

SUPPLEMENTARY FIGURES

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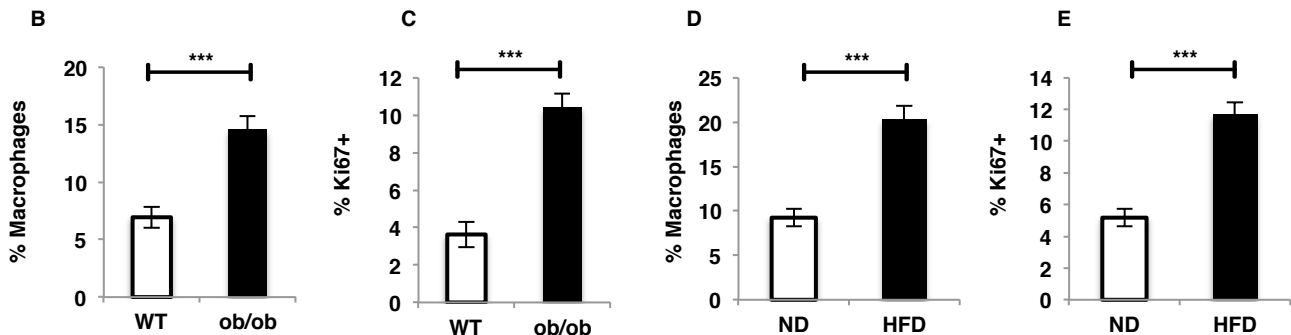
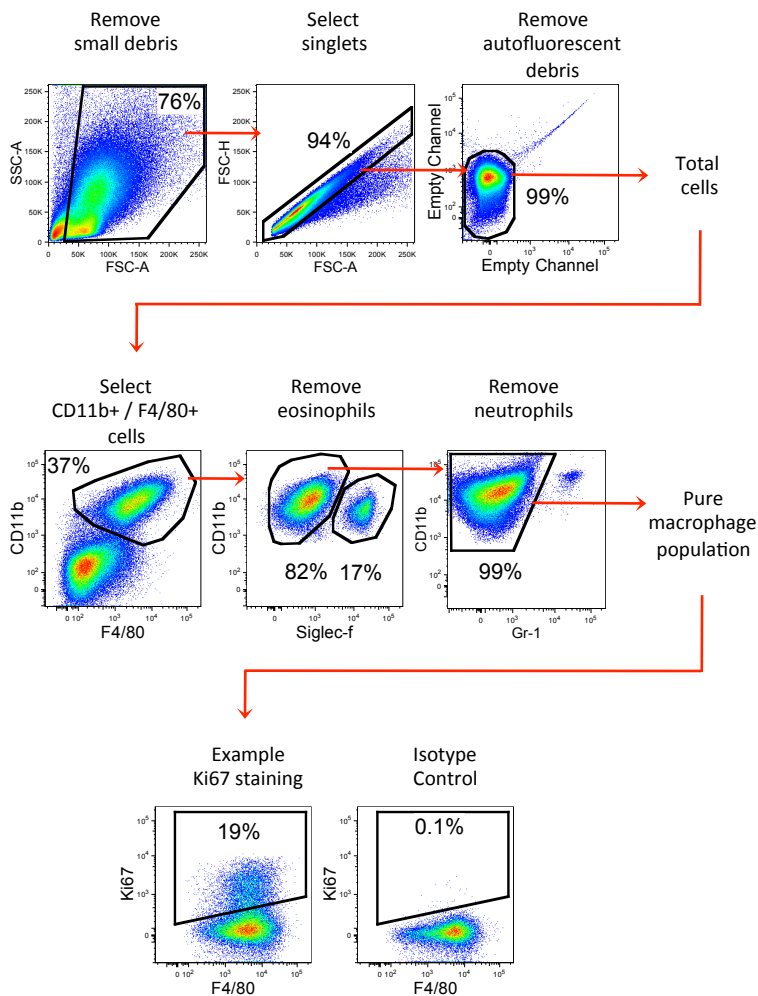


Figure S1, related to Figure 1. (A) Flow cytometry gating scheme for identifying macrophages in adipose tissue SVF. **(B)** Percentage of macrophages in SVF from SAT of WT and *ob/ob* mice. **(C)** Percentage of macrophages expressing Ki67 in SAT of WT and *ob/ob* mice. n=10. **(D)** Percentage of macrophages in SVF from SAT of mice fed a ND or a HFD for 10 weeks. **(E)** Percentage of macrophages expressing Ki67 in SAT of mice fed a ND or a HFD. n=18. . All graphs are expressed as mean ± s.e.m. Statistical significance was determined by Student's t-test. ***p<0.001.

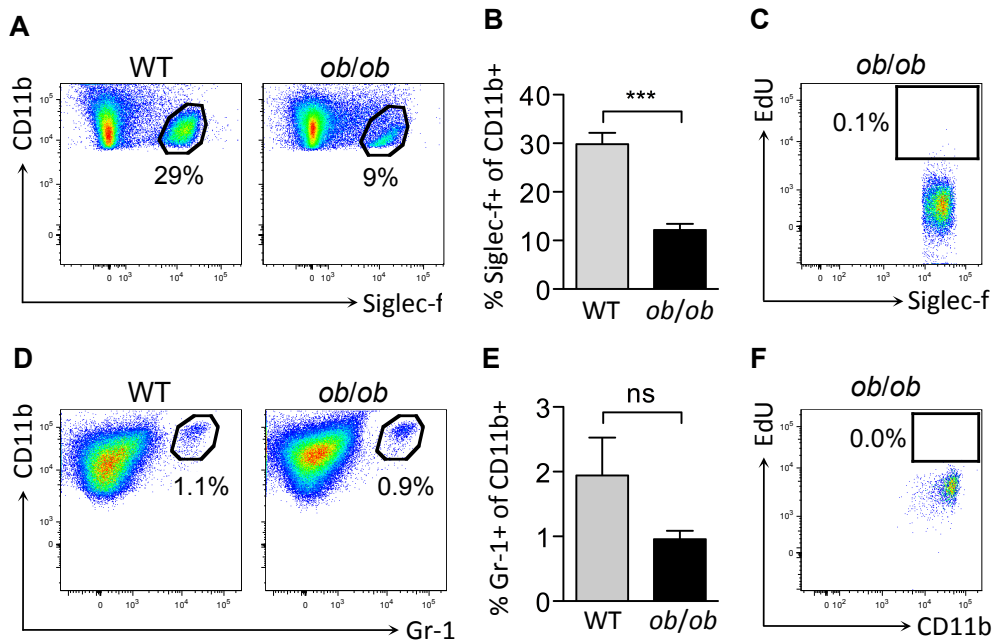


Figure S2, related to Figure 2. Effect of obesity on proliferation of diverse immune cell types in adipose tissue. **(A)** Representative dot plots of CD11b+ SVF cells stained for the eosinophil marker Siglec-f. **(B)** Percentages of CD11b+ cells that are eosinophils in WT and *ob/ob* mice. $n = 48-51$ mice/group from 10 independent experiments. **(C)** Representative dot plot of SVF eosinophils 3 hours after EdU injection. **(D)** Representative dot plots of CD11b+ SVF cells stained with the neutrophil marker Gr-1. **(E)** Percentages of CD11b+ cells that are neutrophils. $n = 14-15$ mice/group from 3 independent experiments. **(F)** Representative dot plot of SVF neutrophils 3 hours after EdU injection. All graphs are expressed as mean \pm s.e.m. Statistical significance was determined by Student's t-test. ns = not significant. *** $p < 0.001$.

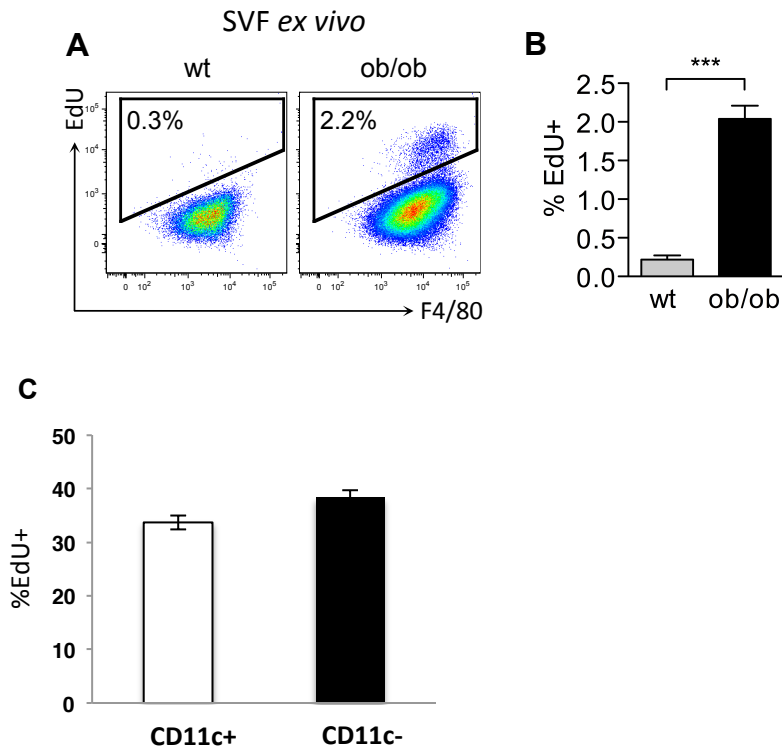


Figure S3, related to Figure 3. (A) SVF was isolated and treated with EdU. Representative dot plots of *ex vivo* macrophage EdU incorporation and (B) corresponding rates. (C) Percentage of pro- (CD11c+) and anti- (CD11c-) inflammatory macrophages that incorporated EdU (EdU+) or not (EdU-) during a 80-hour EdU exposure in VAT of *ob/ob* mice. n=9-10 mice/group. All graphs are expressed as mean \pm s.e.m. Statistical significance was determined by Student's t-test. ns = not significant. ***p<0.001.

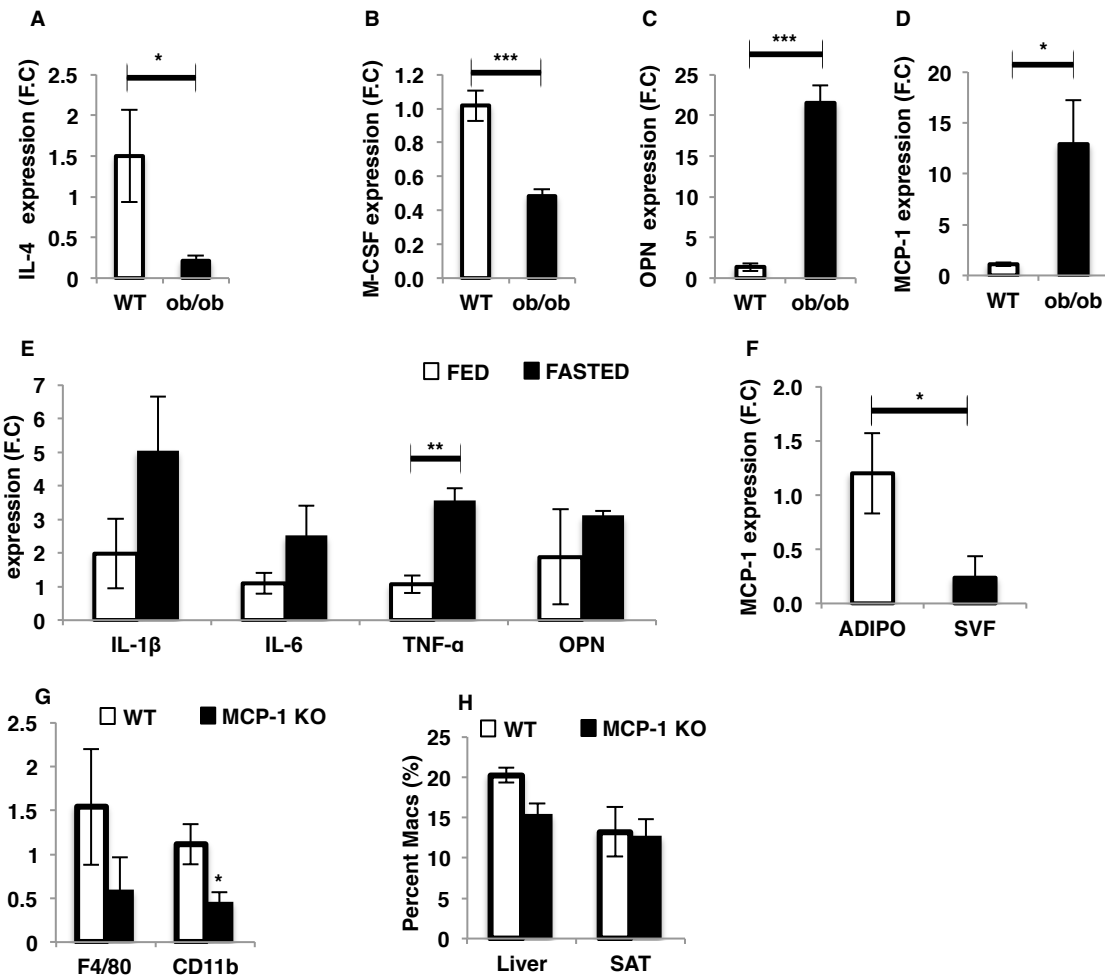


Figure S4, related to Figure 4. VAT was isolated from WT and *ob/ob* (A) IL-4, (B) M-CSF, (C) OPN and (D) MCP-1 expression was measured by RT-PCR. n=5. (E) Expression of interleukin 1 (IL-1 β), interleukin 6 (IL-6), tumor necrosis factor (TNF- α) and osteopontin in epididymal AT of HFD fed mice fasted for 24 hours. (F) Expression of MCP-1 in adipocytes and SVF from epididymal AT of HFD fed mice. (G) F4/80 and CD11b expression in VAT of WT and MCP-1 KO fed a HFD for 6 weeks. n=5. (H) Percentage of macrophages in Liver and SAT of WT and MCP-1 KO fed a HFD for 6 weeks. n=5. Statistical significance was determined by Student's t-test. ns = not significant. ***p<0.001; **p<0.01; *p<0.05.

Supplementary Experimental Procedures

Antibodies used for flow cytometry on murine cells: F4/80-APC (clone Cl:A3-1, AbD Serotec), CD11b-PerCP-Cy5.5 (clone M1/70), Gr-1-APC-Cy7 (clone RB6-8C5), Siglec-f-PE (clone E50-2440), Ly6C-PE-Cy7 (clone AL-21), Ki67-FITC (clone B56) and IgG1 κ -FITC (clone MOPC-21, BD Pharmingen), and CD11c-V450 (clone HL-3, BD Horizon).

Antibodies used for flow cytometry on human cells: CD14-FITC, CD11c-APC, CD11b-PE and CD45-PerCP; BD Bioscience