Figure S1. C3aR mRNA expression in mouse tissues (C57BL6; normal diet). Total RNA was extract from tissues and C3aR expression examined by semi-quantitative RT-PCR. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) was used as a loading control, increasing amount of mouse genomic DNA as a positive control and water as a negative control. PCR products were resolved on agarose gel and visualized by UV shadowing after ethidium bromide staining.

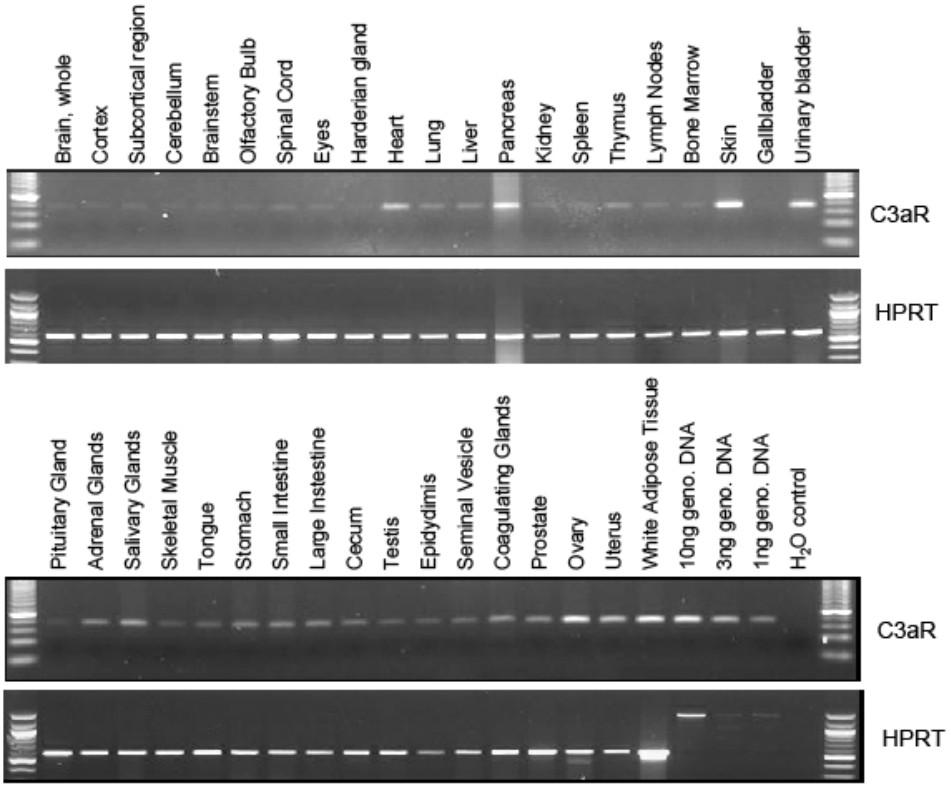

Figure S2. Relative C3aR mRNA expression normalized to eWAT ND. Total RNA was extracted from various tissues from lean (n=10) and obese mice (n=10, 8-16 weeks on HFD). Quantitative RT-PCR for C3aR mRNA expression was performed and normalized to cyclophilin B. Each PCR reaction was performed in duplicate.

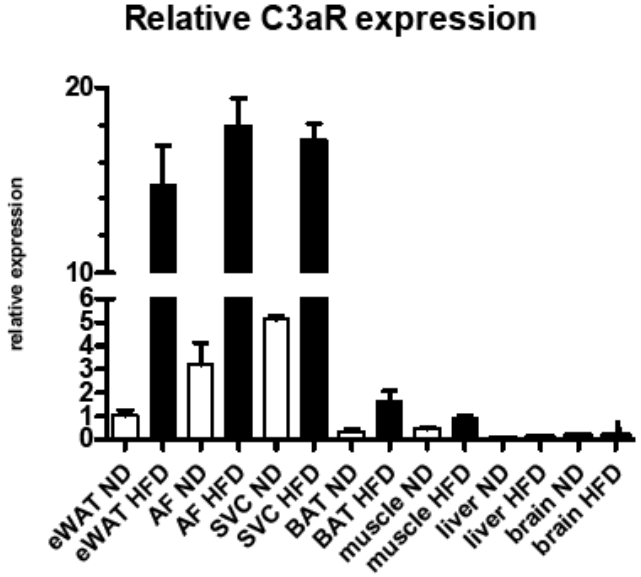

Figure S3. Generation of the C3aR$^{-/-}$ mice. (A) Schematic representation of the mouse C3aR gene. The C3aR gene is on the reverse strand of mouse chromosome 6. It is composed of 2 exons; exon 1 is a small non-coding exon and exon 2 contains the complete open reading frame, followed by a 3' untranslated region (UTR). The C3aR gene was disrupted by homologous recombination where part of the coding region was deleted (73-270bp) and a LacZ-neo cassette inserted. (B) Genotyping of the knockout mice by PCR using 3 primers (2 primers for the C3aR gene and 1 primer for the LacZ-neo cassette) confirmed proper targeting of the C3aR gene. PCR products were resolved on agarose gel with a 100bp DNA ladder. (C) Quantitative RT-PCR for C3aR mRNA expression in eWAT and muscle from WT and C3aR$^{+/-}$ mice (n=6 per group). No C3aR mRNA expression was detected in the C3aR$^{-/-}$ tissues (data not shown). All reactions were performed in duplicate. C3aR mRNA expression was normalized to cyclophilin B. **P < 0.01 by unpaired t test.

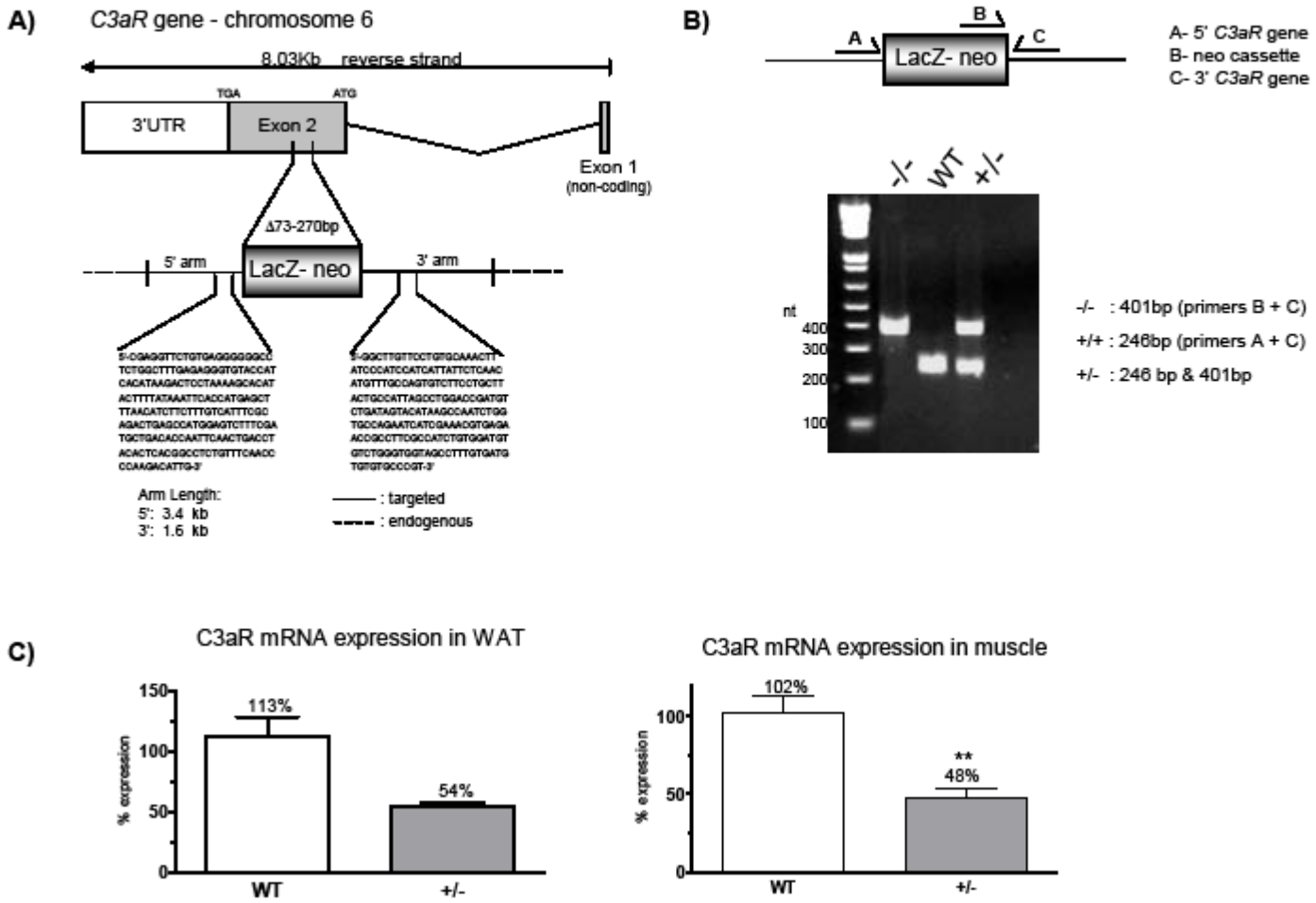

Figure S4. Plasma cytokine expression in WT and C3aR1$^{-/-}$ mice on ND and HFD after 8 weeks HFD. Plasma expression of MCP-1, IL-6, TNFα, IL-1β, IL-12/ IL-23p40, RANTES, IL-2 and IL-10 was assessed in WT and C3aR-/- mice on ND and HFD. Data presented as mean +/- SEM (n = 6 per group). No changes in plasma IL-4 and KC/GRO expression was seen in all 4 groups and plasma GM-CSF and IFNγ were below detection limits (data not shown). All measurements were done in triplicate. *P < 0.05, **P < 0.01, ***P < 0.001 by ANOVA.

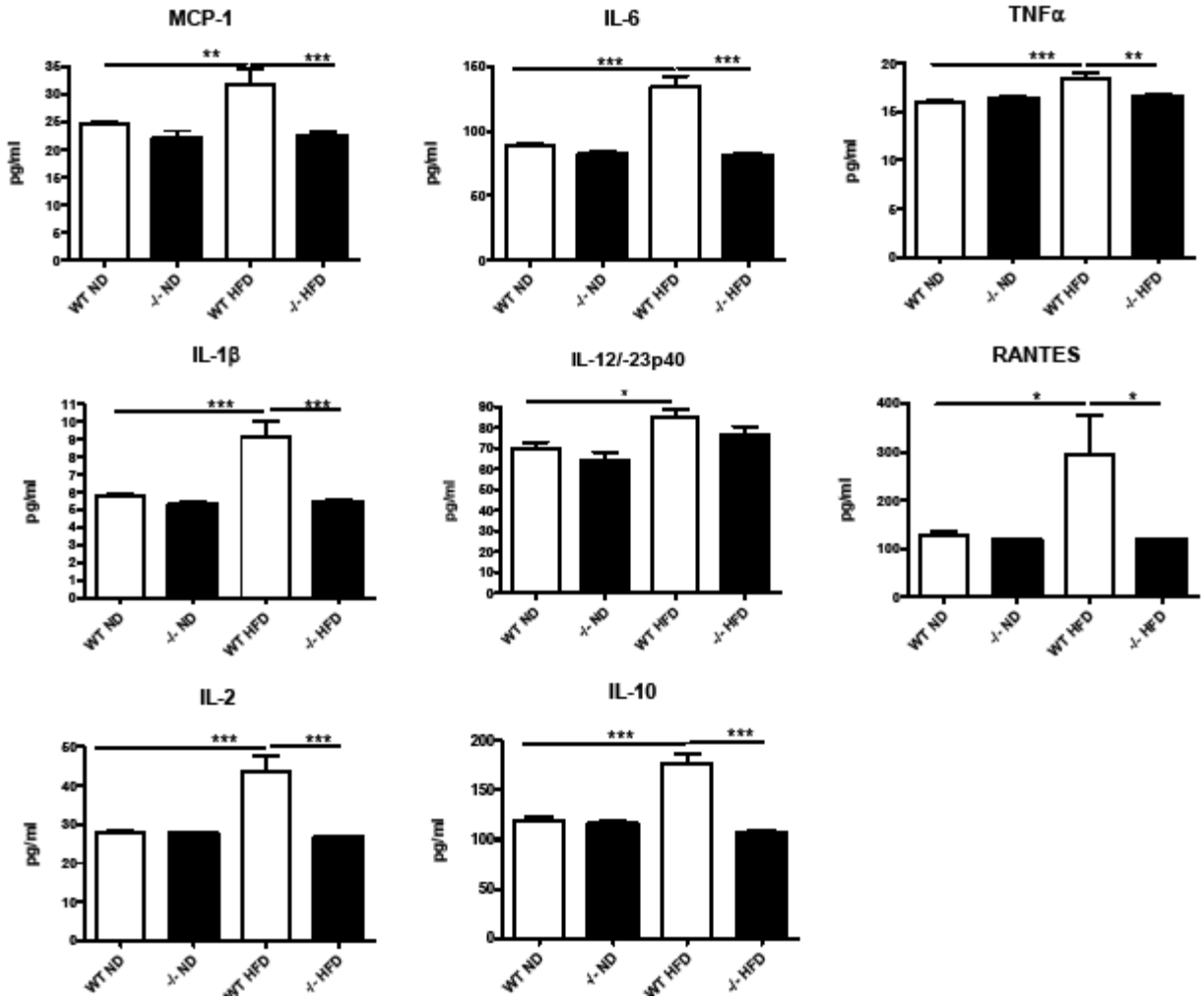

Figure S5. The effect of C3aR deletion on BMDM functions. (A) Bone marrow cells from femurs were collected and differentiated for 5 days in the presence of murine M-CSF. Adherent BMDMs from WT, C3aR$^{+/-}$ and C3aR$^{-/-}$ were untreated (M0), treated with LPS + IFNγ (M1 polarization) or IL-4 + IL-13 (M2 polarization) for 2 days (n=6 per condition). Cytokine secretion (IL-1β, IL-6, TNFα, RANTES and MCP-1) was assessed in cell supernatants. No change in KC/GRO and GM-CSF was seen in any of the groups. Furthermore, both IFNγ and IL-4 (used for polarization) were detected at similar levels in M1 and M2, respectively (data not shown). (B) RNA was extracted from BMDMs and quantitative RT-PCR performed to assess the expression level of M1- (Nos1, Nos2, Marco) and M2- (MRC2) specific genes. F4/80 expression was also determined in these cells. All measurements were done in duplicate. Data presented as mean +/- SEM (n = 6 per group). The following probes from Applied Biosystems were used: cyclophilin B: Mm00478295_m1, F4/80: Mm00802530_m1, CD68: Mm00839636_g1, MCP-1/ CCL2: Mm00441242_m1, IL-6: Mm00446190_m1, Arg2:Mm00477592_m1, Arg1: Mm00475988_m1, C3aR: Mm02620006_s1 and Mm01184110_m1, Nos1: Mm00435175_m1, Nos2: Mm00440485_m1, Marco: Mm00440265_m1, MRC2: Mm00485184_m1. *P < 0.05, **P < 0.01, ***P < 0.001 by ANOVA.

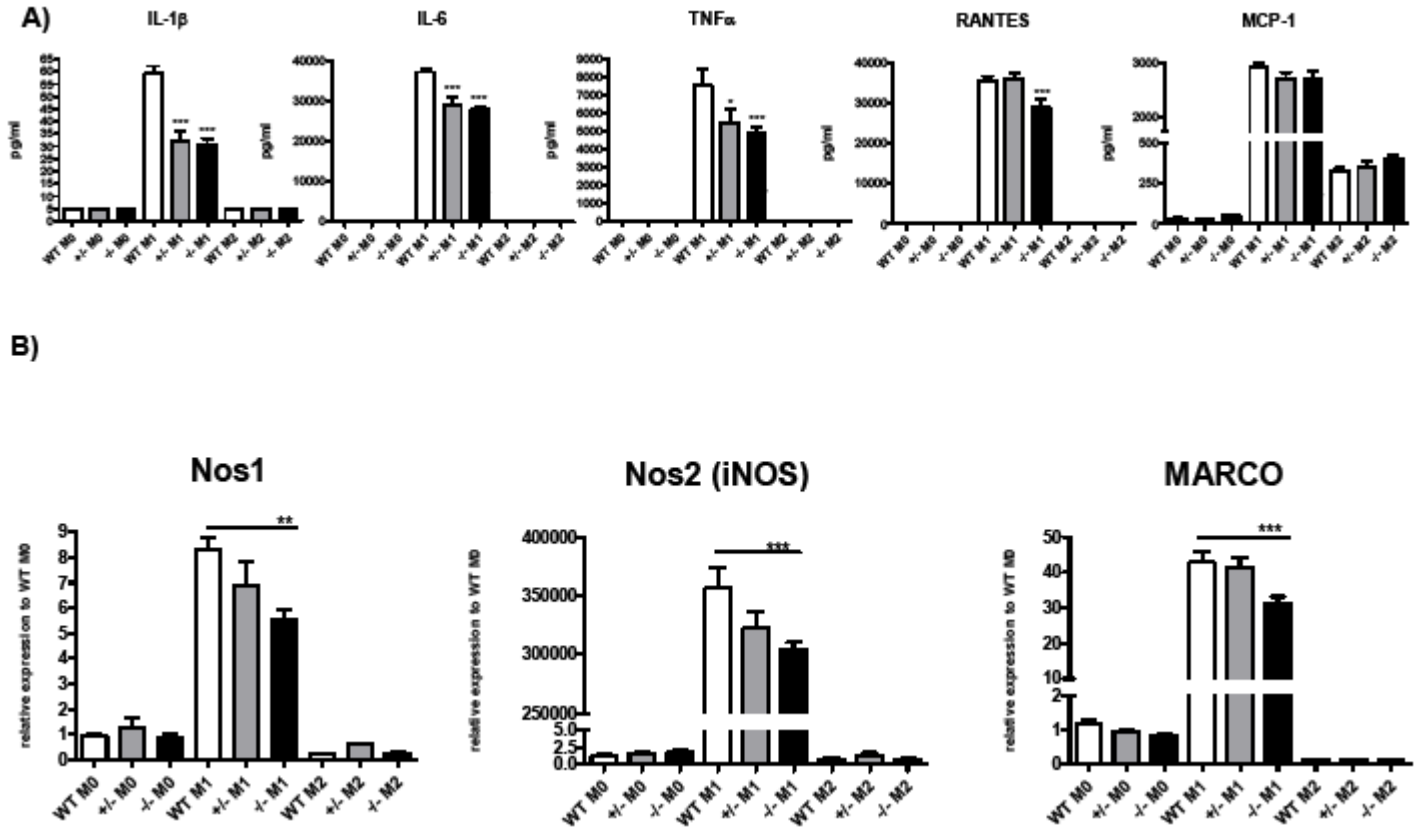

Figure S6. The effect of C3aR deletion on thioglycollate elicited peritoneal macrophages functions. Five week old male WT and C3aR$^{-/-}$ mice (n=5 per group) were injected with 1ml of a sterile 6% thioglycollate solution in the mouse peritoneal cavity. Four days later, mice were euthanized and cells collected by washing peritoneal cavity. Cell were resuspend in complete media and plated at 5 X 10^5 macrophages/6 well (n=5 per condition). (A) Cells from peritoneal lavages were plated and allowed to adhere for 2hrs. Adherent cells (peritoneal macrophages) from WT and C3aR-/- were untreated (M0), treated with LPS + IFNγ (M1 polarization) or IL-4 + IL-13 (M2 polarization) for 2 days (n=5 per condition). Cytokine secretion (IL-6, TNFα, RANTES and MCP-1) was assessed in cell supernatants. (B) RNA was extracted from peritoneal macrophages and quantitative RT-PCR performed to assess the expression level of M1- (Nos1, Nos2, Marco) specific genes. All measurements were done in duplicate. Data presented as mean +/- SEM (n = 5 per group). Probes used described in Figure S6 legend. *P < 0.05, **P < 0.01, ***P < 0.001 by ANOVA.

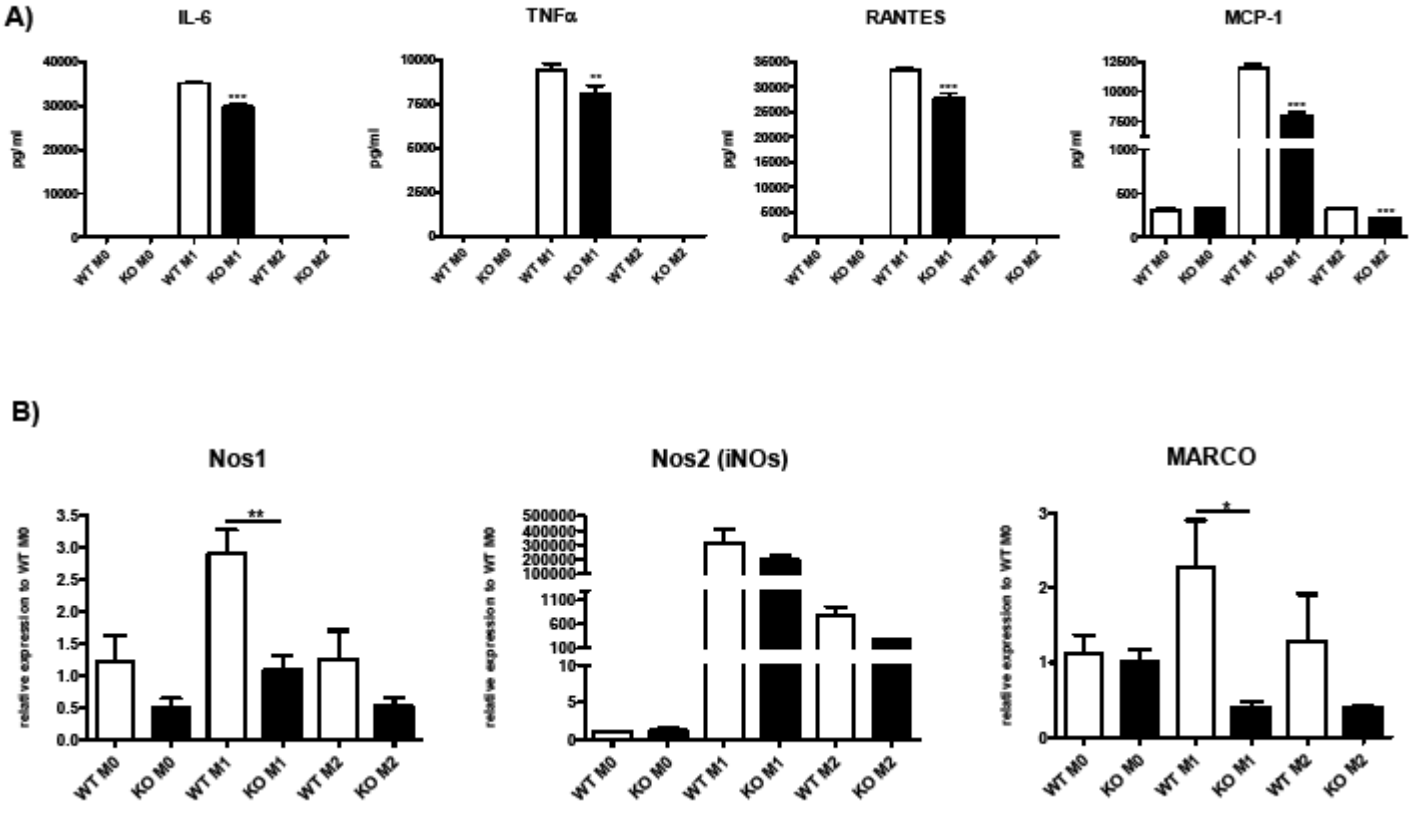

Figure S7. Body weight and food intake in C3aR-/- mice on ND and HFD in a second DIO study. A second cohorts of C3aR$^{-/-}$ and WT mice (n=6 per group) were initially age-matched (5-7 weeks old). Absolute body weight and food intake in grams over the 6 week period was measured.

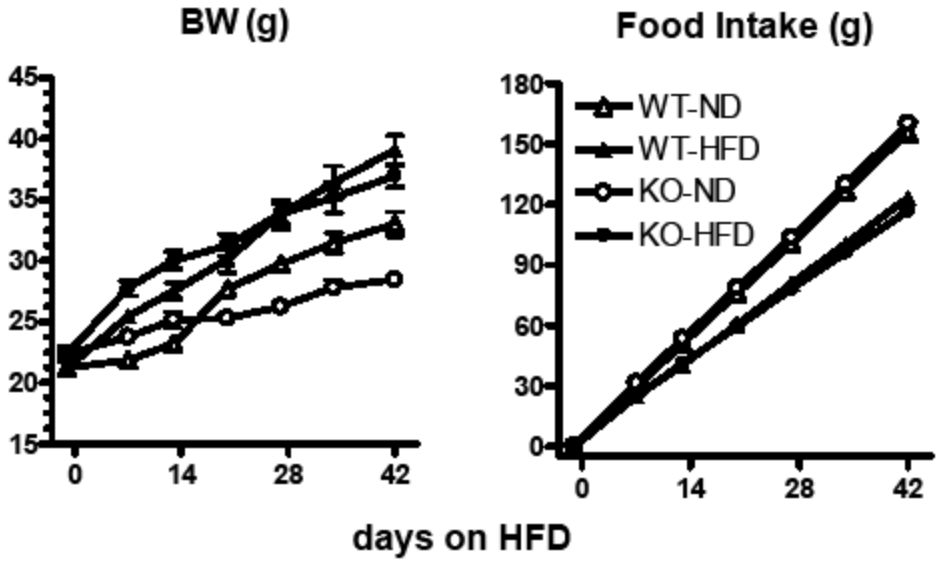

Figure S8. C3aR mRNA expression in mouse cell lines. Total RNA was extracted from 3T3-L1 adipocytes, macrophage cell lines (J774, RAW264.7, MHS) and SVC fraction from HFD WAT. Quantitative RT-PCR for C3aR mRNA expression was performed and normalized to cyclophilin B. Each PCR reaction was performed in duplicate.