Obesity alters B cell and macrophage populations in brown adipose tissue

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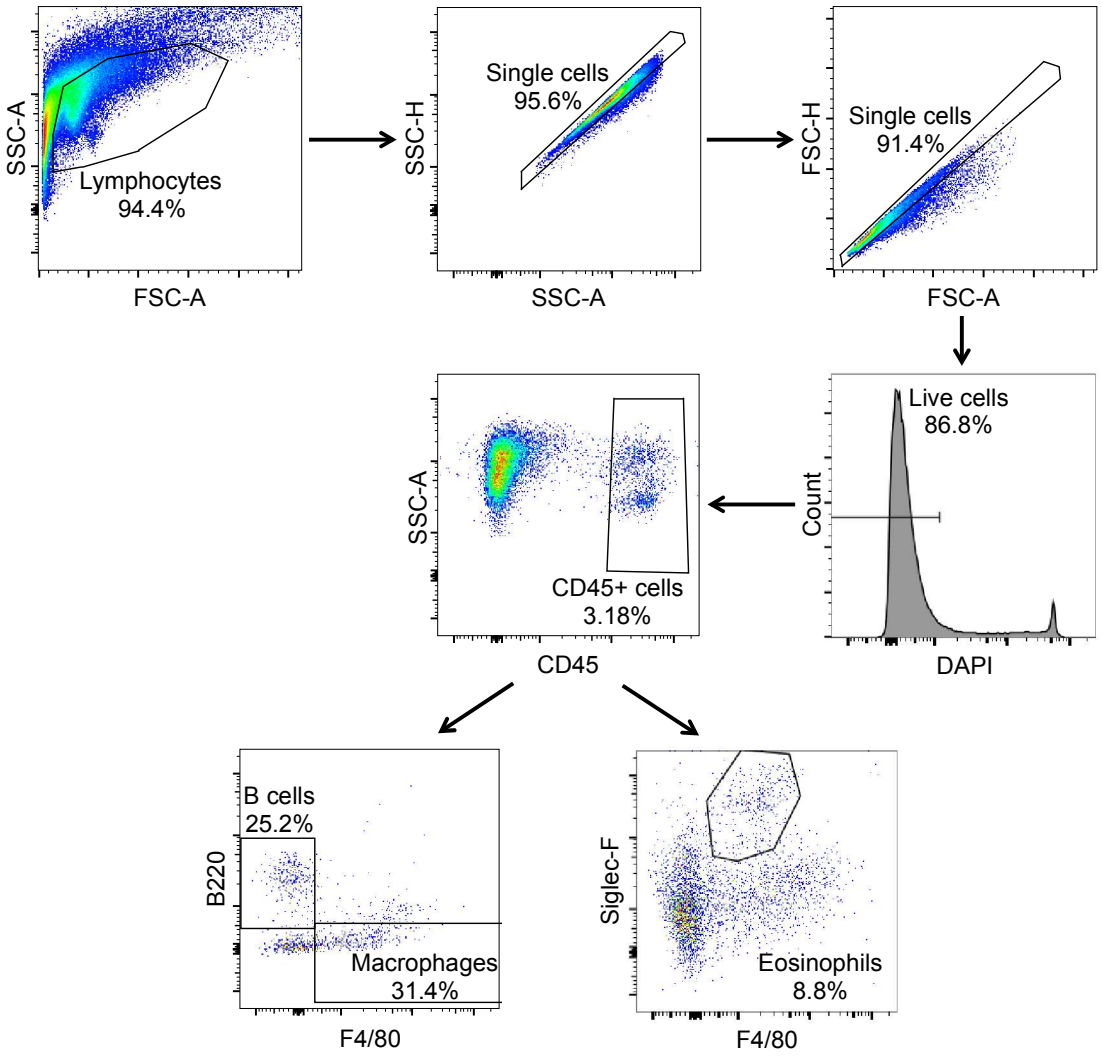
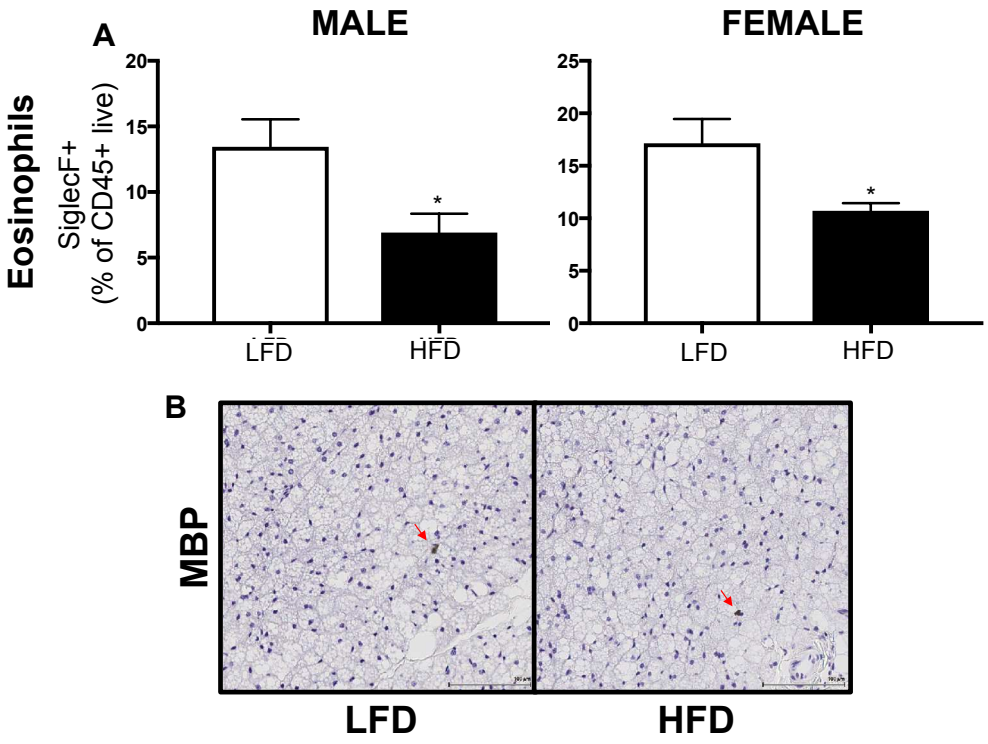


Supplementary Figure S1. Flow cytometry gating scheme.

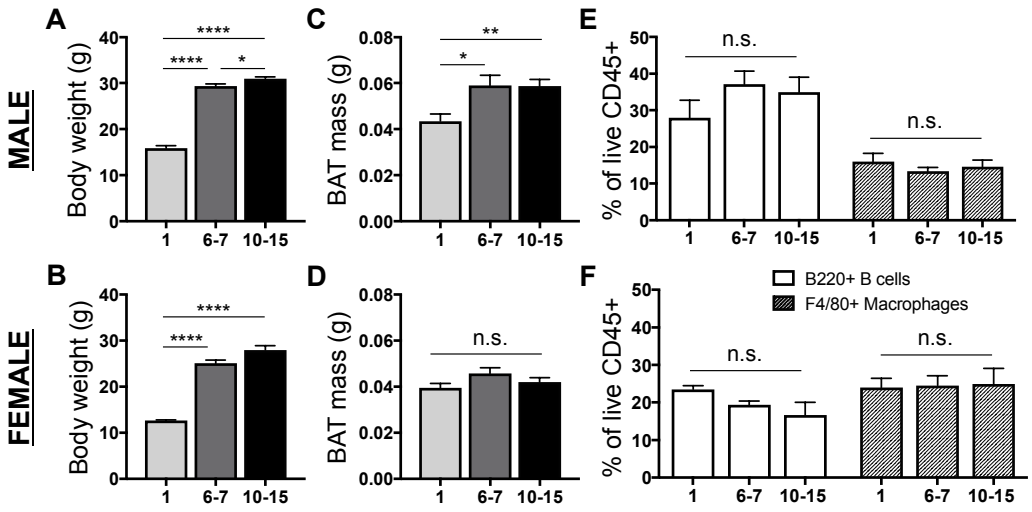
Leukocytes were identified initially by their forward scatter (FSC) and side scatter (SSC) properties. Subsequent FSC and SSC pulse geometry gates were used to eliminate aggregating cells. Viable leukocytes were identified as DAPI-, CD45+ cells. From CD45+ cells, the analysis of B cells (B220+, F4/80-) and macrophages (B220-, F4/80+) was achieved by using a dot plot. Eosinophils were gated from CD45+ leukocytes as F4/80+ and SiglecF+.



Supplementary Figure S2. Flow cytometry and immunohistochemistry of brown adipose tissue (BAT) for eosinophils.

BAT was collected from wild type C57BL/6J mice after 8 weeks of 10% low fat diet (LFD, white bars) or 60% high fat (HFD, black bars). **A.** SVF from BAT was processed and analyzed by flow cytometry for SiglecF+ eosinophils and presented as percent of CD45+ live cells. Data are presented as mean ± SEM of 5 samples per group. **B.** BAT was fixed in 10% formalin before further processing and staining for MBP. Data presented are representative images from 3 samples per group.

* $P<0.05$



Supplementary Figure S3. **Analysis of brown adipose tissue (BAT) immune cells with aging.**

Male and female wild type C57BL/6J mice were aged to 1, 6-7, or 10-15 months. Their final body weight was measured (**A**: male, **B**: female), and BAT was collected and weighed (**C**: male, **D**: female). BAT stromal vascular fraction was processed and analyzed by flow cytometry for B220+ B cells (white bars) and F4/80+ macrophages (hatched bars) as a % of CD45+ live cells (**E**: male, **F**: female). Data are presented as mean ± SEM of 4-18 samples per group.

* P<0.05

** P<0.01

**** P<0.0001

n.s. P>0.05