Supplemental Figures

A Small Molecule, UAB126, Reverses Diet-Induced Obesity and Its Associated Metabolic Disorders

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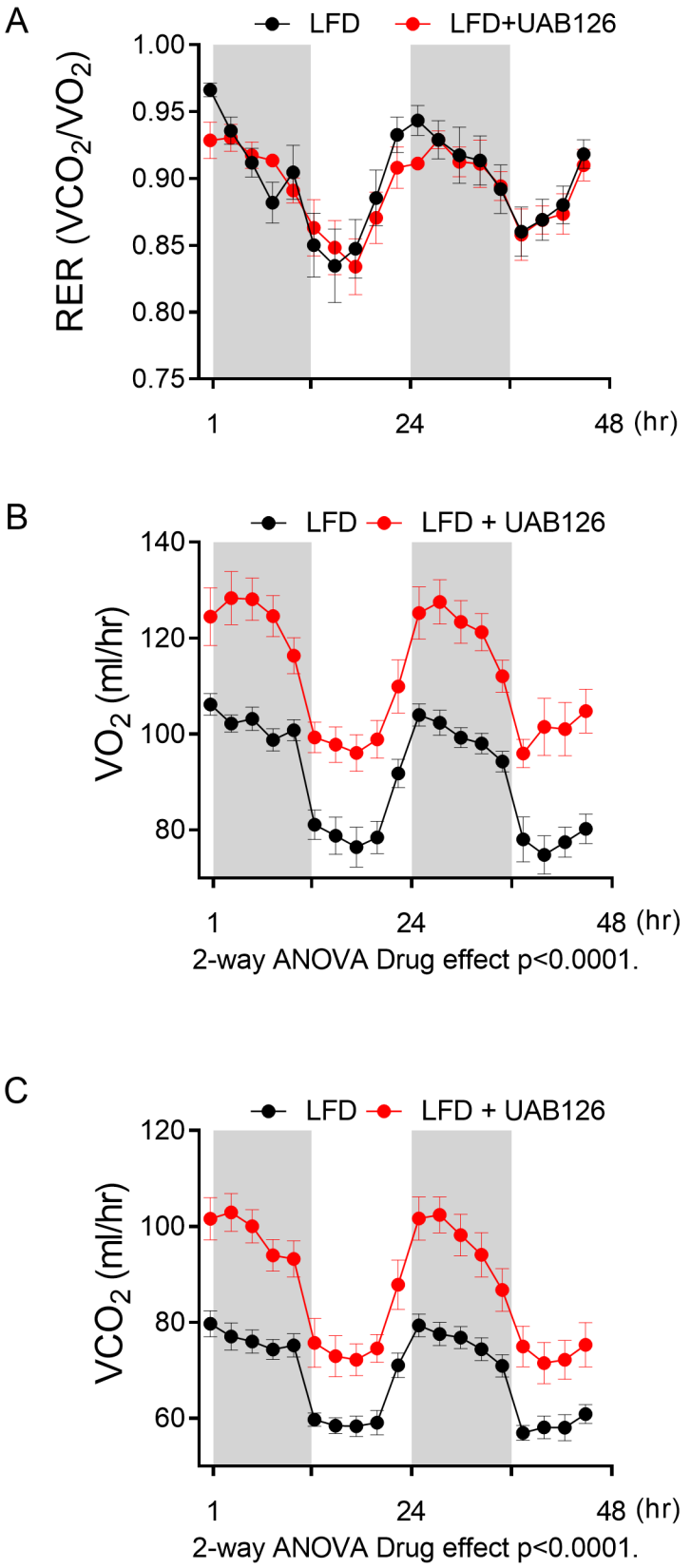
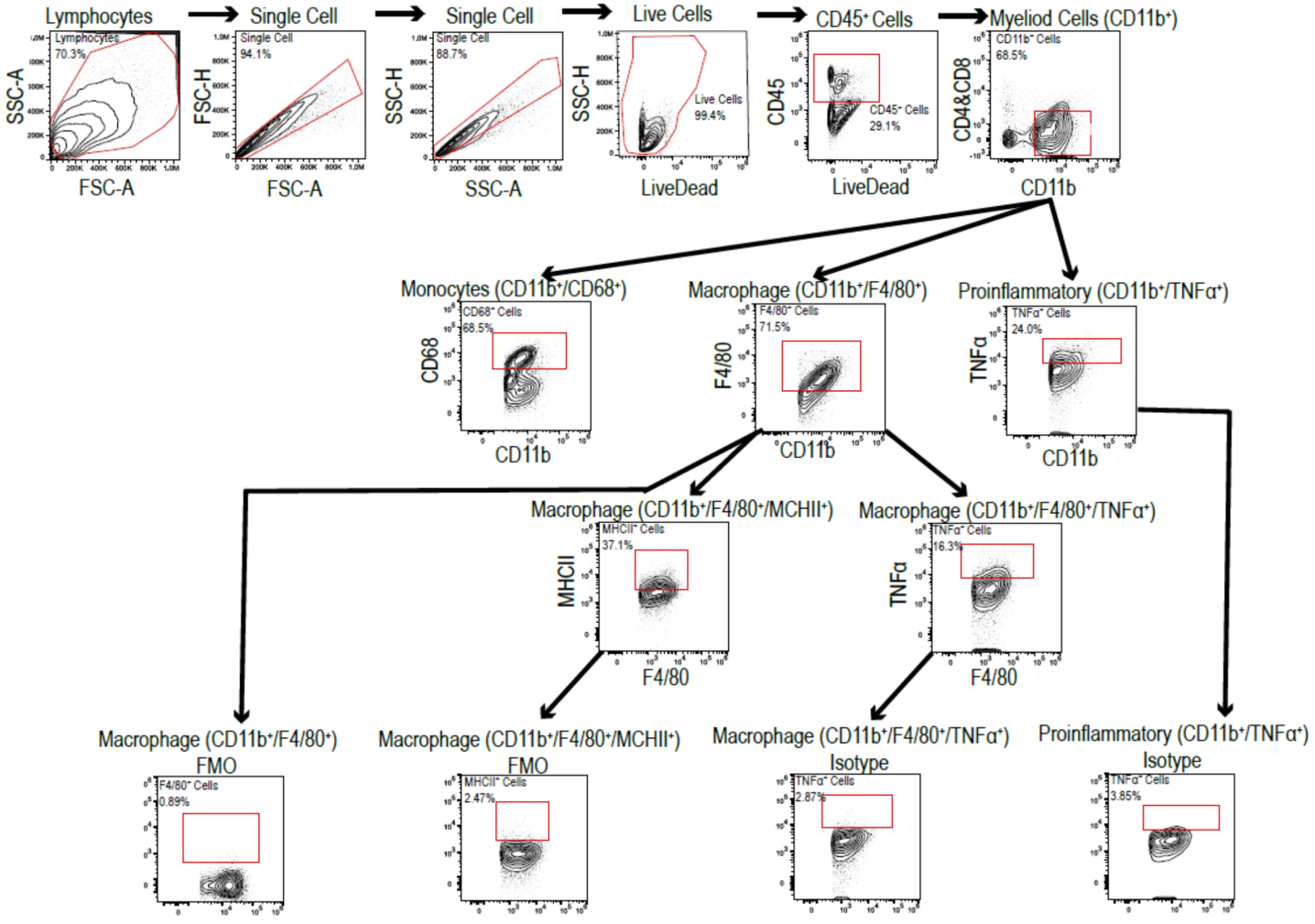
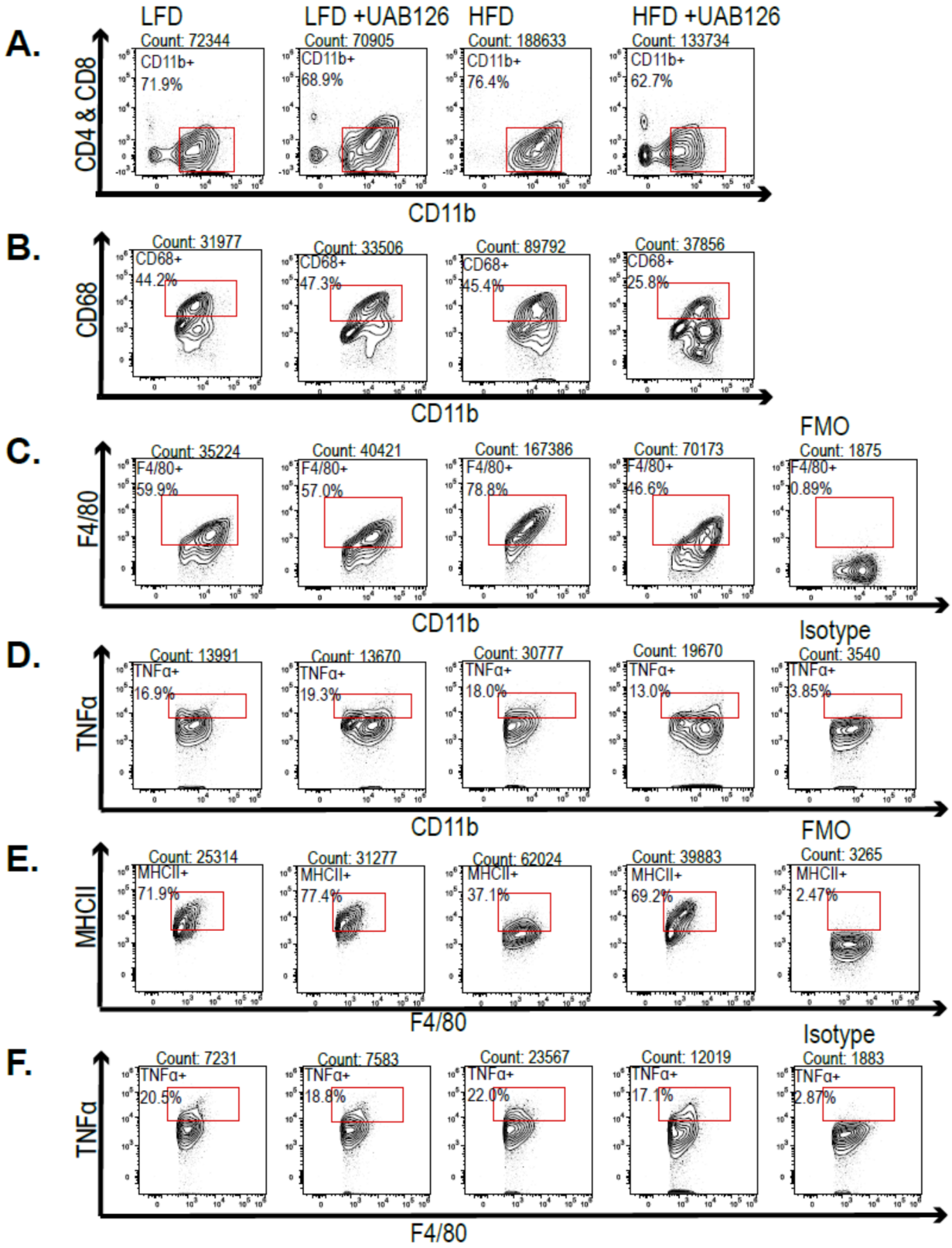
Supplementary Figure 1. UAB126 does not affect RER in LFD-fed mice, but increases oxygen consumption. Indirect calorimetry was used to measue RER (A), VO_2 (B), and VCO_2 (C) in LFD-fed mice. UAB126 increases oxygen consumption, which suggests that metabolic rate is increased. (n=5-6).



Supplementary Figure 2. Flow cytometry gating strategy. Stromal vascular cells were pre-gated on single cells, live cells, CD45+, and a CD4- and CD8- dump gate was used to immunophenotype myeloid cells (CD11b) and macrophages (F4/80). Monocytes were identified as CD11b+ CD68+and macrophages were CD11b+ F4/80+. Pro-inflammatory monocytes were identified as CD11b+ TNFα+, pro-inflammatory macrophages were identified as CD11b+ F4/80+ MHCII+ and CD11b+ F4/80+ TNFα+. Fluorescence minus one (FMO) and isotype control is also shown for F4/80, MHCII, and TNFα.



Supplementary Figure 3. Representative FACs plots of myeloid cells, monocytes, and macrophages from SVC of mice on a high fat diet, low fat diet, and treated with or without UAB126. Flow cytometry analysis of stromal vascular cells isolated from epididymal adipose tissue for the detection of CD11b+ myeloid cells (A), CD11b+ CD68+ monocytes (B), CD11b+ F4/80+ macrophages (C), proinflammatory CD11b+ TNFα+ myeloid cells (D), activated CD11b+ F4/80+ MHCII+ macrophages (E), and proinflammatory CD11b+ F4/80+ TNFα+ M1 macrophages (F). (n=5-7)



Supplemental Fig. 4 UAB126 requires in vivo metabolism for its activity. Human embryonic kidney 293 (HEK293) cellls were transiently transfected with a luciferase construct containing RXR responsive element, a plasmid expressing RXRα and pRL-tk (Promega), and then treated with UAB30 (1 μM), or UAB126 (10 μM) for overnight prior to a dual luciferase assays **(A)**. Stromal vascular cells (SVC) were isolated from inguinal fat tissue of C57BL/6J mice, and then the SVCs were incubated with brown adipocyte differentiation media with or without UAB126 or UAB30, and uncoupling protein (Ucp-1) gene expression was assessed by qPCR **(B)**. Serum from vehicle-treated mice was spiked with UAB126 and run as a positive control in the HPLC analysis **(C)**. UAB126 was dissolved in 1:9 (Ethanol: PEG400), and then UAB126 (100 mg/kg body weight/day) was orally administered by gavage for 5 days. One hour after the final dose, serum was collected and subjected to HPLC analysis **(D)**.