGB2, MDFIC, BCL2, ADAM19, TRIM21, TAGLN2, LTB, CCR7, NLRP1, PTPRC, CTSZ, SORL1, TXK, PARP8, TTN, SLA2, GATA3, SAMHD1, CCL5, AP3M2, IQGAP2, FCGBP, CXCL8, SPN, E2F3, TNFSF10, CAMK2N1, CTSH, SITI, JAK3, PIM1, PRF1, EOMES
3	Antigen processing and presentation	27/54	7.67E-19	1.10E-16	0	27
						HLA-DRA, HLA-DPA1, HLA-DPB1, PSMB9, HLA-E, CD74, HLA-DRB1, B2M, TAP1, PSME1, HLA-DRB5, HLA-DQA1, CIITA, HLA-DMB, CD8A, CTSS, PSMB8, HLA-B, HLA-F, CD4, HLA-DQB1, HLA-DOA, PSME2, TAP2, HLA-A, HLA-C, HSPA5
4	Allotransplant rejection	47/172	1.47E-18	2.03E-16	0	47
						HLA-DRA, HLA-E, CD74, B2M, CCR2, TAP1, GZMA, CD96, IL2RA, KRT1, HLA-DQA1, GBP2, HLA-DMB, CD8A, CCR5, CD40LG, FYB1, RPS3A, CD2, IL2RG, CTSS, IL4R, RPL39, CAPG, CD3D, CCR1, IL2RB, CD4, HLA-DOA, CRTAM, ITGAL, TAP2, ST8SIA4, LCP2, HLA-A, RPL9, ETS1, DARS1, CD3E, ITGB2, LTB, CFP, PTPRC, CCL5, NLRP3, SITI, PRF1

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Supplemental Table 1 (h) (partial) [continued]

Group Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated
5 Interferon Gamma Response	47/181	1.39E-17	1.80E-15	0	47
					PSMB9, CD74, HLA-DRB1, B2M, CD69, TAP1, GZMA, PSME1, HLA-DQA1, CIITA, CASP1, SAMD9L, OAS2, TXNIP, IL4R, MT2A, SRI, VAMP5, PSMB8, HLA-B, XAF1, IL2RB, BTG1, NLRCS, PSME2, ST8SIA4, LCP2, HLA-A, ZBP1, IRF1, IFIT1, TRIM21, IL10RA, TRIM14, PNP, SLAMF7, OAS3, PARP12, CFH, SAMHD1, CCL5, TNFSF10, FGL2, ST3GAL5, PIMI1, RTP4, SPI10
6 Cell adhesion molecules (CAMs)	28/84	6.41E-14	6.94E-12	0	28
					HLA-DRA, HLA-DPA1, HLA-DPB1, HLA-E, HLA-DRB1, CD226, ITGB1, ITGA4, ITGAM, HLA-DRB5, HLA-DQAI, HLA-DMB, CD8A, CD40LG, CD2, HLA-B, HLA-F, SELL, CD4, HLA-DQB1, HLA-DOA, ITGAL, HLA-A, PECAM1, ITGB2, HLA-C, PTPRC, SPN
7 Immunoregulatory interactions between a lymphoid and a non-lymphoid cell	26/77	3.63E-13	3.73E-11	0	26
					HLA-E, B2M, CD226, IFITM1, CD96, ITGB1, ITGA4, LAIR1, NCR3, CD8A, CD40LG, CD3D, HLA-B, HLA-F, SELL, HCST, CRTAM, ITGAL, HLA-A, TYROBP, CD3E, CD300A, ITGB2, HLA-C, SLAMF7, CD200R1
8 Cap-dependent translation initiation	25/72	5.04E-13	4.92E-11	0	25
					RPL35A, RPL30, RPL34, RPL23, RPL31, RPL21, RPL37, RPL23A, RPL11, RPL32, RPL27, RPL39, RPL38, RPL12, RPL4, RPL10A, RPL41, RPL37A, RPL9, RPL5, RPL24, RPL6, RPL19, UBA52, RPL7
9 T cell receptor regulation of apoptosis	76/529	3.50E-12	3.34E-10	0	76
					VIM, RPS24, PSMB9, LGALS1, GBP1, IFITM1, CCR2, TAP1, IL2RA, PSME1, PTMA, RPL23, RPS29, S100A6, RPS20, CASP1, GADD45B, FCER1G, S100A4, RPL37, MCL1, CCR5, CD40LG, MAL, APOL3, RPS3A, CD2, CYBB, PSMB8, HLA-B, CCR1, TNFRSF1B, TRAF1, HLA-DQB1, RAC2, BTG1, ITGAL, RPL10A, PSME2, RPL41, RC3TB2, HSP90B1, TRAF5, PDE7A, HMGB1, TNFRSF25, CD3E, IRF1, ARHGAP2, CLIC5, ITGB2, BCL2, ATM, CDC42, RPL6, LTB, CCR7, NLRP1, PTPRC, RHOH, PNP, TUBB, HSPA5, GATA3, CCL5, IQGAP2, VIPR1, CXCL8, SPN, S100A10, NLRP3, CNBP, DYNLL1, PIMI, RPS14, SH3BGR1
10 Viral myocarditis	20/50	5.29E-12	4.93E-10	0	20
					HLA-DRA, HLA-DPA1, HLA-DPB1, HLA-E, HLA-DRB1, HLA-DRB5, HLA-DQA1, HLA-DMB, CD40LG, FYN, HLA-B, HLA-F, HLA-DQB1, RAC2, HLA-DOA, ITGAL, HLA-A, ITGB2, HLA-C, PRF1

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Supplemental Table 1 | (h) (partial) [continued]

Group Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated
11 Interferon gamma signaling	24/76	1.53E-11	1.28E-09	0	24
					HLA-DRA, HLA-DPA1, HLA-DPB1, HLA-E, HLA-DRB1, B2M, GBP1, HLA-DRB5, HLA-DQA1, CIITA, GBP2, OAS2, GBP5, MT2A, HLA-B, HLA-F, HLA-DQB1, HLA-A, IRF1, TRIM21, HLA-C, CAMK2G, TRIM14, OAS3
12 Immune system	127/1169	9.49E-11	7.04E-09	0	127
					HLA-DRA, HLA-DPA1, HLA-DPB1, PSMB9, HLA-E, SOCS2, CD74, IL32, TRIM69, LGALS3, CISH, HLA-DRB1, RPS27A, B2M, GBP1, CD226, IFITM1, CCR2, TAP1, CD96, IL2RA, ITGB1, ITGA4, PSME1, NEFL, ITGAM, HLA-DRB5, HLA-DQA1, CIITA, GBP2, TNFSF12, CASP1, HLA-DMB, LAIR1, TNFSF13B, FCER1G, NCR3, CD8A, OAS2, CD40LG, CTSS, GZMM, FYN, TLR5, IL2RG, TXNIP, CTSS, C5AR2, GBF5, P2RX7, MT2A, PTPRJ, CYBB, PSMB8, CD3D, CALM1, HLA-B, HLA-F, SELL, XAF1, TNFRSF1B, GRAP2, IL2RB, HCST, CD4, HLA-DQB1, CNKSR2, LAT, HLA-DOA, CRTAM, ITGAL, NLRCS, CD27, PSME2, TAP2, LCP2, HLA-A, TYROBP, SYNGAP1, MYLIP, HSP90B1, PEBP1, EDAR, ZBP1, HMGB1, TNFRSF25, CD3E, IRF1, ATP6V0E2, IFIT1, CD300A, ITGB2, BCL2, CDC42, TRIM21, HLA-C, CFL1, LTB, THEM4, CAMK2G, ARRBI, NLRP1, PTPRC, TRIM14, SLAMF7, RPS6KA5, ARPC2, TXK, ARPC3, HSPA5, OAS3, FGF9, UBA52, CFH, SAMHD1, UBE2F, FCN1, NUP37, NLRP3, CD200R1, CTSH, AIM2, SKP1, DYNLL1, JAK3, RPS6KA1, BTLA
13 Phosphorylation of CD3 and TCR zeta chains	12/19	1.14E-10	8.28E-09	0	12
					HLA-DRA, HLA-DPA1, HLA-DPB1, HLA-DRB1, HLA-DRB5, HLA-DQA1, PTPRJ, CD3D, CD4, HLA-DQB1, CD3E, PTPRC
14 Formation of the ternary complex, and subsequently, the 43S complex	18/49	3.31E-10	2.29E-08	0	18
					RPS24, RPS25, RPS27A, RPS12, RPS15A, RPS6, RPS29, RPS7, RPS21, RPS20, RPS23, RPS8, RPS13, RPS27, RPS3A, RPS11, RPS18, RPS14
15 Asthma	11/17	4.81E-10	3.16E-08	0	11
					HLA-DRA, HLA-DPA1, HLA-DPB1, HLA-DRB1, HLA-DRB5, HLA-DQA1, HLA-DMB, FCER1G, CD40LG, HLA-DQB1, HLA-DOA
16 Acute phase in atopic dermatitis	14/30	7.29E-10	4.71E-08	0	14
					IL2RA, MAF, FCER1G, CD40LG, FYN, IL2RG, IL4R, CD3D, IL2RB, CD4, CD3E, PTPRC, GATA3, JAK3

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Supplemental Table 1 (th) (partial) [continued]

Group Term	Overlap	P value	Adjusted P value	Up-regulated genes	Down-regulated genes
17 CD8 ⁺ naive T-cell → CD4 ⁺ naive T-cell surface expression markers	15/35	7.87E-10	5.01E-08	0	15 CD69, IL2RA, CCR5, CD40LG, IL4R, SELL, CD4, ITGAL, CD27, HLA-A, PECAM1, CCR7, PTPRC, BTLA, PRF1
18 Hematopoietic cell lineage	20/70	5.53E-09	3.12E-07	0	20 HLA-DRA, HLA-DPA1, HLA-DPB1, HLA-DRB1, IL2RA, ITGA4, ITGAM, HLA-DRB5, HLA-DQA1, HLA-DMB, CD8A, MS4A1, CD2, IL4R, CD3D, CD4, HLA-DQB1, HLA-DOA, CD3E, GP5
19 Disease	70/553	7.92E-09	4.35E-07	0	70 RPS24, RPS25, PSMB9, RPS27A, B2M, RPL35A, LYZ, RPS12, RPS15A, IGFBP4, PSME1, RPL30, RPL34, RPS6, RPL23, RPS29, RPL31, RPS7, RPS21, RPS20, RPS23, RPS8, RPS13, RPL21, RPL37, CCR5, RPL23A, RPL11, RPS27, RPL32, RPL27, RPS3A, FYN, APOBEC3G, RPL39, CYBB, RPL38, PSMB8, CALM1, RPL12, CD4, NM12, IGFBP3, RPL4, RPL10A, PSME2, RPL41, HLA-A, RPL37A, HSP90B1, PPIA, RPL9, PDIA6, ATP6V0E2, RPL5, CDC42, RPL24, RPL6, THEM4, RPL19, HSPA5, FGF9, UBA52, RPS1, CXCL8, NUP37, RPL7, SKP1, RPS18, RPS14
20 Interferon alpha response	23/93	8.59E-09	4.65E-07	0	23 PSMB9, CD74, B2M, IFITM1, TAP1, PSME1, GBP2, CASP1, SAMD9L, LPAR6, TXNIP, IL4R, PSMB8, SELL, PSME2, IRF1, TRIM21, HLA-C, TRIM14, PARP12, LAMP3, RTP4, SPI10

Supplemental Table 1 | (i) (partial) Pathway enrichment analysis of all upregulated EPA DEGs generated using *clusterProfiler* using 10 human pathway databases. Enriched pathways are shown with information on which group that term clusters into based on the Jaccard index > 0.70, pathway term, number of DEGs that overlap with that database gene set for that pathway, p value, adjusted p value, number of DEGs in that pathway that were upregulated, number of DEGs in that pathway that were downregulated, and gene names of all DEGs included in that pathway. Only the top term of the 19 groups are shown here.

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	regulated Genes
1	NRF2 pathway	20/86	6.08E-11	1.90E-07	20	0	NQO1, GCLC, MAFG, HMOX1, FTL, SLC5A3, SLC6A9, TXNRD1, SQSTM1, GSR, PRDX1, GCLM, G6PD, PGD, KEAP1, GSTP1, SLC39A7, SLC7A11, SLC6A6, SLC39A6
2	Amino acid transport across the plasma membrane	9/18	6.40E-09	6.65E-06	9	0	SLC7A5, SLC43A2, SLC7A1, SLC3A2, SLC38A2, SLC1A5, SLC1A4, SLC7A11, SLC6A6
3	Glutamine in cancer metabolism	9/24	1.40E-07	8.73E-05	9	0	SLC7A5, PSAT1, GCLC, SLC3A2, SLC1A5, PYCR1, GCLM, SLC7A11, PSPH
4	Xenobiotic metabolism	19/136	1.12E-06	0.00044	19	0	NQO1, GCLC, HMOX1, GABARAPL1, SLC1A5, ETFDH, AKR1C3, ARG2, PYCR1, GSR, PGD, EPHX1, UGDH, NPC1, SHMT2, SLC6A6, HES6, LONP1, PINK1
5	mTORC1 signaling	23/195	1.77E-06	0.00061	23	0	SLC7A5, PSAT1, GCLC, EDEM1, PHGDH, SLC1A5, TXNRD1, SQSTM1, MTHFD2, GSR, SLC1A4, PRDX1, DDIT3, G6PD, PPP1R15A, SLC7A11, SHMT2, SLC6A6, GLA, GAPDH, NAMPT, DDX39A, PSPH
6	Metabolic reprogramming in colon cancer	10/40	2.03E-06	0.00063	10	0	PSAT1, SLC1A5, TALDO1, PYCR1, G6PD, PGD, SHMT2, PKM, GAPDH, PSPH
7	Oxidative stress in amyotrophic lateral sclerosis	6/12	2.47E-06	0.00070	6	0	NQO1, GCLC, HMOX1, GSR, GCLM, KEAP1
8	Unfolded protein response	16/110	4.67E-06	0.00112	16	0	SLC7A5, PSAT1, HERPUD1, TARS1, IARS1, CHAC1, VEGFA, EDEM1, CEBPG, MTHFD2, SLC1A4, ATF4, CXXC1, SLC30A5, XPOT, EDC4
9	Trans-sulfuration and one carbon metabolism	8/28	7.44E-06	0.00166	8	0	PSAT1, GCLC, PHGDH, MTHFD2, MTHFD1L, GCLM, SHMT2, PSPH

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Supplemental Table 1 (i) (partial) *(continued)*

Group	Term	Overlap	P value	Adjusted P value	Up-regulated genes	Down-regulated genes	
10	Serine glycine biosynthesis	4/5	1.08E-05	0.00207	4	0	<i>PSAT1, PHGDH, SHMT2, PSPH</i>
11	Oxidative stress, all-trans-retinal and lipofuscin toxicity in AMD	6/15	1.21E-05	0.00210	6	0	<i>NQO1, HMOX1, VEGFA, GCLM, KEAP1, HIF1A</i>
12	SLC-mediated transmembrane transport	19/164	1.84E-05	0.00302	19	0	<i>SLC7A5, SLC43A2, SLC7A1, SLC3A2, SLC38A2, SLC5A3, SLC1A5, SLC6A9, CTNS, SLC12A7, SLC03A1, SLC1A4, SLC35A2, NUP153, SLC39A7, SLC7A11, SLC6A6, SLC30A5, SLC39A6</i>
13	Glutathione metabolism	8/38	8.33E-05	0.01039	8	0	<i>ODC1, GCLC, CHAC1, GSR, GCLM, G6PD, PGD, GSTP1</i>
14	Amino acid biosynthesis and interconversion (transamination)	5/14	0.00013	0.01424	5	0	<i>PSAT1, PHGDH, PYCR1, GPT2, PSPH</i>
15	mTOR signaling activation by amino acids	8/41	0.00015	0.01581	8	0	<i>SLC7A5, SLC3A2, SLC1A5, FNIP1, SESN2, FLCN, ATG4, PPP1R15A</i>
16	Reactive oxygen species pathway	8/45	0.00029	0.02914	8	0	<i>NQO1, GCLC, FTL, TXNRD1, GSR, PRDX1, GCLM, G6PD</i>
17	Ferroptosis	7/35	0.00033	0.03089	7	0	<i>GCLC, HMOX1, SLC3A2, FTL, SAT2, GCLM, SLC7A11</i>
18	Protein nuclear import and export	5/17	0.00036	0.03293	5	0	<i>XPO1, NXF1, RCC1, NUP153, XPOT</i>
19	Metabolic reprogramming in cancer: overview	9/59	0.00040	0.03507	9	0	<i>PSAT1, PHGDH, SLC1A5, PYCR1, SHMT2, PKM, GAPDH, HIF1A, PSPH</i>

Supplemental Table 1 | (j) (partial) Known motif analysis on promoters of down versus upregulated EPA ATAC peaks. Enriched transcription factors are shown in order of significance. Enrichment of transcription factor binding motifs was performed using HOMER. Information on rank, motif, name of transcription factor, p value, log p value, q value (Benjamini), number of target sequences with motif, number of background sequences with motif, percent of targets sequences with motif, and percent of background sequences with motif. Top 20 significant transcription factors are shown here.

Rank	Motif	Name	P value	q value (Benjamini)	# TS with Motif	# BG with Motif	% TS with Motif	% BG with Motif
1		CTCF	1.00E-40	0	41.00	0.90	3.40%	0.14%
2		BORIS	1.00E-32	0	35.00	1.00	2.90%	0.15%
3		OCT:OCT	1.00E-22	0	172.0	42.90	14.27%	6.38%
4		KLF10	1.00E-15	0	110.0	26.30	9.13%	3.92%
5		GRE	1.00E-13	0	35.00	4.80	2.90%	0.72%
6		X-box	1.00E-13	0	19.00	1.60	1.58%	0.24%
7		GRE	1.00E-09	0	21.00	2.90	1.74%	0.43%
8		ARE	1.00E-09	0	29.00	4.30	2.41%	0.64%
9		GATA3	1.00E-09	0	15.00	1.70	1.24%	0.26%
10		ETS:RUNX	1.00E-08	0	14.00	0.60	1.16%	0.10%

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Supplemental Table 1 (j) (partial) [continued]

Rank	Motif	Name	P value	q value (Benjamini)	# TS with Motif	# BG with Motif	% TS with Motif	% BG with Motif
11		RFX1	1.00E-07	0	27.00	4.40	2.24%	0.66%
12		ETV2	1.00E-07	0	111.0	36.30	9.21%	5.40%
13		RFX2	1.00E-07	0	13.00	1.00	1.08%	0.15%
14		ZFP57	1.00E-07	0	13.00	1.60	1.08%	0.24%
15		BRN1	1.00E-06	0	68.00	19.70	5.64%	2.94%
16		GATA2	1.00E-06	0	95.00	32.00	7.88%	4.75%
17		ETV4	1.00E-06	0	101.0	34.60	8.38%	5.15%
18		PSE	1.00E-05	0.0001	97.00	33.50	8.05%	4.98%
19		PGR	1.00E-05	0.0001	43.00	11.80	3.57%	1.75%
20		PU.1	1.00E-05	0.0001	58.00	17.30	4.81%	2.58%

Supplemental Table 1 | (k) (partial) Known motif analysis on promoters of up versus downregulated EPA ATAC peaks. Enriched transcription factors are shown in order of significance. Enrichment of transcription factor binding motifs was performed using HOMER. Information on rank, motif, name of transcription factor, p value, log p value, q value (Benjamini), number of target sequences with motif, number of background sequences with motif, percent of targets sequences with motif, and percent of background sequences with motif. Top 20 significant transcription factors are shown here.

Rank	Motif	Name	P value	q value (Benjamini)	# TS with Motif	# BG with Motif	% TS with Motif	% BG with Motif
1		AP-2α	1.00E-12	0	74.00	34.10	7.91%	3.08%
2		THRb	1.00E-08	0	438.0	413.5	46.84%	37.39%
3		E2F4	1.00E-08	0	14.00	3.00	1.50%	0.27%
4		OCT4:SOX17	1.00E-05	0.0002	17.00	5.30	1.82%	0.48%
5		E2F1	1.00E-05	0.0003	8.00	0.00	0.86%	0.00%
6		ZIC	1.00E-05	0.0005	88.00	64.50	9.41%	5.84%
7		PBX3	1.00E-05	0.0005	21.00	8.90	2.25%	0.81%
8		AR	1.00E-05	0.0005	399.0	396.4	42.67%	35.85%
9		ZNF136	1.00E-04	0.0007	12.00	3.60	1.28%	0.32%
10		REV-ERB	1.00E-04	0.0034	19.00	8.80	2.03%	0.79%

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Supplemental Table 1 | (k) (partial) [continued]

Rank	Motif	Name	P value	q value (Benjamini)	# TS with Motif	# BG with Motif	% TS with Motif	% BG with Motif
11		STAT4	1.00E-03	0.0071	98.00	80.30	10.48%	7.26%
12		TR4	1.00E-03	0.0088	6.00	1.30	0.64%	0.12%
13		GLI3	1.00E-03	0.0088	15.00	6.70	1.60%	0.61%
14		MYB	1.00E-03	0.0088	141.0	125.2	15.08%	11.32%
15		MYNN	1.00E-03	0.0088	27.00	16.00	2.89%	1.44%
16		ZSCAN22	1.00E-03	0.0088	10.00	3.90	1.07%	0.35%
17		THRa	1.00E-03	0.0092	72.00	56.50	7.70%	5.11%
18		TCF7	1.00E-03	0.0092	35.00	22.20	3.74%	2.01%
19		TGIF1	1.00E-03	0.0101	318.0	320.4	34.01%	28.98%
20		HNF4a	1.00E-03	0.0118	49.00	35.40	5.24%	3.20%

Supplemental Table 1 (I) (partial) Pathway enrichment analysis of all downregulated OA DEGs generated using *clusterProfiler* using 10 human pathway databases. Enriched pathways are shown with information on which group that term clusters into based on the Jaccard index > 0.70, pathway term, number of DEGs that overlap with that database gene set for that pathway, p value, adjusted p value, number of DEGs in that pathway that were upregulated, number of DEGs in that pathway that were downregulated, and gene names of all DEGs included in that pathway. Only the top term of the 11 groups are shown here.

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	Genes
1	Prion disease pathway	3/33	9.94E-06	0.00264	0	3	HSP90B1, HSPA5, PDIA3
2	Co-translational ER protein import	2/6	2.48E-05	0.00264	0	2	HSP90B1, HSPA5
3	Endogenous peptide antigen presentation	2/6	2.48E-05	0.00264	0	2	HSPA5, PDIA3
4	Protein folding	2/13	0.00013	0.00401	0	2	HSP90B1, HSP90AA1
5	Electron transport chain (OXPHOS system in mitochondria)	3/87	0.00019	0.00534	0	3	NDUFA12, NDUFB4, ATP5F1C
6	ER stress (unfolded protein response)	2/31	0.00076	0.01277	0	2	HSPA5, HSP90AA1
7	Proteins involved in myocardial ischemia	3/161	0.00113	0.01639	0	3	TMSB4X, TXNIP, HSP90AA1
8	Apoptotic keratinocytes clearance recession in systemic lupus erythematosus	2/38	0.00114	0.01639	0	2	HSP90B1, TXNIP
9	Complex I biogenesis	2/49	0.00189	0.02438	0	2	NDUFA12, NDUFB4
10	Response to elevated platelet cytosolic calcium	2/56	0.00246	0.02878	0	2	TMSB4X, HSPA5
11	T cell receptor regulation of apoptosis	4/529	0.00414	0.03977	0	4	HSP90B1, HSPA5, ARHGDI1B, HSP90AA1

Supplemental Table 1 | (m) (partial) Pathway enrichment analysis of all upregulated OA DEGs generated using *clusterProfiler* using 10 human pathway databases. Enriched pathways are shown with information on which group that term clusters into based on the Jaccard index > 0.70, pathway term, number of DEGs that overlap with that database gene set for that pathway, p value, adjusted p value, number of DEGs in that pathway that were upregulated, number of DEGs in that pathway that were downregulated, and gene names of all DEGs included in that pathway. Only the top term of the first 20 groups are shown here.

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	Genes
1	Lipid and lipoprotein metabolism	13/372	2.86E-09	5.90E-07	13	0	<i>CPT1A, SLC25A20, ACADVL, LPCAT1, DHCR24, FASN, SREBF2, LDLR, HMGCR, UGCG, SPTLC2, KDSR, HMGCS1</i>
2	SREBF and miR-33 in cholesterol and lipid homeostasis	5/15	3.57E-09	5.90E-07	5	0	<i>FASN, SREBF2, LDLR, HMGCR, HMGCS1</i>
3	Fatty acid, triacylglycerol, and ketone body metabolism	8/148	1.90E-07	1.88E-05	8	0	<i>CPT1A, SLC25A20, ACADVL, LPCAT1, FASN, SREBF2, HMGCR, HMGCS1</i>
4	Fatty acid metabolism	7/133	1.45E-06	8.99E-05	7	0	<i>CPT1A, ACADVL, DHCR24, FASN, ACAA2, ETFDH, HMGCS1</i>
5	Cholesterol biosynthesis	4/23	2.61E-06	0.00013	4	0	<i>DHCR24, SREBF2, HMGCR, HMGCS1</i>
6	srebp control of lipid synthesis	3/7	2.68E-06	0.00013	3	0	<i>SREBF2, LDLR, HMGCS1</i>
7	mTORC1 signaling	7/194	1.77E-05	0.00063	7	0	<i>DHCR24, LDLR, HMGCR, SQSTM1, HMGCS1, PPP1R15A, INSI1</i>
8	Androgen response	5/86	3.30E-05	0.00102	5	0	<i>DHCR24, HMGCR, ABHD2, HMGCS1, INSI1</i>
9	Mitochondrial LC-fatty acid beta-oxidation	3/16	4.47E-05	0.00122	3	0	<i>CPT1A, SLC25A20, ACADVL</i>
10	TNF- α signaling via NF- κ B	6/169	8.24E-05	0.00227	6	0	<i>SNN, LDLR, SQSTM1, B4GALT5, KLF10, PPP1R15A</i>
11	HNF3B pathway	3/23	0.00013	0.00263	3	0	<i>CPT1A, ACADVL, HMGCS1</i>

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Supplemental Table 1 (m) (partial) [continued]

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	Genes
12	Proteins involved in non-alcoholic fatty liver disease	5/115	0.00013	0.00263	5	0	<i>CPT1A, FASN, SREBF2, LDLR, INSIG1</i>
13	Ceramide <i>de novo</i> biosynthesis	2/6	0.00028	0.00479	2	0	<i>SPTLC2, KDSR</i>
14	Fatty acid metabolism	3/33	0.00039	0.00563	3	0	<i>CPT1A, ACADVL, ACAA2</i>
15	Import of palmitoyl-CoA into the mitochondrial matrix	2/7	0.00039	0.00563	2	0	<i>CPT1A, SLC25A20</i>
16	Mevalonate pathway	2/7	0.00039	0.00563	2	0	<i>HMGCR, HMGCS1</i>
17	Estrogen response early	5/147	0.00042	0.00591	5	0	<i>FASN, FAM102A, ABHD2, UGCG, KLF10</i>
18	Synthesis of UDP-N-acetyl-glucosamine	2/8	0.00051	0.00688	2	0	<i>RENBP, AMDHD2</i>
19	Sphingolipid metabolism	3/40	0.00068	0.00869	3	0	<i>UGCG, SPTLC2, KDSR</i>
20	Oncostatin M	5/167	0.00075	0.00883	5	0	<i>DHCR24, LDLR, HMGCR, HMGCS1, KLF10</i>

Supplemental Table 1 | (n) Pathway enrichment analysis of all downregulated PA DEGs generated using *clusterProfiler* using 10 human pathway databases. Enriched pathways are shown with information on which group that term clusters into based on the Jaccard index > 0.70, pathway term, number of DEGs that overlap with that databases gene set for that pathway, p value, adjusted p value, number of DEGs in that pathway that were upregulated, number of DEGs in that pathway that were downregulated, and gene names of all DEGs included in that pathway.

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	Genes
1	Cholesterol biosynthesis	2/10	7.41E-05	0.01047	0	2	<i>HMGCR, SQLE</i>
2	Cholesterol biosynthesis pathway	2/15	0.00017	0.01047	0	2	<i>HMGCR, SQLE</i>
3	Statin pathway	2/16	0.00020	0.01047	0	2	<i>HMGCR, SQLE</i>
4	Statin pathway	2/16	0.00020	0.01047	0	2	<i>HMGCR, SQLE</i>
5	Cholesterol biosynthesis	2/22	0.00038	0.01255	0	2	<i>HMGCR, SQLE</i>
6	Cholesterol biosynthesis	2/23	0.00041	0.01255	0	2	<i>HMGCR, SQLE</i>
7	Superpathway of cholesterol biosynthesis	2/23	0.00041	0.01255	0	2	<i>HMGCR, SQLE</i>
8	Activation of gene expression by SREBF (SREBP)	2/42	0.00138	0.03683	0	2	<i>HMGCR, SQLE</i>

Supplemental Table 1 (o) (partial) All significant pathway enrichment analysis of all upregulated PA DEGs generated using *clusterProfiler* using 10 human pathway databases. Enriched pathways are shown with information on which group that term clusters into based on the Jaccard index > 0.70, pathway term, number of DEGs that overlap with that database gene set for that pathway, p value, adjusted p value, number of DEGs in that pathway that were upregulated, number of DEGs in that pathway that were downregulated, and gene names of all DEGs included in that pathway. Only the top term of the first 20 groups are shown here.




Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	regulated genes
1	Estrogen deficiency in female obesity	3/6	6.12E-08	1.44E-05	3	0	<i>SREBF1</i> , <i>SCD</i> , <i>ACACA</i>
2	Fatty acid, triacylglycerol, and ketone body metabolism	6/175	1.37E-07	1.44E-05	6	0	<i>CPT1A</i> , <i>SLC25A20</i> , <i>ACADVL</i> , <i>SREBF1</i> , <i>SCD</i> , <i>ACACA</i>
3	Import of palmitoyl-CoA into the mitochondrial matrix	3/10	3.66E-07	2.52E-05	3	0	<i>CPT1A</i> , <i>SLC25A20</i> , <i>ACACA</i>
4	Mitochondrial LC-fatty acid beta-oxidation	3/16	1.70E-06	6.94E-05	3	0	<i>CPT1A</i> , <i>SLC25A20</i> , <i>ACADVL</i>
5	Ghrelin pathway	3/19	2.93E-06	9.31E-05	3	0	<i>SREBF1</i> , <i>ABCG1</i> , <i>ACACA</i>
6	Fatty acid biosynthesis	3/22	4.64E-06	0.00011	3	0	<i>ACAA2</i> , <i>SCD</i> , <i>ACACA</i>
7	Liver X receptor pathway	2/5	2.28E-05	0.00028	2	0	<i>SREBF1</i> , <i>SCD</i>
8	Import of palmitoyl-CoA into the mitochondrial matrix	2/7	4.77E-05	0.00055	2	0	<i>CPT1A</i> , <i>SLC25A20</i>
9	reversal of insulin resistance by leptin	2/8	6.36E-05	0.00063	2	0	<i>CPT1A</i> , <i>ACACA</i>
10	AMPK related catabolism deceleration in glucose insufficiency	2/8	6.36E-05	0.00063	2	0	<i>SREBF1</i> , <i>ACACA</i>
11	HNF3B pathway	2/23	0.00057	0.00345	2	0	<i>CPT1A</i> , <i>ACADVL</i>
12	RORA activates circadian expression	2/25	0.00067	0.00400	2	0	<i>CPT1A</i> , <i>SREBF1</i>
13	Fatty acyl-CoA biosynthesis	2/31	0.00103	0.00580	2	0	<i>SCD</i> , <i>ACACA</i>

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Supplemental Table 1 | (o) (partial) [continued]

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	
					regulated	regulated	
					Genes	Genes	
14	Circadian rhythm related genes	3/135	0.00109	0.00599	3	0	<i>CPT1A, SREBF1, KLF10</i>
15	PPAR signaling pathway	2/40	0.00172	0.00895	2	0	<i>CPT1A, SCD</i>
16	Activation of chaperones by IRE1 alpha	2/45	0.00217	0.01072	2	0	<i>ACADVL, HYOU1</i>
17	Oleate biosynthesis	1/3	0.00470	0.01975	1	0	<i>SCD</i>
18	Metabolism	6/1142	0.00520	0.02155	6	0	<i>CPT1A, ACADVL, LPCAT3, ABCG1, ACAA2, ACACA</i>
19	Beta-oxidation of myristoyl-CoA to lauroyl-CoA	1/4	0.00626	0.02451	1	0	<i>ACADVL</i>
20	VEGFR -> CTNND signaling	1/4	0.00626	0.02451	1	0	<i>COL18A1</i>

Supplemental Table 1 (p) Known motif analysis on promoters of down versus upregulated OA ATAC peaks. All enriched transcription factors are shown in order of significance. Enrichment of transcription factor binding motifs was performed using HOMER. Information on rank, motif, name of transcription factor, p value, log p value, q value (Benjamini), number of target sequences with motif, number of background sequences with motif, percent of targets sequences with motif, and percent of background sequences with motif.

Rank	Motif	Name	P value	q value (Benjamini)	# TS with Motif	# BG with Motif	% TS with Motif	% BG with Motif
1		ZNF41	1.00E-02	0.9003	5.00	6.40	0.54%	0.10%
2		RAR:RXR	1.00E-02	1	5.00	8.30	0.54%	0.13%
3		WT1	1.00E-02	1	48.00	228.8	5.15%	3.60%

Supplemental Table 1 | (q) (partial) [continued]

Rank	Motif	Name	P value	q value (Benjamini)	# TS with Motif	# BG with Motif	% TS with Motif	% BG with Motif
12		OCT4:SOX17	1.00E-09	0	48.00	4.50	1.26%	0.50%
13		PTF1A	1.00E-09	0	1198.0	240.9	31.38%	27.06%
14		ZNF692	1.00E-08	0	93.00	11.50	2.44%	1.29%
15		PPARα	1.00E-08	0	412.0	72.20	10.79%	8.11%
16		ELF4	1.00E-08	0	351.0	60.70	9.19%	6.82%
17		DMC1	1.00E-08	0	21.00	0.00	0.55%	0.00%
18		RUNX2	1.00E-08	0	370.0	64.60	9.69%	7.26%
19		Nur77	1.00E-07	0	59.00	6.20	1.55%	0.69%
20		ETS1	1.00E-07	0	118.0	16.70	3.09%	1.87%
21		SMAD4	1.00E-07	0	600.0	113.1	15.72%	12.70%
22		PU.1	1.00E-07	0	188.0	30.00	4.92%	3.37%
23		IRF8	1.00E-07	0	70.00	8.30	1.83%	0.93%

Supplemental Table 1 | (r) Known motif analysis on promoters of down versus upregulated PA ATAC peaks. All enriched transcription factors are shown in order of significance. Enrichment of transcription factor binding motifs was performed using HOMER. Information on rank, motif, name of transcription factor, p value, log p value, q value (Benjamini), number of target sequences with motif, number of background sequences with motif, percent of targets sequences with motif, and percent of background sequences with motif.

Rank	Motif	Name	P value	q value (Benjamini)	# TS with Motif	# BG with Motif	% TS with Motif	% BG with Motif
1		IRF8	1.00E-04	0.022	25.00	27.00	4.62%	1.96%
2		PBX3	1.00E-03	0.143	18.00	19.10	3.33%	1.38%
3		NFKB	1.00E-03	0.143	4.00	0.80	0.74%	0.06%
4		ZNF382	1.00E-03	0.143	4.00	0.90	0.74%	0.06%
5		ETS:E-box	1.00E-02	0.143	11.00	9.20	2.03%	0.67%
6		GATA2	1.00E-02	0.147	48.00	79.60	8.87%	5.77%
7		NKX3-2	1.00E-02	0.185	174.0	369.4	32.16%	26.78%
8		GATA3	1.00E-02	0.234	9.00	8.20	1.66%	0.60%
9		IRF2	1.00E-02	0.234	9.00	8.80	1.66%	0.64%
10		p63	1.00E-02	0.234	25.00	37.00	4.62%	2.68%
11		ZNF264	1.00E-02	0.234	34.00	54.70	6.28%	3.97%

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Supplemental Table 1 (r) [continued]

Rank	Motif	Name	P value	q value (Benjamini)	# TS with Motif	# BG with Motif	% TS with Motif	% BG with Motif
12		ZNF317	1.00E-02	0.234	6.00	5.00	1.11%	0.36%
13		GATA4	1.00E-02	0.234	67.00	125.9	12.38%	9.12%
14		GATA1	1.00E-02	0.237	41.00	70.20	7.58%	5.09%
15		IRF3	1.00E-02	0.242	20.00	28.20	3.70%	2.04%
16		TLX	1.00E-02	0.253	25.00	38.70	4.62%	2.80%
17		FOXM1	1.00E-02	0.253	72.00	139.5	13.31%	10.11%
18		NFY	1.00E-02	0.253	48.00	86.70	8.87%	6.28%
19		ELF4	1.00E-02	0.253	53.00	97.10	9.80%	7.04%

Supplemental Table 1 | (s) (partial) Known motif analysis on promoters of up versus downregulated PA ATAC peaks. All enriched transcription factors are shown in order of significance. Enrichment of transcription factor binding motifs was performed using HOMER. Information on rank, motif, name of transcription factor, p value, log p value, q value (Benjamini), number of target sequences with motif, number of background sequences with motif, percent of targets sequences with motif, and percent of background sequences with motif. Top 20 significant transcription factors are shown here.

Rank	Motif	Name	P value	q value (Benjamini)	# TS with Motif	# BG with Motif	% TS with Motif	% BG with Motif
1		PITX1	1.00E-09	0	30.00	3.10	2.16%	0.57%
2		ZBTB12	1.00E-08	0	63.00	11.80	4.54%	2.19%
3		HOXC9	1.00E-07	0	95.00	20.30	6.84%	3.75%
4		Pdx1	1.00E-07	0	183.0	47.10	13.17%	8.74%
5		Nur77	1.00E-07	0	22.00	2.70	1.58%	0.49%
6		HOXA1	1.00E-05	0.0001	37.00	6.90	2.66%	1.27%
7		DR4	1.00E-05	0.0003	32.00	5.20	2.30%	0.97%
8		CHOP	1.00E-04	0.0008	46.00	9.60	3.31%	1.77%
9		REV-ERB	1.00E-04	0.0009	22.00	3.70	1.58%	0.68%
10		EBF	1.00E-04	0.0012	17.00	2.90	1.22%	0.53%

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Supplemental Table 1 | (s) (partial) [continued]

Rank	Motif	Name	P value	q value (Benjamini)	# TS with Motif	# BG with Motif	% TS with Motif	% BG with Motif
11		COUP-TFII	1.00E-04	0.0012	230.0	69.50	16.56%	12.88%
12		IRF-BATF	1.00E-04	0.0033	16.00	2.50	1.15%	0.46%
13		MEF2B	1.00E-03	0.0036	171.0	51.00	12.31%	9.45%
14		PBX2	1.00E-03	0.0037	153.0	44.30	11.02%	8.21%
15		PAX5	1.00E-03	0.0046	53.00	12.80	3.82%	2.38%
16		ZNF415	1.00E-03	0.0067	75.00	19.60	5.40%	3.62%
17		SOX9	1.00E-03	0.0067	112.0	31.20	8.06%	5.79%
18		STAT4	1.00E-03	0.0067	130.0	37.40	9.36%	6.92%
19		COUP-TFII	1.00E-03	0.0067	248.0	78.20	17.85%	14.50%
20		PBX1	1.00E-03	0.0074	10.00	0.00	0.72%	0.00%

