Supporting Information

Appendix S1: Supplementary Materials and Methods

Reagents

Hapten 2,4,6-trinitrochlorobenzene (TNCB) was from Chemical Alta (Edmonton, Canada); bovine serum albumin (BSA), hexadecyltrimethylammonium bromide, lysozyme, magnesium chloride, JumpStart™ Taq ReadyMix™ for qPCR, o-dianisidine dihydrochloride, sodium dodecyl sulfate, Tris-EDTA, hydrogen peroxide, *phenol:chloroform:isoamyl alcohol* (25:24:1), Bicinchoninic Acid Solution, Copper(II) Sulfate Pentahydrate 4% Solution, Protein Standard, collagenase type II, $CaCl_2$, RPMI media and supplements were from Sigma-Aldrich Co. (St Louis, MO); biotinylated anti-mouse IgG1 mAb, biotinylated anti-mouse IgG2a mAb, and TMB substrate were from BD Biosciences (San Jose, CA); TNP-BSA was from Biosearch Technologies (Petaluma, CA); streptavidin-horseradish peroxidase was from Vector Laboratories (Burlingame, CA). Heat-inactivated fetal bovine serum (FBS) was from Gemini Bio-Products (West Sacramento, CA). Silica beads were from BioSpec Products (USA); proteinase K was from Roche Diagnostics (Germany). Mouse ELISA Sets for IFN-γ, TNF-α, IL-6 and IL-10 were from BD Biosciences (San Jose, CA). Mouse IL-17A (homodimer) ELISA Ready-SET-Go-Kit was from eBioscience (San Diego, CA). Acetone, ethanol 99,8%, H_2SO_4, Na_2HPO_4 x 12 H2O were from Chempur, Piekary Śląskie, Poland. Mouse immunoglobulins (Ig) were prepared from mouse sera and conjugated with TNP hapten. A single preparation with a level of substitution of 40 TNP per Ig molecule (TNP40-Ig) was used throughout the study [E1, E2].

High-fat diet induced obesity (HFDIO)

To induce obesity, male C57BL/6 mice (5-6 wk old) were fed with HFD [(composed of the following energy sources: 20% is provided by carbohydrates, 60% by fat, and 20% by protein,

25 overall calories: 5.21 kcal/g) from Research Diets, NJ] *ad libitum* for 8 weeks. Age-matched
26 control male C57BL/6 mice were maintained on a normal diet (ND) [(composed of the
27 following energy sources: 53% is provided by carbohydrates, 11% by fat, and 36% by protein,
28 overall calories: 4 kcal/g) for the same period of time. The bodyweight (BW) gain was
29 monitored once a week using a digital scale (Scout Pro balances Ohaus), prior to CHS induction
30 and experimentation.

31 **Cytokine production**

32 Cytokine production by auricular lymph node cells (ELNC) was determined in HFD and ND
33 fed mice that were sensitized and challenged with TNCB as described above. ELNC were
34 isolated from auricular lymph nodes, 24 h after challenge, and cultured ($3x10^6$/ml) with 100 µg
35 TNP_{40}-Ig antigen (mouse immunoglobulins conjugated with TNP) in 1 ml complete RPMI-
36 1640 medium. To determine cytokine secretion by cells, infiltrating adipose tissue (AT),
37 subcutaneous adipose tissue (scAT) was harvested 4 days after TNCB sensitization from HFD
38 and ND fed mice, cut into fine pieces, and placed (2 g) in 1 ml complete RPMI-1640 medium.
39 Processed scAT samples were then cultured in the presence of 100 µg antigen. After 48 h, the
40 culture supernatants of ELNC and scAT were collected and tested for cytokine concentration
41 using an ELISA Kit.

42 **Antibody measurement**

43 After 8 weeks of feeding with HFD or ND, the mice were sensitized and challenged with TNCB
44 as described above. The serum samples were collected 24 h after challenge and TNP-specific
45 antibodies were measured by ELISA as previously described [E3].

46 **Myeloperoxidase (MPO) assay and ear weight evaluation**

47 The ears were removed 24 h post challenge and the ear tissue biopsies (6 mm diameter punch)
48 were weighed with an analytical balance (Sartorius, Germany). The biopsies were homogenized

49 in 0.5 ml buffer (0.5% hexadecyltrimethylammonium bromide, pH = 6.0). The homogenates
50 were freeze-thawed 3 times, followed by centrifugation at 15,000 g for 30min. Twenty µl
51 aliquots were mixed with 200 µl phosphate buffer (pH = 6.0) containing 0.167 mg/ml o-
52 dianisidine dihydrochloride and $5x10^{-4}$% H_2O_2, and incubated at 25˚C for 20 min in 96-well
53 flat-bottomed plates. The absorbance was measured at 460 nm (colorimetric detection). MPO
54 activity was expressed in units per protein concentration (units/mg of protein).

55 **Isolation of cell infiltrates from AT tissue**

56 To determine the phenotype of the cell infiltrates in AT tissue, scAT were isolated, as
57 previously reported for mice fed with HFD or ND before TNCB sensitization.[E4] In brief, 4 days
58 after TNCB sensitization, scAT from a subcutaneous part of the groin area was extracted and
59 weighed using the analytical balance (Sartorius, Germany) after previous inguinal lymph node
60 removal, and was minced, and incubated in PBS with 0.5% FBS 5 mM $CaCl_2$, together with 2
61 mg/mL of collagenase type II, at 37ᴼC for 40 min in a water bath. During incubation, samples
62 were shaken intermittently every 5 min on a vortex mixer. Next, 10 ml of PBS with 0.5%
63 FBS was added, and the suspension was filtered through a 100 µm nylon filter and centrifuged
64 at 4ᴼC at 500 g for 10 min. The supernatant was decanted, and the remaining cell pellets were
65 resuspended with PBS with 0.5% FBS . The number of viable cells was counted based on trypan
66 blue exclusion.

67 **Staining of lymphoid cells and flow cytometry analysis**

68 To determine the phenotype of immune cells, single cell suspensions from ALN (ALNC) and
69 SPL (SPLC), and scAT were isolated, 4 days post TNCB sensitization, from the mice fed with
70 HFD and ND. Cells were washed and pre-incubated with Fc blocker (0.5 µg of anti-mouse
71 CD16/CD32 mAb, eBioscience, San Diego, CA) for 15 min at 4°C followed by incubation with
72 mAbs conjugated with appropriate fluorochrome, for 30min. Surface markers were stained with

73 mAbs (all from BioLegend unless indicated): anti-TCRβ-FITC (H57-597): anti-CCR7-APC
74 (4B12), anti-CD4-PerCP-Cy5.5 (GK1.5), anti-CD11c-APC-Cy7 (N418), anti-CD25-APC
75 (PC61), anti-CD80-PerCP-Cy5.5 (16-10A1), anti-CD86-PE-Cy7 (GL-1), anti-CD103-APC
76 (2E7), anti-CD207-PE (4C7) and anti-CD4-PE-Cy7 (RM4-5, from BD Biosciences). To detect
77 intracellular cytokines, ALNC and SPLC ($5x10^6$/ml) were first stimulated for 4 h with PMA (5
78 μl of 10 μg/ml solution, Sigma Chemical Co.) and ionomycin (2.5 μl of 200 μg/ml solution
79 Sigma Chemical Co.) in the presence of Golgi Plug (1 μl, eBioscience), followed by surface
80 marker staining. After washing, the cells were fixed and permeabilized according to
81 manufacturer's instructions and stained with anti-IL-17A-PE (TC11-18H10), anti-IFN-γ-APC
82 (XMG1.2), anti-IL-4-PE (11B11) and anti-IL-10-APC (JES5-16E3). FoxP3 positive cells in
83 scAT cell infiltrates were determined by staining with anti-FoxP3-PE mAb (FJK-16s, BD
84 Biosciences) using the mouse Treg staining kit (eBioscience) according to manufacturer's
85 instructions. The cells were analyzed with a FACS Canto II (Becton Dickinson) and data were
86 analyzed using FlowJo software. Gating information for all the flow cytometry analysis is
87 presented as Supplementary Information.

88 **Extraction of bacterial DNA from the gut contents**

89 The contents of large intestine were collected when the mice were terminated (8 weeks after
90 feeding with HFD or ND). The bacterial DNA was isolated using the protocol as previously
91 published [E5]. qPCR was used to evaluate the alteration of gut bacteria after HFD feeding as
92 previously described [E6].

93 **Transfer of gut microbiota**

94 To test the influence of HFD-induced GM dysbiosis on CHS reaction, the experiment of
95 "adoptive microbiota transfer" (FMT- fecal microbiota transplantation) was performed by oral
96 gavage with the fecal material from the donors that were fed with ND or HFD. The luminal

97 contents of large intestine were harvested from the donors after 8 weeks of feeding with ND or
98 HFD. The fecal materials were suspended into 4 ml of sterile PBS and centrifuged, to remove
99 undigested food, after mixing vigorously for at least 1 min, centrifuged at 4 min, 300 x g. The
100 fecal supernatant (300 μl) was orally inoculated into the recipient mice, twice a week for two
101 weeks, prior to CHS induction. To test the efficacy of FMT the gut content of recipients were
102 harvested 14 days after first FMT. The bacterial DNA was isolated using the protocol as
103 previously published [E5] . qPCR was used to evaluate the alteration of gut bacteria after HFD
104 feeding as previously described [E6].

105 **Statistical analysis**

106 Results are presented as mean ± SEM. Student's t-test, Mann Whitney's test and one-way
107 ANOVA, followed by Tukey's test, were used for statistical analysis with Prism software.
108 $p<0.05$ was considered statistically significant.

109 **SUPPLEMENTARY REFERENCES**

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111 trinitrophenyl group. *Biochemistry*. (1966) 5:3385–3395.
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118 75:e50707. doi: 10.3791/50707.
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121 E6. Strzępa A, Majewska-Szczepanik M, Lobo FM, Wen L, Szczepanik M. Broad spectrum
122 antibiotic enrofloxacin modulates contact sensitivity through gut microbiota in a murine
123 model. *J Allergy Clin Immunol.* (2017) 140:121-33.e3. doi: 10.1016/j.jaci.2016.11.052.

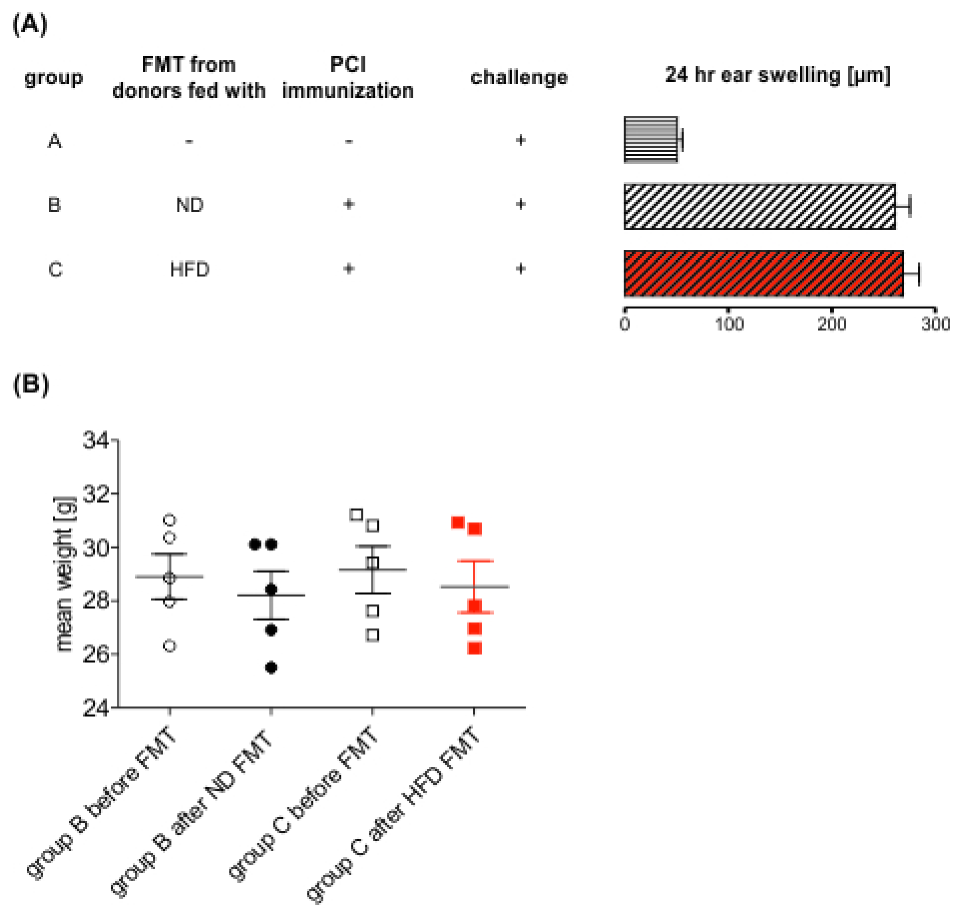
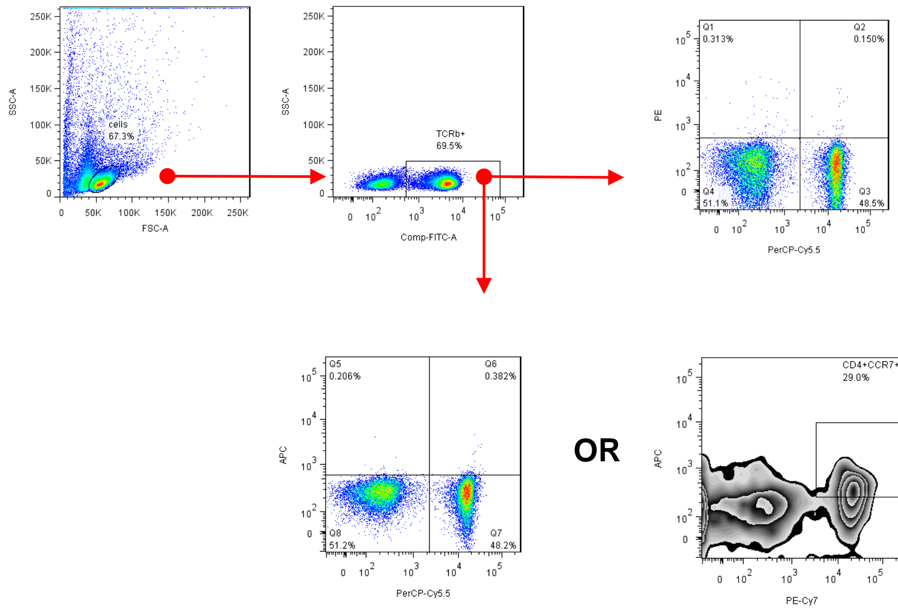


FIGURE S1

Gating strategy for FIGURE 4A and FIGURE 4B



Gating strategy for FIGURE 4C and FIGURE 4D

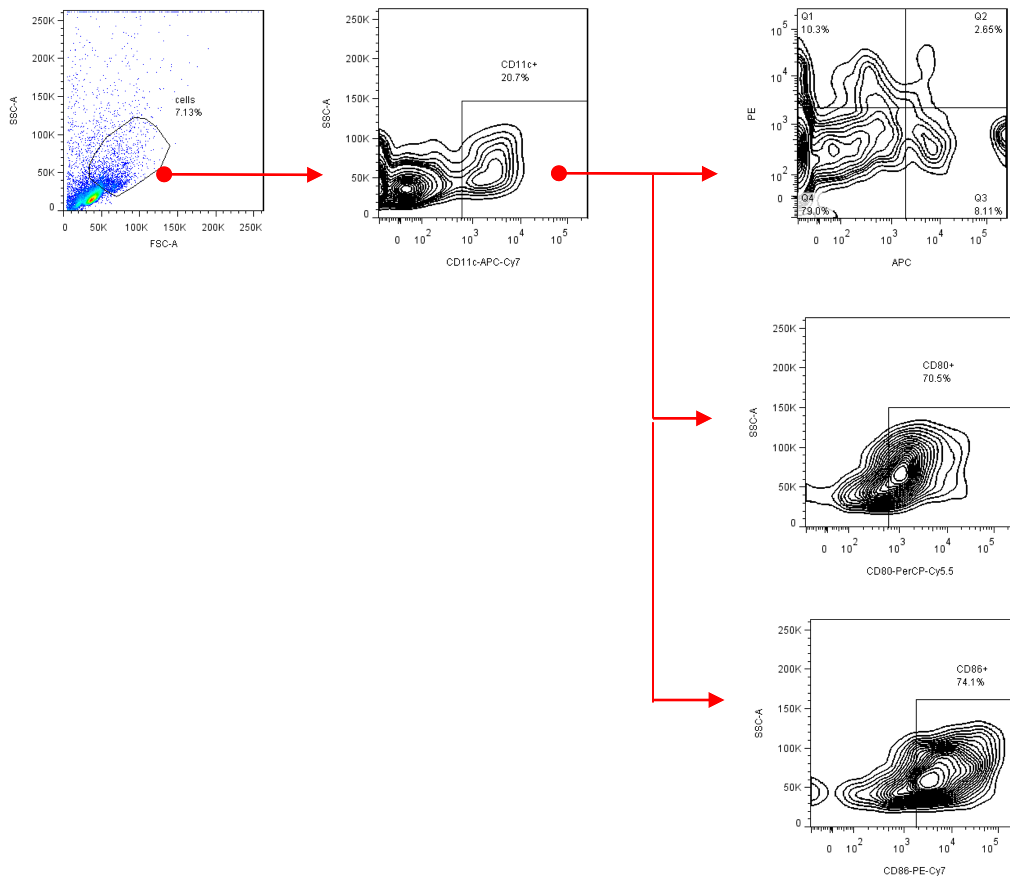


FIGURE S2.

FMO staining and representative flow plots for ALNC presented in FIGURE 4A

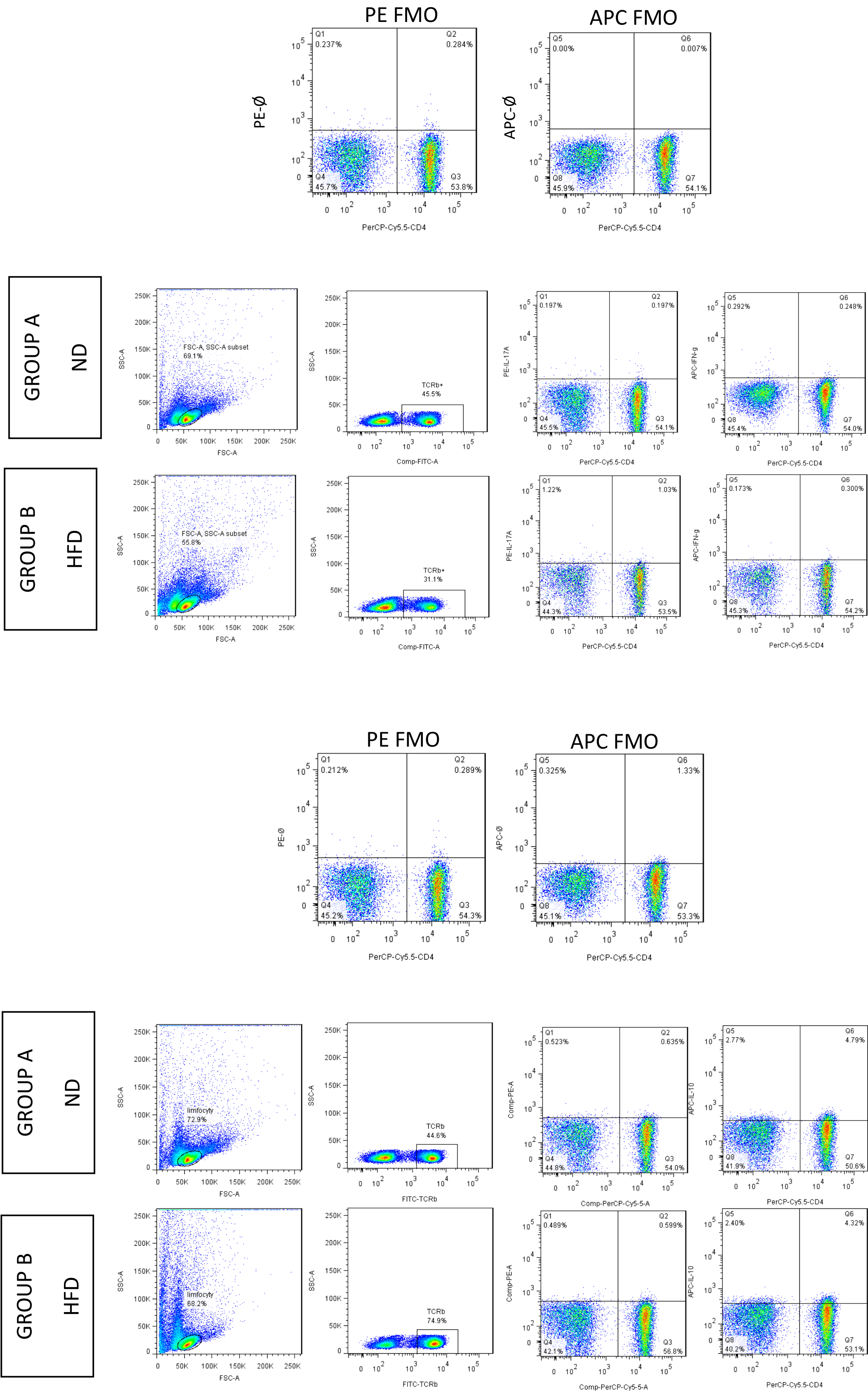


FIGURE S3

FMO staining and representative flow plots for SPLC presented in FIGURE 4B

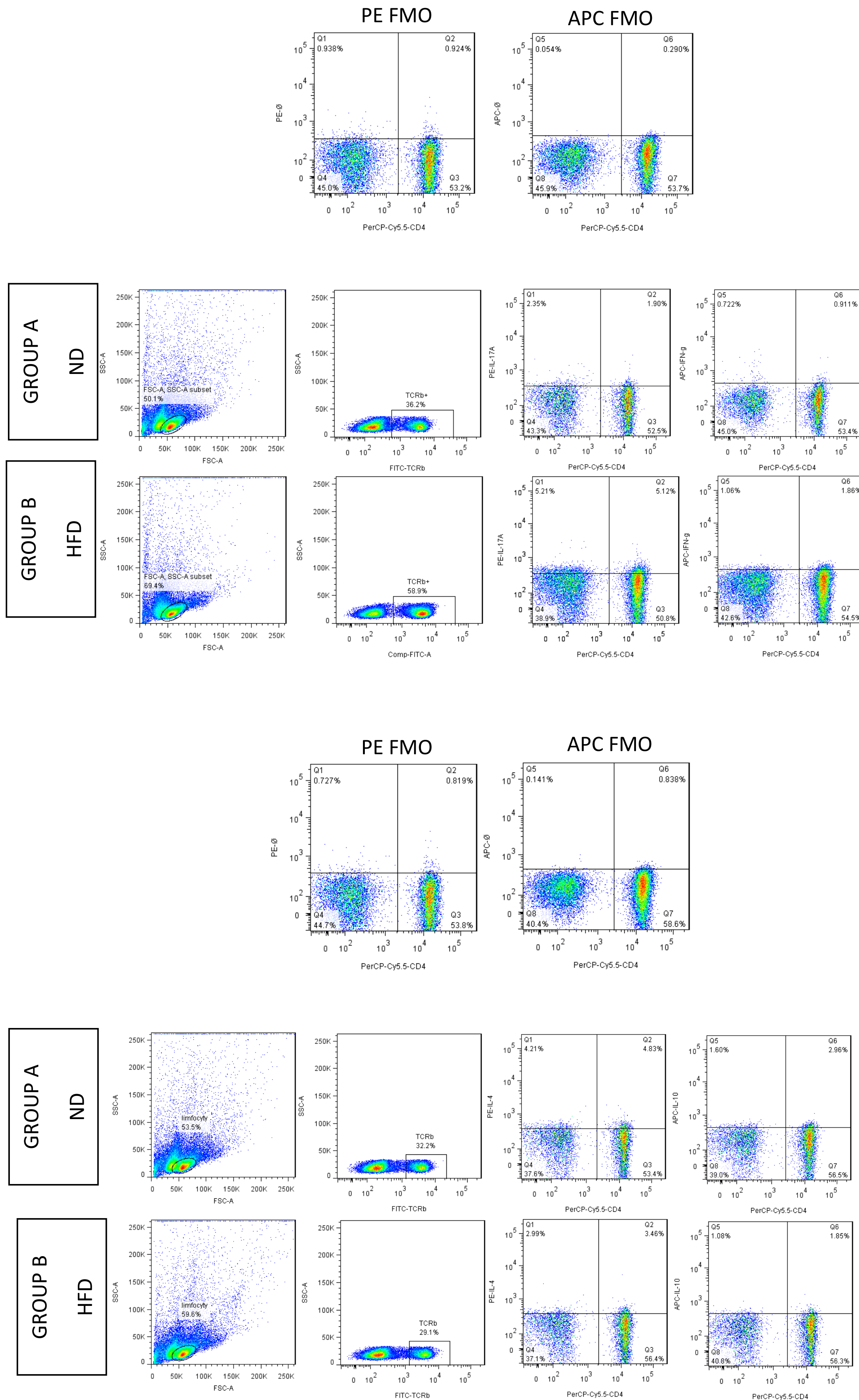
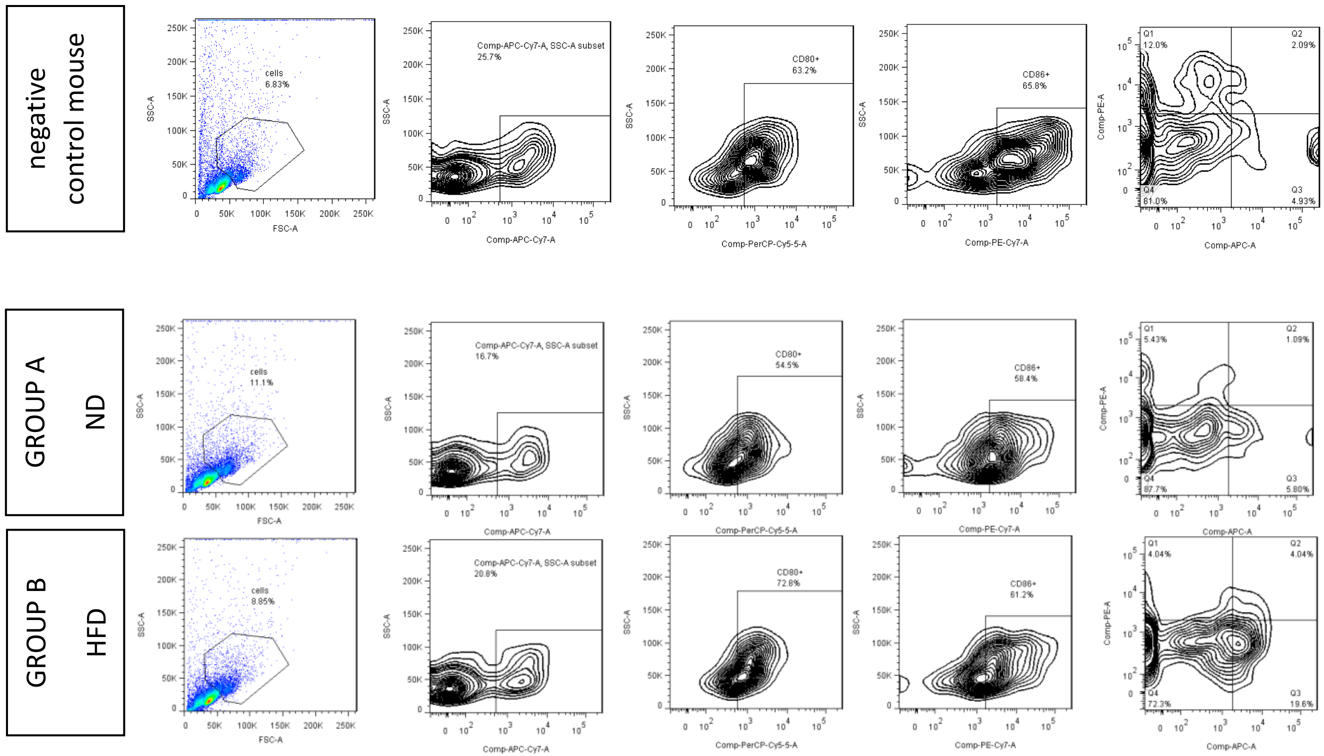


FIGURE S4

Representative flow plots for ALNC presented in FIGURE 4C



Representative flow plots for SPLC presented in FIGURE 4D

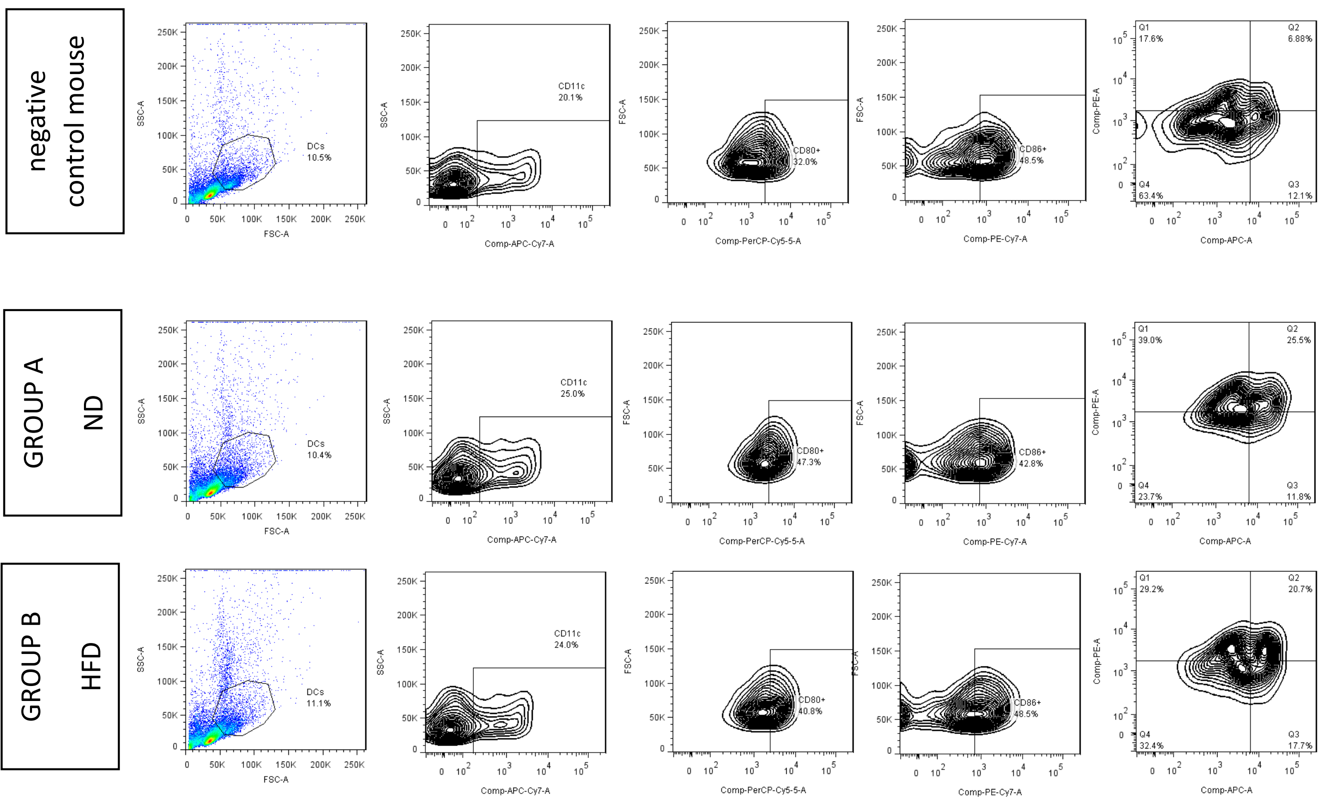
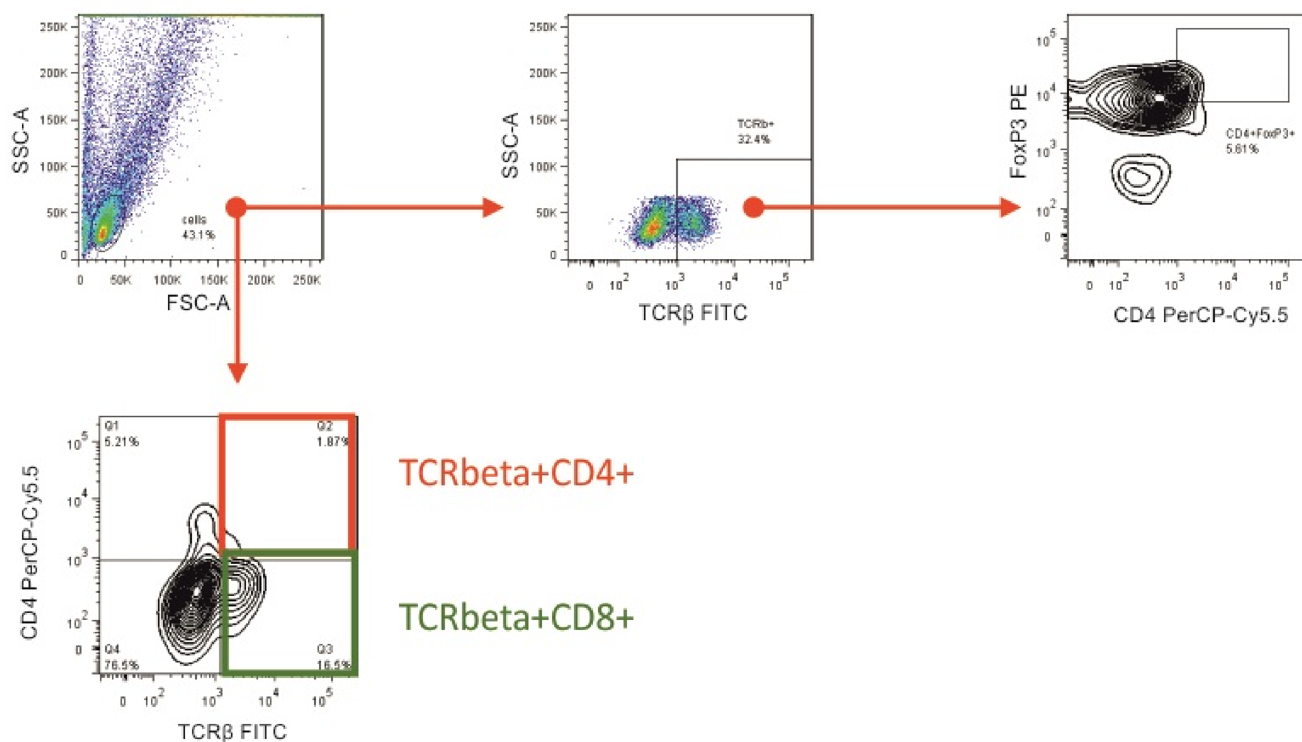


FIGURE S5

Gating strategy for FIGURE 5C



Gating strategy for FIGURE 5D

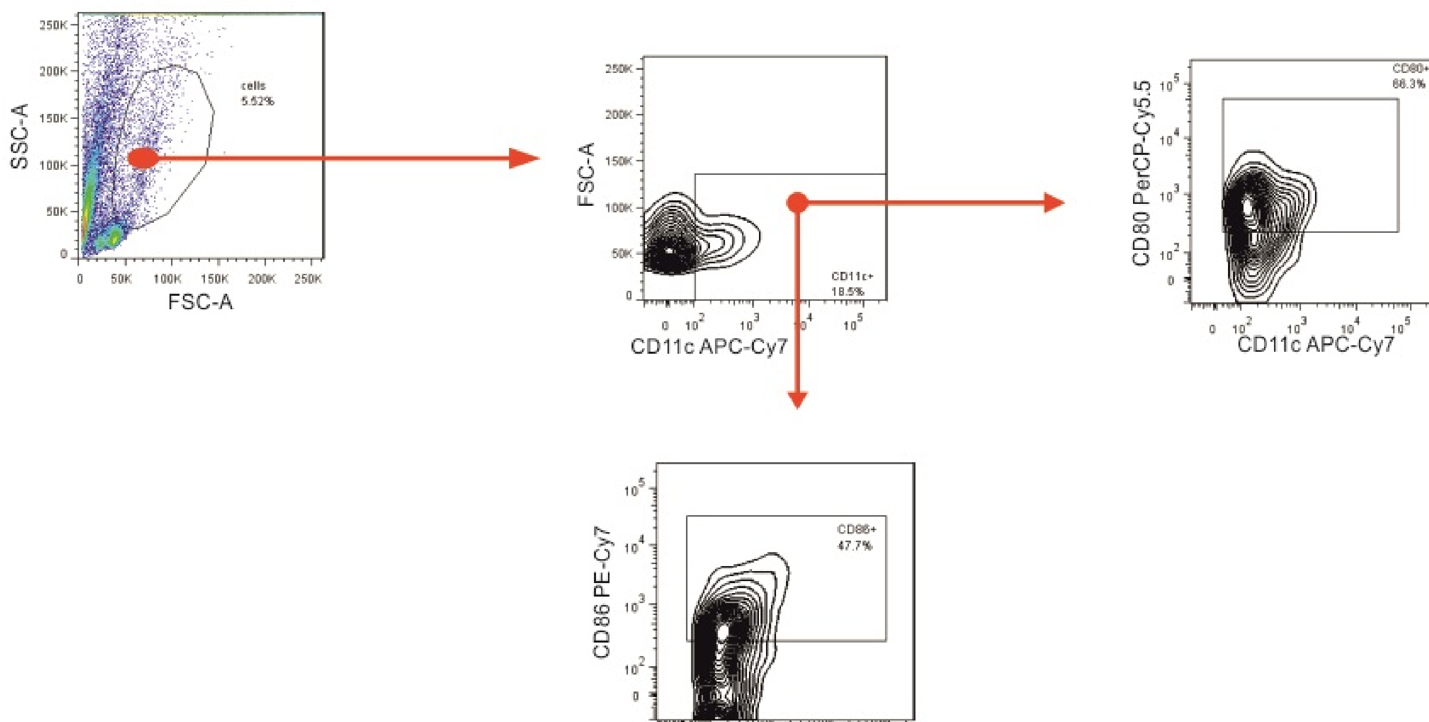


FIGURE S6

0 10^2 10^3 10^4 10^5
CD11c APC-Cy7

FMO staining and representative flow plots for cells presented in FIGURE 5C .

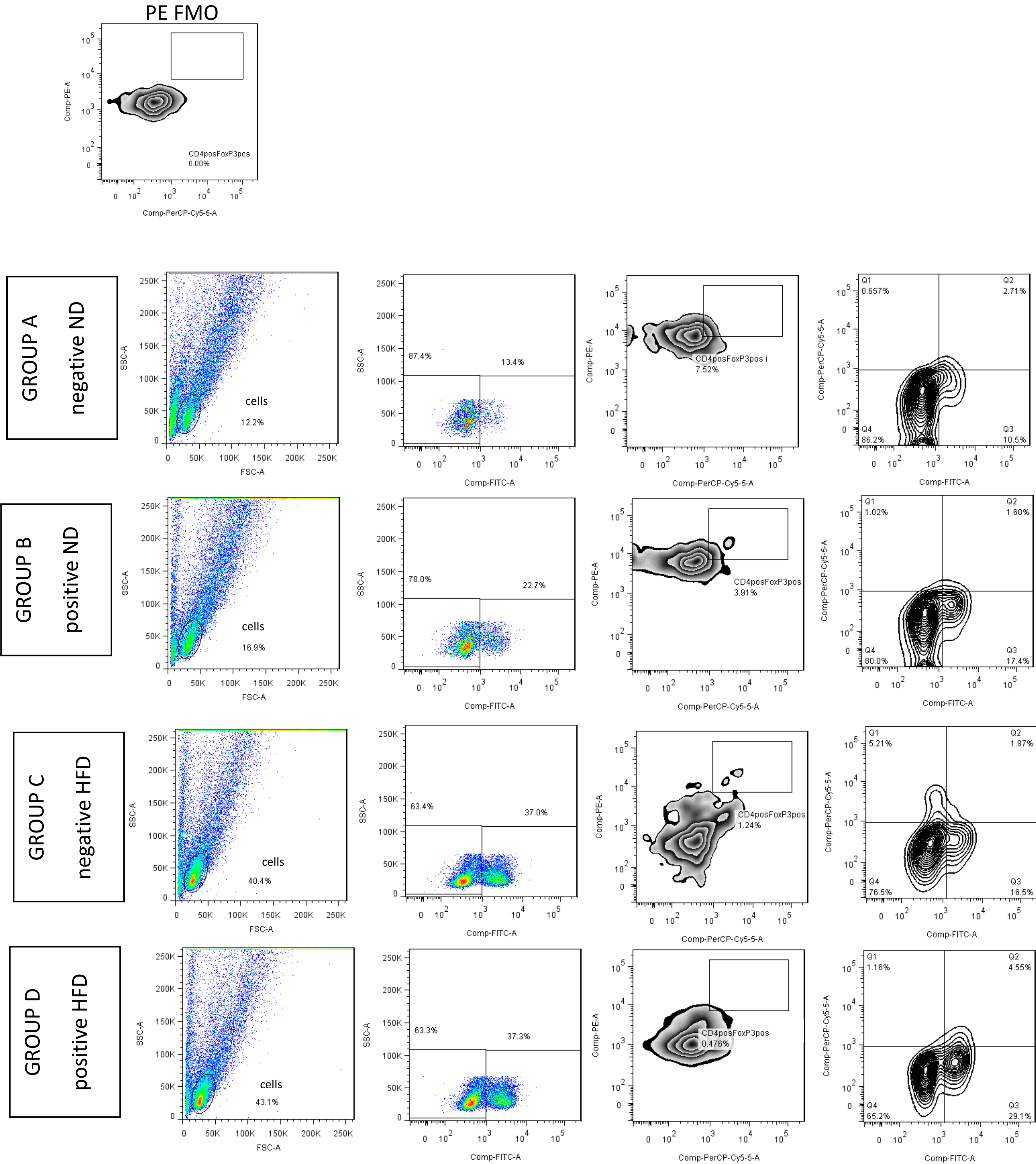


FIGURE S7

Representative flow plots for cells presented in FIGURE 5D.

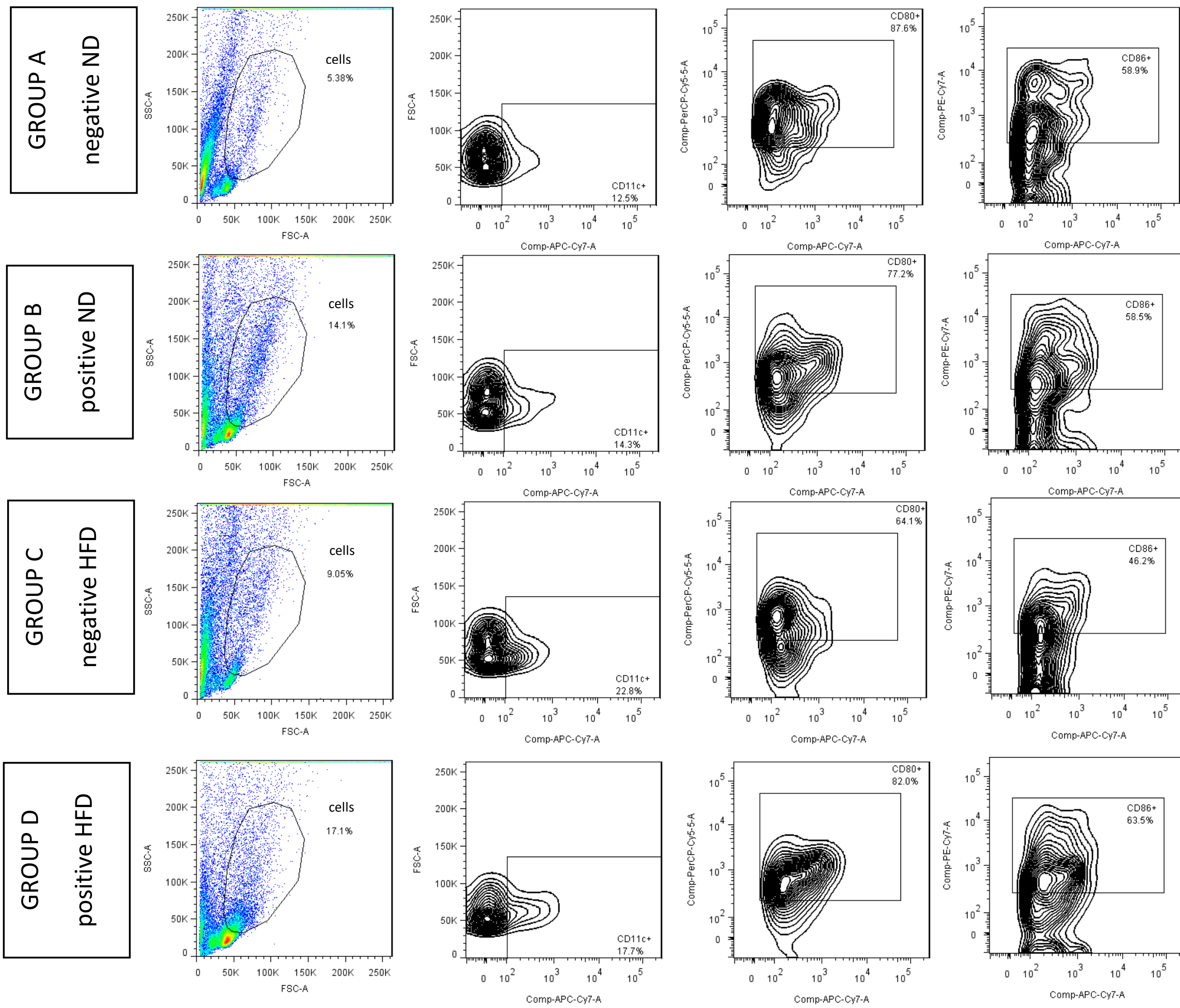


FIGURE S8