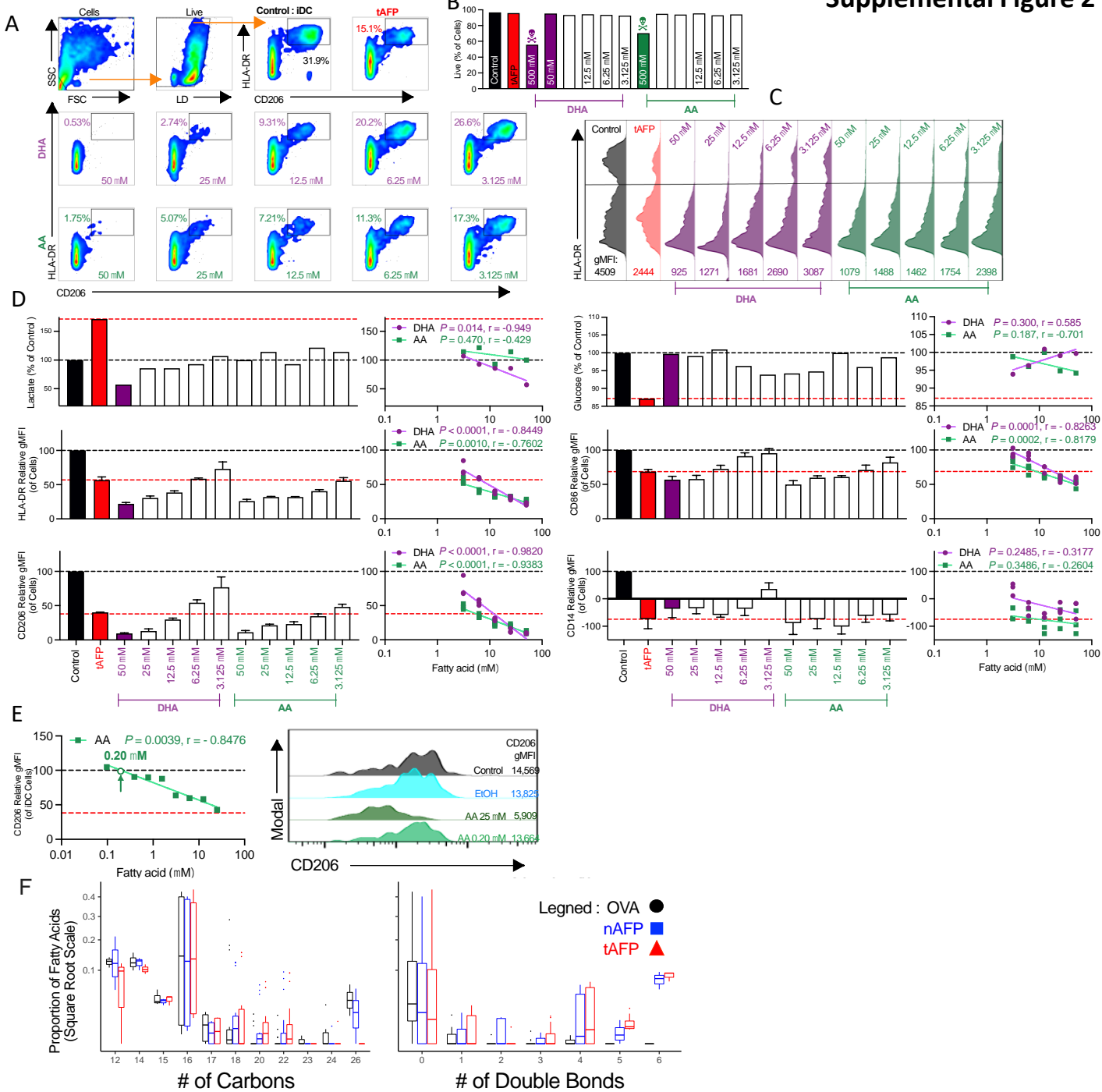## SUPPLEMENTAL FIGURE 1: Representative puromycin histograms

Representative puromycin histograms are shown of **A**) OVA, **B**) nAFP, and **C**) tAFP-treated iDCs treated with the metabolic inhibitors 2-deoxy-glucose (DG, purple) or oligomycin (O, red) or both (DGO, gray) and control samples (Co, black) (C). The statistics shown are the result of a Spearman's rank correlation test.

**SUPPLEMENTAL FIGURE 2: PUFAs inhibit *in vitro* DC differentiation**

Shown are **A**) representative flow cytometry plots of iDCs identified by (top, left to right) complexity and size (SSC, FSC), viability (Live dead negative), and (middle and bottom of A) by co-expression of HLA-DR and CD206. **B**) Shown are bar graphs of cell viability of control (black), tAFP (red), or fatty acid treated (iDCs). **C**) Cells were treated with various concentrations of docosahexaenoic acid (DHA, purple) or arachidonic acid (green). **D**) Displayed are the supernatant lactate and glucose concentrations and the relative gMFIs (as a percent of control-treated cells) of HLA-DR, CD206, CD86, and CD14 of control, tAFP, and DHA or AA-treated cells. Correlations between the fatty acid concentration and the corresponding analyte are shown. **E**) Additional titrations were performed to identify a concentration of AA that alone did not result in a statistically decreasing CD206 (green arrow, 0.20 micromolar). Black and red dashed lines indicate average control and tAFP levels, respectively. The statistics shown are the result of Spearman's rank correlation tests. **F**) Shown are box and whisker plots of the proportion of fatty acids grouped based on either the number of total carbons or the double bonds bound to OVA, nAFP, or tAFP.

| Pt. Number | Gender | HBTB Case Patient Race | HBTB Case Patient Ethnicity | Age at time of blood draw | Serum AFP closest to sample collection (ug/L) | Stage at enrollment | Histologic grade at enrollment |
|---|---|---|---|---|---|---|---|
| 1 | Male | Asian | Non-Hispanic | 76 | 3790.8 | Stage IV | Moderately differentiated |
| 2 | Male | Asian | Non-Hispanic | 71 | 432.6 | Stage IIIB | Unknown |
| 3 | Female | Black or African American | Non-Hispanic | 75 | 26.1 | Stage IVA | Poorly differentiated |
| 6 | Female | Black or African American | Non-Hispanic | 61 | 2.6 | Stage IVB | Poorly differentiated |
| 7 | Male | White | Non-Hispanic | 67 | 1752.5 | Stage IVB | Moderately differentiated |
| 8 | Male | White | Non-Hispanic | 68 | <2.0 | Stage IVB | Moderately differentiated |
| 9 | Male | White | Non-Hispanic | 72 | <2.0 | Stage IVA | Unknown |
| 10 | Male | White | Non-Hispanic | 83 | 7287.9 | Stage IVB | Moderately differentiated |

| Presence of NAFLD Diagnosed Pathologically (i.e. Surgical Pathology or Core Biopsy) | Fibrosis | Medical History |
| --- | --- | --- |
| No | F0 | Hepatitis B infection (HBsAg positive and/or viral load positive); Smoking History; Hyperlipidemia; H. Pylori |
| Unknown | Unknown | Smoking History; Diabetes; Hyperlipidemia; Hepatitis B carrier (core Ab positive); Hypertension |
| Unknown | Unknown | H. Pylori; Hepatitis B infection (HBsAg positive and/or viral load positive); Hypertension; Cholangitis |
| No | F1 | Hepatitis C; Smoking History |
| No | F0 | Hepatitis B carrier (core Ab positive); Hepatitis C; Hypothyroidism; Smoking History |
| No | F1 | Hepatitis C; Hepatitis B carrier (core Ab positive) |
| Unknown | Unknown | Hypertension; Hepatitis B carrier (core Ab positive); Hepatitis C; Smoking History |
| Unknown | Unknown | Hyperlipidemia; Smoking History; Hypertension |

Supplementary Table 3: Polyunsaturated fatty acids tested

| Fatty Acid | Common Name | Class |
|---|---|---|
| 12:0 | Lauric acid or dodecanoic acid | Saturated |
| 14:0 | Myristic acid or tetradecanoic acid | Saturated |
| 15:0 | Pentadecanoic acid | Saturated |
| 16:0 | Palmitic acid or hexadecanoic acid | Saturated |
| 17:0 | Margaric acid or heptadecanoic acid | Saturated |
| 18:0 | Stearic acid or octadecanoic acid | Saturated |
| 20:0 | Arachidic acid or eicosanoic acid | Saturated |
| 22:0 | Behenic acid or docosanoic acid | Saturated |
| 23:0 | Tricosanoic acid | Saturated |
| 24:0 | Lignoceric acid or tetracosanoic acid | Saturated |
| 26:0 | Cerotic acid or hexacosanoic acid | Saturated |
| 16:1 | Palmitoleic acid, or hexadecenoic acid (n7) | Monounsaturated |
| 17:1 | Heptadecanoic acid n(7) | Monounsaturated |
| 18:1 | Oleic acid or octadecenoic acid (n9) | Monounsaturated |
| 20:1 | Gadoleic acid or eicosenoic acid | Monounsaturated |
| 22:1 | Erucic acid or docosenoic acid (n9) | Monounsaturated |
| 24:1 | Nervonic acid or tetracosenoic acid (n9) | Monounsaturated |
| 18:2 | Linoleic acid or octadecadienoic acid (n9) | Polyunsaturated |
| 20:2 | Eicosadienoic acid (n6) | Polyunsaturated |
| 22:2 | 13,16-docosadienoic acid (n6) | Polyunsaturated |
| 18:3 N3 | Alpha-linolenic acid or octatrienoic acid (n3) | Polyunsaturated |
| 18:3 N6 | Gamma-linolenic acid or octatrienoic acid (n6) | Polyunsaturated |
| 20:3 N3 | 11,14,17-eicosatrienoic acid (n3) | Polyunsaturated |
| 20:3 N6 | Bishomo-gamma-linolenic acid (n6) | Polyunsaturated |
| 20:3 N9 | 5,8,11-eicosatrienoic acid (n9) | Polyunsaturated |
| 22:3 | 13,16,19-docosatrienoic acid (n3) | Polyunsaturated |
| 18:4 | Stearidonic acid or octatetraenoic acid (n3) | Polyunsaturated |
| 20:4 | Arachidonic acid or eicosatetraenoic acid (n6) | Polyunsaturated |
| 22:4 | Adrenic acid or 7,10,13,16-docosatetraenoic acid (n6) | Polyunsaturated |
| 20:5 | Eicosapentaenoic acid (n3) | Polyunsaturated |
| 22:5 N3 | 7,10,13,16,19-docosapentaenoic acid (n3) | Polyunsaturated |
| 22:5 N6 | 4,7,10,13,16-docosapentaenoic acid (n6) | Polyunsaturated |
| 22:6 | Docosahexaenoic acid (n3) | Polyunsaturated |

**Table 1. HCC Patient Characteristics**

| Characteristic (N=8 patients) | Number or median (range) | % |
|---|---|---|
| Gender (n) | | |
|     Male | 6 | 75% |
|     Female | 2 | 25% |
| Age (years) | | |
|     Median (range) | 72 (61-83) | |
| Race | | |
|     African-American | 2 | 25% |
|     Asian | 2 | 25% |
|     Caucasian | 4 | 50% |
| Ethnicity | | |
|     Non-Hispanic/Latino | 8 | 100% |
|     Hispanic/Latino | 0 | 0% |
| Liver Disease Etiology | | |
|     Hepatitis C (cAb+) | 4 | 50% |
|     Hepatitis B (sAg+) | 2 | 25% |
| Child Pugh Score at Enrollment | | |
|     Child Pugh A | 7 | 87% |
|     Child Pugh B | 1 | 13% |
| Serum AFP (ug/L) | | |
|     Median (range) | 229 (<2.0-7287.9) | |
| Disease stage | | |
|     Stage IIIB | 1 | 13% |
|     Stage IVA | 3 | 38% |
|     Stage IVB | 4 | 50% |
| Histologic grade | | |
|     Moderately differentiated | 4 | 50% |
|     Poorly differentiated | 2 | 25% |
|     Unknown | 2 | 25% |

**Supplementary Table 2.** scMEP panel and antibody information

| Metal | Myeloid/DC | clone | Column2 | Group |
|-------|-----------|-------|---------|-------|
| 89 | CD45 | HI30 | surface | DC |
| 113 | CD11c | Bu15 | surface | DC |
| 115 | CD11b | ICRF44 | surface | DC |
| 127 | IdU | | other | DNA_RNA_PROT |
| 140 | CD3 | UCHT1 | surface | DC |
| 141 | CD98 | UM7F8 | surface | AA |
| 142 | HADHA | EPR17940 | intracellular | FAO |
| 143 | GSS | EPR6563 | intracellular | ROS |
| 144 | XBP1 | polyclonal | intracellular | SIGNAL |
| 145 | GLS | polyclonal | intracellular | AA |
| 146 | ATF4 | EPR18111 | intracellular | SIGNAL |
| 147 | GAPDH | 6C5 | intracellular | GLYC |
| 148 | CD14 | RMO52 | surface | DC |
| 149 | CytC | 6H2.B4 | intracellular | ETC_TCA |
| 150 | SDHA | 2E3GC12FB2AE2 | intracellular | ETC_TCA |
| 151 | Puromycin | 12D10 | intracellular | DNA_RNA_PROT |
| 152 | ENO1 | EPR10863(B) | intracellular | GLYC |
| 153 | CS | EPR8067 | intracellular | ETC_TCA |
| 154 | BrU | 3D4 | intracellular | DNA_RNA_PROT |
| 155 | CD163 | GHI/61 | surface | HEME |
| 156 | PFKFB4 | polyclonal | intracellular | GLYC |
| 157 | PDK1 | 2H3AA11 | intracellular | SIGNAL |
| 158 | ATP5A | 15H4C4 | intracellular | ETC_TCA |
| 159 | CD86 | IT2.2 | surface | DC |
| 161 | TOMM20 | EPR15581-54 | intracellular | MITO |
| 162 | G6PD | EPR20668 | intracellular | PPP |
| 163 | CD36 | 5-271 | surface | FAO |
| 164 | CD1c | L161 | surface | DC |
| 165 | PGC1a | polyclonal | intracellular | MITO |
| 166 | GLUT1 | EPR3915 | surface | GLYC |
| 167 | CD303 | 201A | surface | DC |
| 168 | CD206 | 15-2 | surface | DC |
| 169 | LDHA | EP1566Y | intracellular | GLYC |
| 170 | IDH2 | EPR7577 | intracellular | ETC_TCA |
| 171 | HK2 | 3D3 | intracellular | GLYC |
| 172 | MCT1 | P14612 | surface | GLYC |
| 173 | CPT1A | 8F6AE9 | intracellular | FAO |
| 174 | ASCT2 | CAL33 | surface | AA |
| 175 | PDL1 | 29E.2A3 | surface | DC |
| 176 | HIF1A | EP1215Y | intracellular | SIGNAL |
| 196 | S6_p | A17020B | intracellular | SIGNAL |
| 198 | dead | | | |
| 209 | HLA-DR | L243 | surface | DC |

Listed are metal conjugates and protein targets used in scMEP profiling.

## Supplementary Table 1. SCENITH panel and antibody information

| Marker | Fluorophore | Clone | Host | Company | Catalogue # |
|---|---|---|---|---|---|
| **Antibodies used in matured DC SCENITH profiling panel** | | | | | |
| CD14 | BUV395 | MφP9 | Mouse | BD | 563561 |
| AMPK | Dyligtht 350 | 2B7 | Mouse | Novus Bio | NBP2-22127UV |
| CD276 | BUV496 | 7-517 | Mouse | BD | 749897 |
| CD274 | BUV563 | MIH1 | Mouse | BD | 741423 |
| CD303 | BUV615 | V24-785 | Mouse | BD | 751078 |
| HLA-DR | BUV805 | G46-6 | Mouse | BD | 748338 |
| CD1c | BV421 | L161 | Mouse | BioLegend | 331525 |
| iNOS | eFluor 450 | CXNFT | Rat | eBioscience/TF | 48-5920-80 |
| CCR7 | BV480 | 3D12 | Rat | BD | 566170 |
| CD80 | BV510 | 2D10 | Mouse | BioLegend | 305233 |
| CD141 | BV605 | M80 | Mouse | BioLegend | 344117 |
| CD206 | BV711 | 15-2 | Mouse | BioLegend | 321135 |
| ILT-3 | BV750 | ZM3.8 | Mouse | BD | 747371 |
| CD86 | BV785 | IT2.2 | Mouse | BioLegend | 305441 |
| anti-Puromycin | AF488 | 12D10 | Mouse | Millipore Sigma | MABE343-AF488 |
| CD11c | PerCP | BU15 | Mouse | Thermo Fisher | A15803 |
| CD273 | BB700 | MIH18 | Mouse | BD | 746072 |
| CD36 | PE | 5-271 | Mouse | BioLegend | 336206 |
| p-mTOR1 | PE | O21-404 | Mouse | BD | 563489 |
| PPARg | AF594 | NA | Rat | Bioss Inc | bs-4590R-A594 |
| pS6K | PE-Cy5.5 | OTI1G4 | Mouse | Novus | NBP2-73209PECY55 |
| p-mTOR1 (Ser 2448) | PE-Cy7 | MRRBY | Mouse | eBioscience/TF | 50-112-3458 |
| CD98 | PE-Vio770 | REA387 | Rat | Myltenyi Biotec | 130-126-200 |
| p-AMPKa-1/2 (Thr183/Thr172 | AF647 | NA | Rat | Bioss Inc | bs-4002R-A647 |
| Zombie NIR Fixable Viability Kit | | | | BioLegend | 423105 |
| GLUT1 | APC/Cy7 | not specified | Rat | Bioss Inc | SPC-1295D-APCCY7 |
| | | | | | |
| **Antibodies used in fresh PBMC SCENITH profiling panel** | | | | | |
| FceRI | BUV805 | AER-37 | Mouse | BD | 749337 |
| CD14 | BV605 | 63D3 | Mouse | Bioloegend | 367125 |
| CD16 | BV785 | 3G8 | Mouse | Bioloegend | 302045 |
| CD11c | PE-Cy5 | B-ly6 | Mouse | BD | 551077 |
| CD206 | BV711 | 15.2 | Mouse | Bioloegend | 321135 |
| HLA-DR | BV650 | L243 | Mouse | Bioloegend | 307649 |
| CD141 (BDCA-3) | BV480 | 1A4 | Mouse | BD | 746604 |
| CD3 | NovaFluor Blue 61 | UCHT1 | Mouse | eBioscience/TF | H002T02B05 |
| CD88 | APC-Fire 750 | S5/1 | Mouse | Bioloegend | 344315 |
| CD89 | APC-Fire 750 | A59 | Mouse | Bioloegend | 354115 |
| CD56 | Alexa Fluor 532 | NCAM1/795 | Mouse | Novus Biological | NBP2-47826AF532 |
| CD19 | V450 | HIB19 | Mouse | BD | 560353 |
| CD123 | BUV496 | 6H6 | Mouse | BD | 751836 |
| CD45RA | BV570 | HI100 | Mouse | Bioloegend | 304132 |
| CD1c | BV421 | L161 | Mouse | Bioloegend | 331526 |

Listed are protein targets used in conjunction with SCENITH functional metabolic profiling of DC differentiation states.