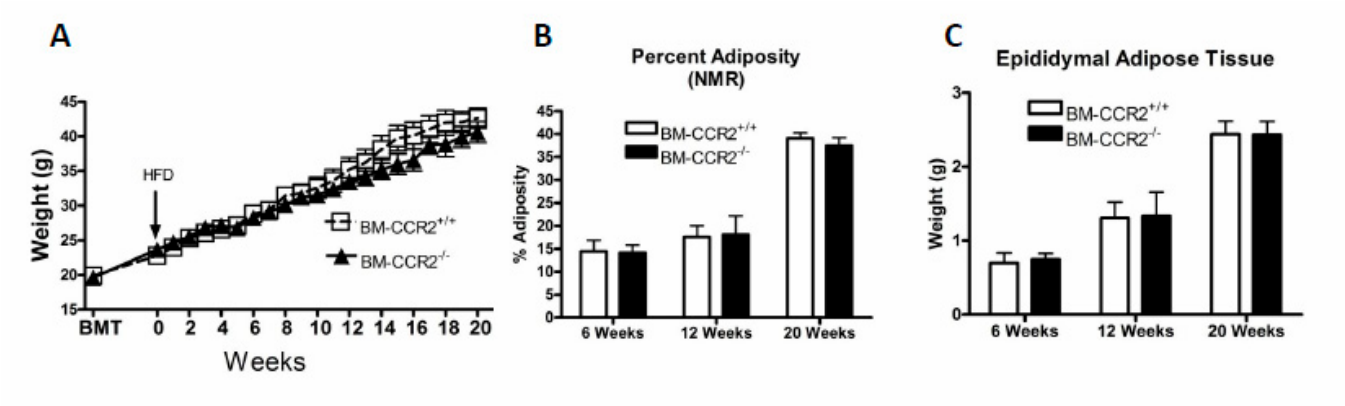
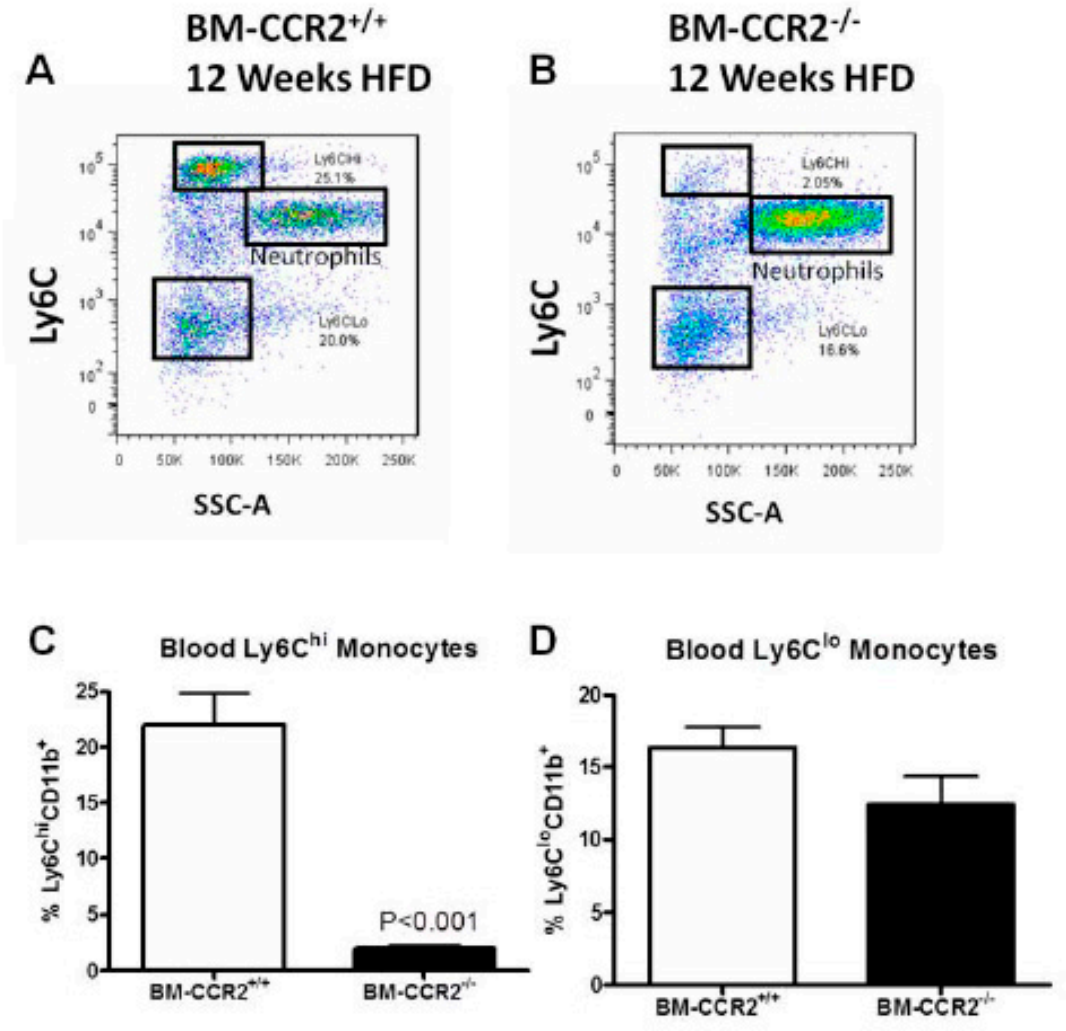
SUPPLEMENTARY DATA

Supplementary Figure 1. BM-CCR2$^{-/-}$ and BM-CCR2$^{+/+}$ mice have similar metabolic Parameters. A) Weight gain curves of BM-CCR2$^{+/+}$ and BM-CCR2$^{-/-}$ mice during 20 weeks of HFD feeding (mean ± SEM; n = 9 mice per group). B) Percent adiposity in BM-CCR2$^{+/+}$ and BM-CCR2$^{-/-}$ mice after 6, 12 and 20 weeks on a HFD (mean ± SEM; n = 7-10 mice per group). Percent adiposity was calculated by dividing total AT mass, as assessed by NMR, by body weight. C) Epididymal AT pad weight after 6, 12 and 20 weeks on a HFD (mean ± SEM; n = 7-10 mice per group).



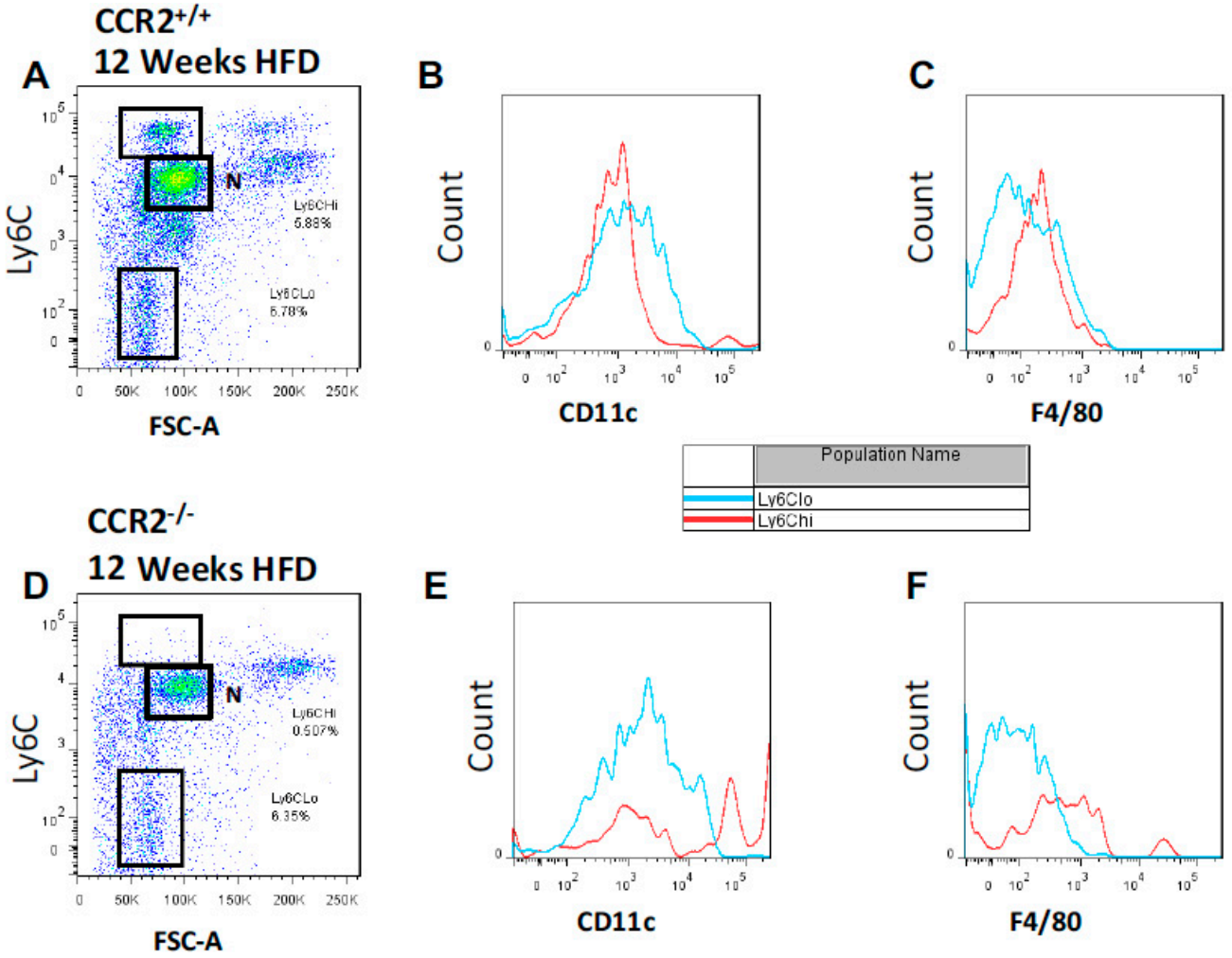
SUPPLEMENTARY DATA

Supplementary Figure 2. BM-CCR2$^{-/-}$ mice have decreased levels of circulating inflammatory monocytes. White blood cells were isolated from BM-CCR2$^{+/+}$ and BM-CCR2$^{-/-}$ mice fed an HFD for 12 weeks. Circulating monocyte levels were assessed by flow cytometry by first gating on all live CD11b^{+} cells. Representative pseudo-color flow cytometry plot of Ly6C expression in (*A*) BM-CCR2$^{+/+}$ controls and (*B*) BM-CCR2$^{-/-}$ mice. Quantification of the percentage of circulating (*C*) Ly6C^{hi} monocytes and (*D*) Ly6C^{lo} monocytes in BM-CCR2$^{+/+}$ and BM-CCR2$^{-/-}$ mice (mean ± SEM; n = 4 mice per group).



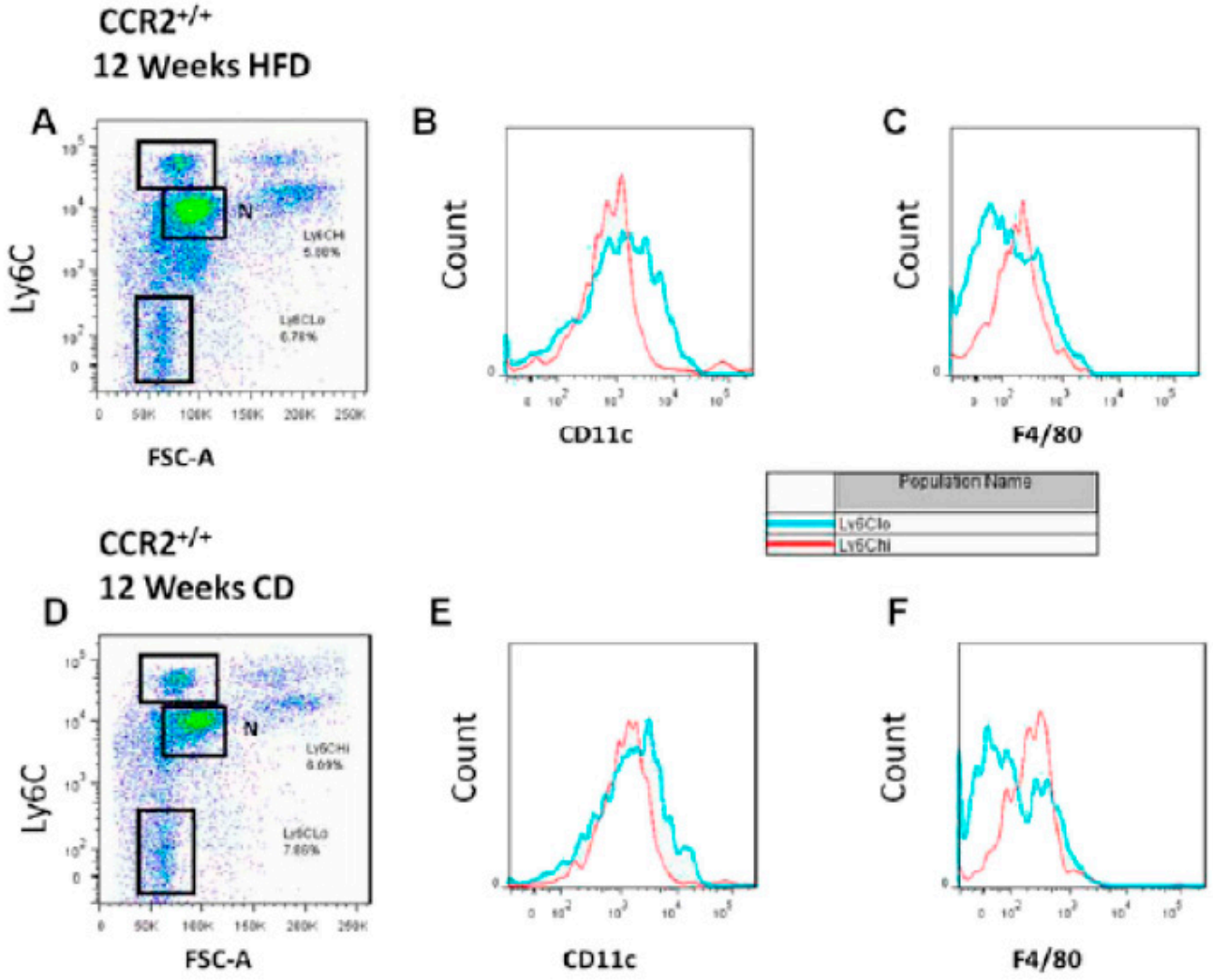
SUPPLEMENTARY DATA

Supplementary Figure 3. CCR2$^{-/-}$ mice have decreased levels of blood Ly6C^{hi} monocytes and increased expression of CD11c in Ly6C^{lo} monocytes. White blood cells were isolated from CCR2$^{+/+}$ and CCR2$^{-/-}$ mice fed an HFD for 12 weeks. Circulating monocyte levels were assessed by flow cytometry by first gating on all live CD11b^{+} cells. *A*: Gates on Ly6C^{hi} and Ly6C^{lo} populations in CCR2$^{+/+}$ mice. *B*: Histogram of CD11c expression in Ly6C^{hi} (red line) and Ly6C^{lo} (blue line) monocytes in CCR2$^{+/+}$ mice. *C*: Histogram of F4/80 expression in Ly6C^{hi} (red line) and Ly6C^{lo} (blue line) monocytes in CCR2$^{+/+}$ mice. *D*: Gates on Ly6C^{hi} and Ly6C^{lo} populations in CCR2$^{-/-}$ mice. *E*: Histogram of CD11c expression in Ly6C^{hi} (red line) and Ly6C^{lo} (blue line) monocytes in CCR2$^{-/-}$ mice. *F*: Histogram of F4/80 expression in Ly6C^{hi} (red line) and Ly6C^{lo} (blue line) monocytes in CCR2$^{-/-}$ mice. "N" in panels *A* and *D* indicates neutrophil populations.



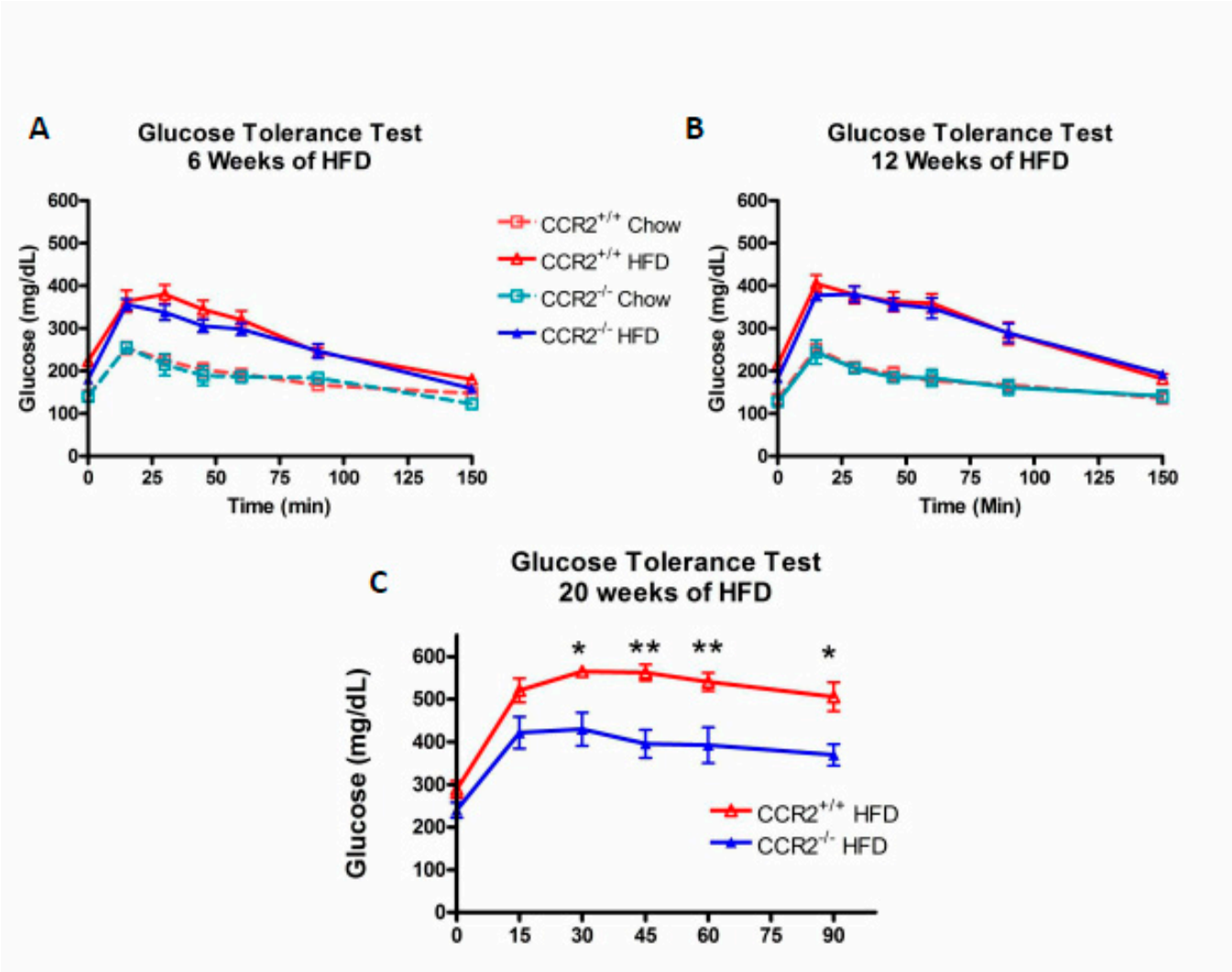
SUPPLEMENTARY DATA

Supplementary Figure 4. HFD feeding increases the numbers and the CD11c expression of Ly6C^{hi} monocytes in CCR2$^{+/+}$ mice. White blood cells were isolated from CCR2$^{+/+}$ mice fed an HFD or CD for 12 weeks. Circulating monocyte levels were assessed by flow cytometry by first gating on all live CD11b^{+} cells. *A*: Gates on Ly6C^{hi} and Ly6C^{lo} populations in HFD-fed CCR2$^{+/+}$ mice. *B*: Histogram of CD11c expression in Ly6C^{hi} (red line) and Ly6C^{lo} (blue line) monocytes in HFD-fed CCR2$^{+/+}$ mice. *C*: Histogram of F4/80 expression in Ly6C^{hi} (red line) and Ly6C^{lo} (blue line) monocytes in HFD-fed CCR2$^{+/+}$ mice. *D*: Gates on Ly6C^{hi} and Ly6C^{lo} populations in CD-fed CCR2$^{+/+}$ mice. *E*: Histogram of CD11c expression in Ly6C^{hi} (red line) and Ly6C^{lo} (blue line) monocytes in CD-fed CCR2$^{+/+}$ mice. *F*: Histogram of F4/80 expression in Ly6C^{hi} (red line) and Ly6C^{lo} (blue line) monocytes in CD-fed CCR2$^{+/+}$ mice. "N" in panels *A* and *D* indicates neutrophil populations.



SUPPLEMENTARY DATA

Supplementary Figure 5. HFD fed CCR2$^{-/-}$ mice have delayed improvements in glucose tolerance. Glucose tolerance curves of mice injected with 1 g of dextrose per kg of lean body mass. A) GTT curve of CCR2$^{+/+}$ and CCR2$^{-/-}$ mice after 6 weeks on either a HFD or CD (mean ± SEM; n = 3-9 mice per group). Both CD groups are significantly more glucose tolerant than both HFD groups ($P<0.05$). B) GTT curve of CCR2$^{+/+}$ and CCR2$^{-/-}$ mice after 12 weeks on either a HFD or CD (mean ± SEM; n = 2-8 mice per group). Both CD groups are significantly more glucose tolerant than both HFD groups ($P<0.05$). C) GTT curve of CCR2$^{+/+}$ and CCR2$^{-/-}$ mice after 20 weeks on either a HFD (mean ± SEM; n = 4 mice per group).

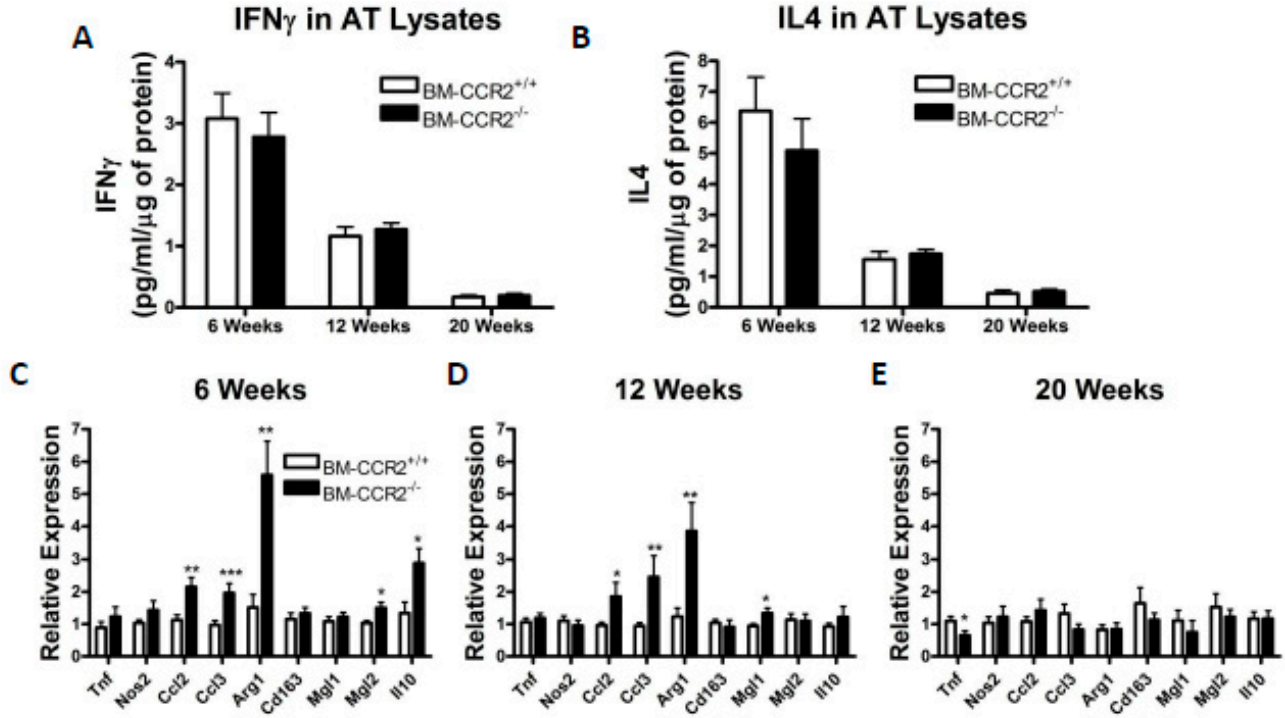


*P<0.05, **P<0.01 compared to CCR2$^{+/+}$ at respective time point

SUPPLEMENTARY DATA

Supplementary Figure 6. BM-CCR2$^{-/-}$ mice have similar levels of IFNγ and IL4 protein in AT to those of BM-CCR2$^{+/+}$ controls after all periods of HFD

A) Levels of IFNγ protein in AT lysates were measured by ELISA (mean ± SEM; n = 7-10 mice per group). B) Levels of IL4 protein in AT lysates were measured by ELISA (mean ± SEM; n = 6-7 mice per group). C-E) RNA was isolated from AT and used for gene expression analysis by real-time RT-PCR. The mRNA expression of *Tnf*, *Nos2*, *Ccl2*, *Ccl3*, *Arg1*, *Cd163*, *Mgl1*, *Mgl2* and *Il10* was assessed after 6, 12 and 20 weeks of HFD feeding and plotted as expression relative to the BM-CCR2$^{+/+}$ group of each time point (mean ± SEM; n = 7-10 mice per group).

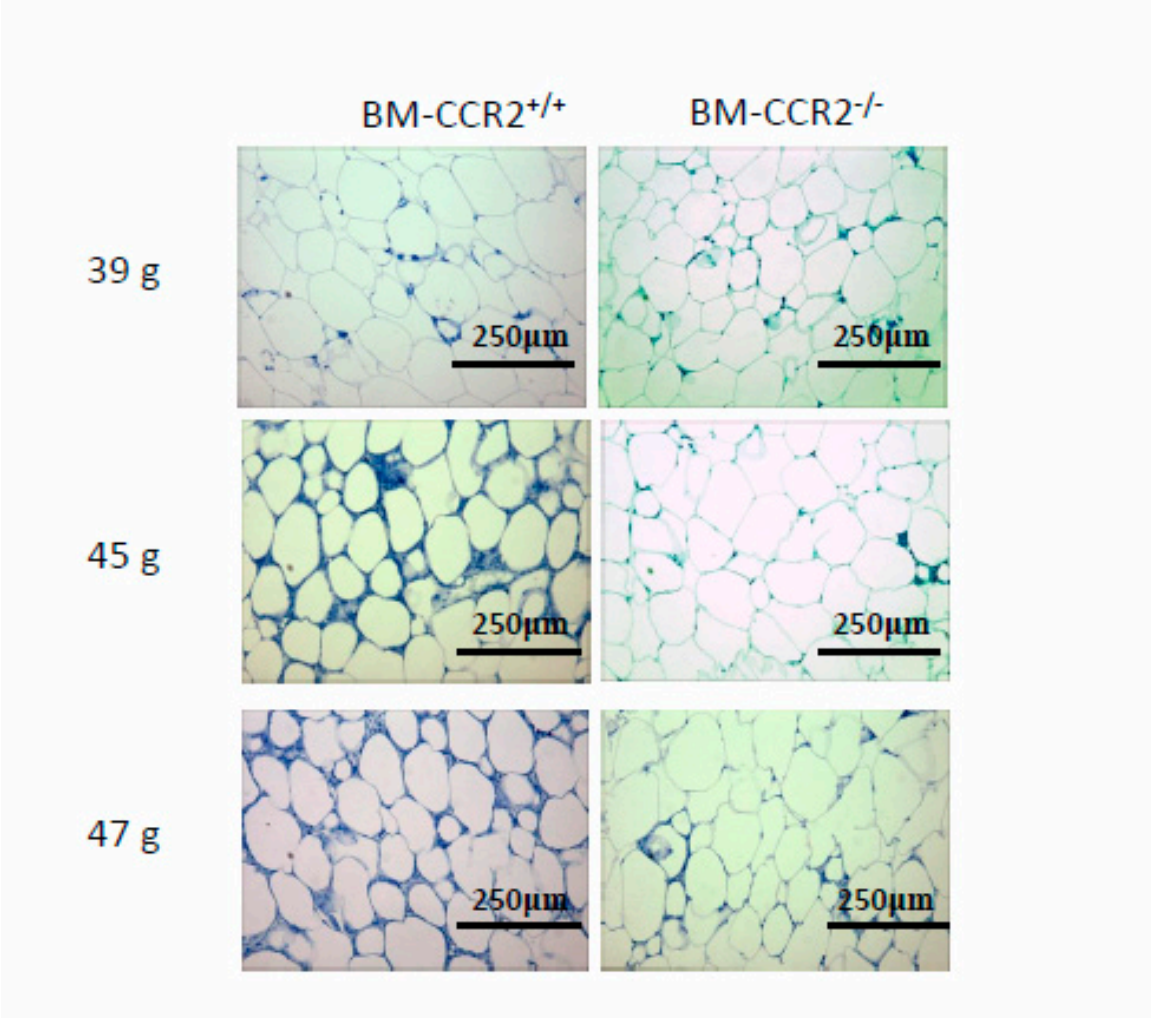


*P<0.05 compared to the BM-CCR2$^{+/+}$ group
**P<0.01 compared to the BM-CCR2$^{+/+}$ group
***P<0.001 compared to the BM-CCR2$^{+/+}$ group

SUPPLEMENTARY DATA

Supplementary Figure 7. BM-CCR2$^{-/-}$ mice have lower immune infiltration than weight-matched BM-CCR2$^{+/+}$ mice.

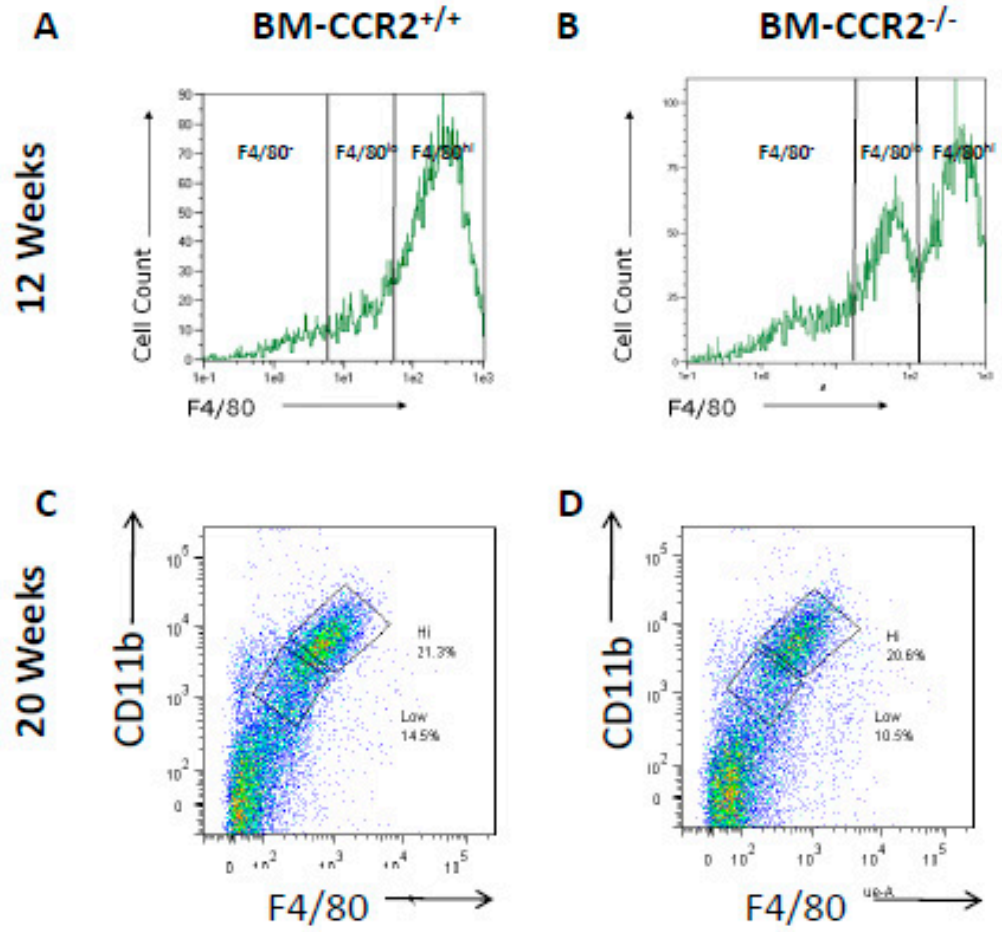
AT was collected from weight matched BM-CCR2$^{+/+}$ and BM-CCR2$^{-/-}$ mice fed HFD for 20 weeks and used to assess macrophage infiltration by histological analysis. Representative TBO-stained AT pictures of weight-matched BM-CCR2$^{+/+}$ and BM-CCR2$^{-/-}$ mice are presented.



SUPPLEMENTARY DATA

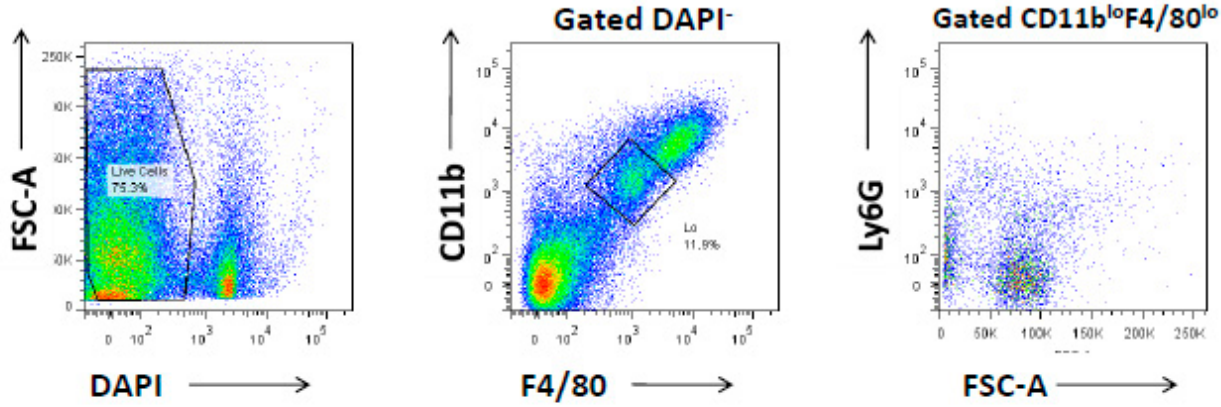
Supplementary Figure 8. BM-CCR2$^{-/-}$ mice have a unique CD11bF4/80^{lo} population after 12 weeks of HFD feeding.

A) Representative flow cytometry histogram of F4/80-expressing populations in A) BM-CCR2$^{+/+}$ and B) BM-CCR2$^{-/-}$ mice after 12 weeks of HFD feeding. Representative flow cytometry pseudo-color plot for C) BM-CCR2$^{+/+}$ controls and D) BM-CCR2$^{-/-}$ mice after 20 weeks of HFD gated on all live cells.



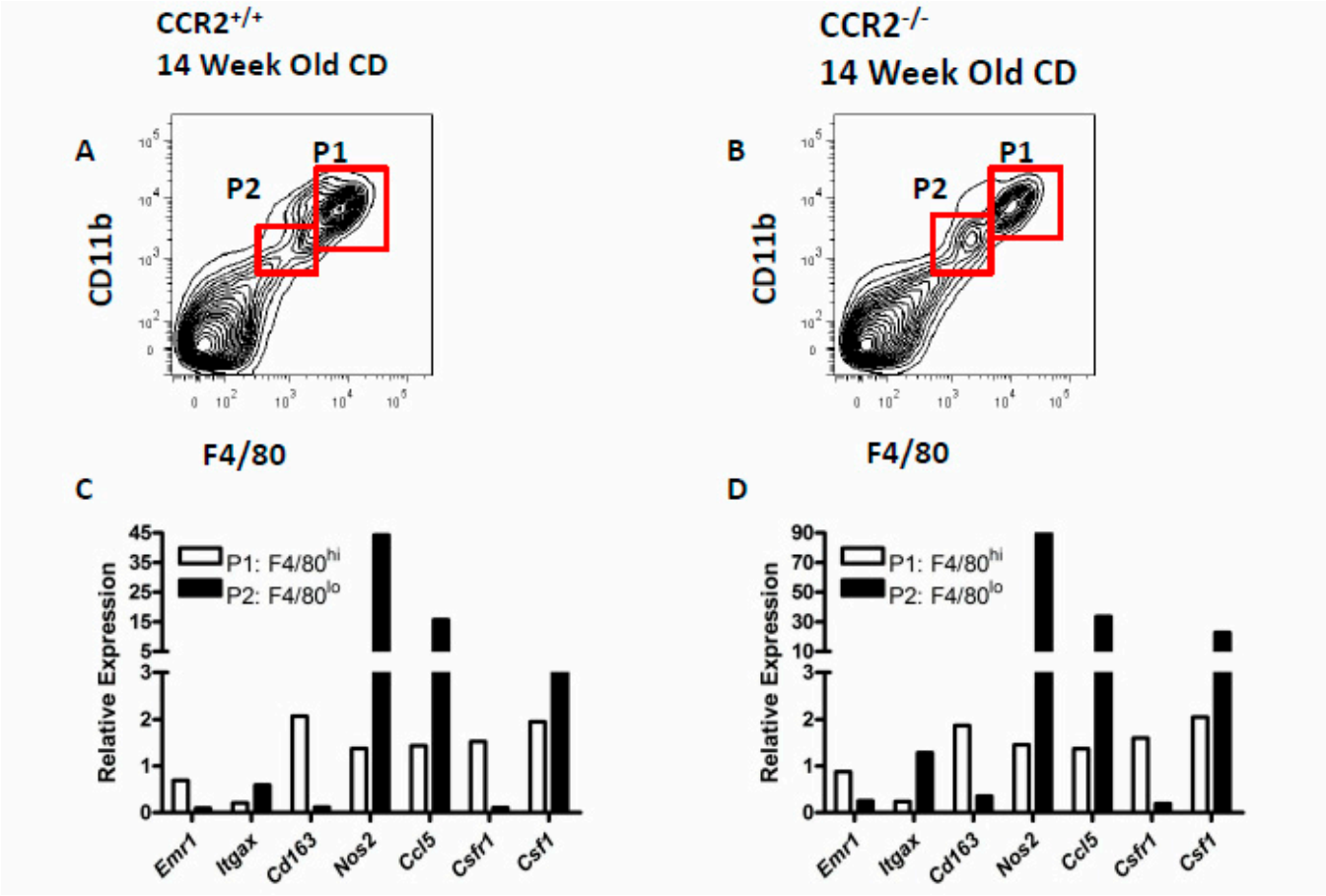
SUPPLEMENTARY DATA

Supplementary Figure 9. **CD11b^{lo}F4/80^{lo} cells have a Ly6G^{-} immunophenotype**
SVCs were isolated from the AT of CCR2$^{-/-}$ mice fed a HFD for 10 weeks. Representative image of gating strategy used to assess the expression of Ly6G in the CD11b^{lo}F4/80^{lo} cells.



SUPPLEMENTARY DATA

Supplementary Figure 10. CD11b^{lo}F4/80^{lo} cells are found in the AT of CD fed CCR2$^{+/+}$ and CCR2$^{-/-}$ mice. SVCs were isolated from 14 weeks old CD fed CCR2$^{+/+}$ and CCR2$^{-/-}$ mice. Gating strategy used for FACS sorting of CD11b^{lo}F4/80^{lo} and CD11b^{hi}F4/80^{hi} cells in A) CCR2$^{+/+}$ mice and B) CCR2$^{-/-}$ mice. B) Gene expression of key CD11b^{lo}F4/80^{lo} genes relative to CD11b^{hi}F4/80^{hi} cells in C) CCR2$^{+/+}$ mice and D) CCR2$^{-/-}$ mice (n = 3 pooled biological samples).



SUPPLEMENTARY DATA

Supplementary Figure 11. CD11b^{lo}F4/80^{lo} cells accumulate in the peritoneum of thioglycollate-injected mice.

Peritoneal cells were isolated from CCR2$^{-/-}$ and CCR2$^{+/+}$ mice 72 h post-thioglycollate injection. These cells were analyzed by flow cytometry. A) Flow cytometry plot of CD11b and F4/80 expression in the peritoneal cells of CCR2$^{-/-}$ mice (representative image of 5 different biological samples). B) Flow cytometry plot of CD11b and F4/80 expression in the peritoneal cells of CCR2$^{+/+}$ mice (representative image of 5 different biological samples).