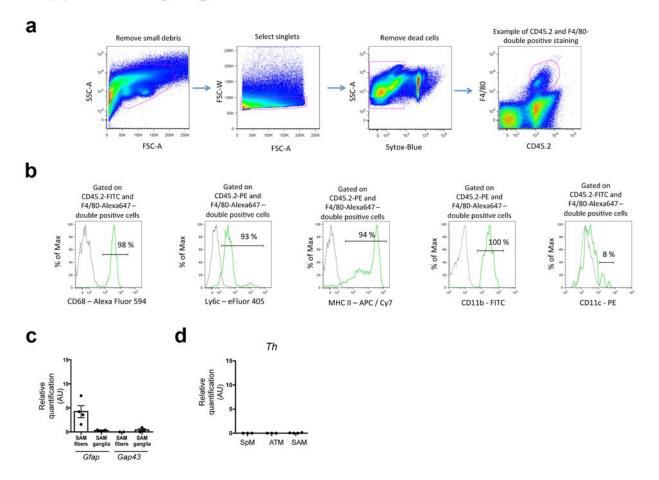
Supplementary Figure 5



Supplementary Figure 5. Gating strategy for identification of macrophages. Myeloid and glial marker expression by macrophages. Absence of machinery for NE biosynthesis in SAMs.

(a) Flow cytometry gating scheme for identification of CD45.2, F4/80-double positive macrophage populations. Dot plots represent gating strategy for macrophages in subcutaneous adipose tissue. (b) Representative flow cytometry histograms for CD68, Ly6c, MHC II, CD11b and CD11c expression in SAMs. Cells were gated on CD45.2, F4/80-double positive population. Histograms are representative of 3 experiments. (c) CD45.2-PE, F4/80-Alexa Fluor 647 -- double positive macrophages were isolated from sympathetic nerve fibers (SAM fibers) and superior cervical ganglia (SAM ganglia). Expression of mRNA for Gfap and Gap43 was determined by gRT-PCR and is presented relative to Gapdh expression, n = 4 experiments for SAM fibers (Gfap), n = 3 experiments for SAM ganglia (Gfap and Gap43), n = 2 experiments SAM fibers (Gap43). (d) CD45.2-PE, F4/80-Alexa Fluor 647 - double positive macrophages were isolated from spleen (SpM), adipose tissue (ATM), and sympathetic nerve fibers (SAM). Expression of mRNA for TH was determined by qRT-PCR and is presented relative to Gapdh expression. Tissues were pooled from 10 mice. n = 3 experiments for SpM and ATM and n = 4experiments for SAM. Data were analyzed in panel c by two-tailed Student's t-test and panel d by one-way ANOVA followed by Tukey's multiple comparison test. Data are shown as average ± SEM.