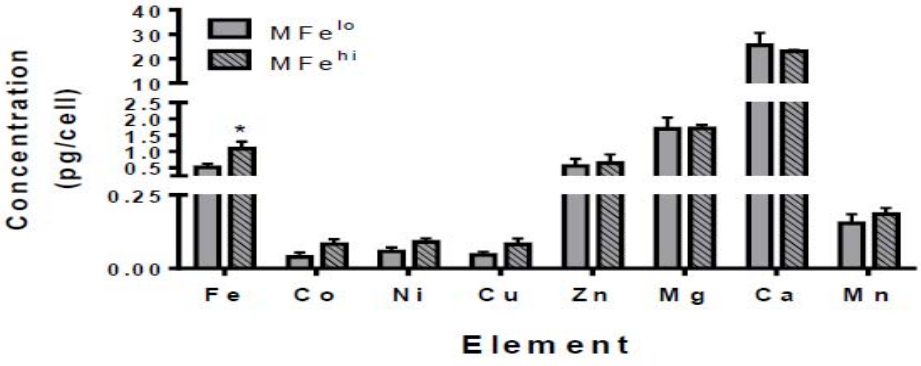
SUPPLEMENTARY DATA

**Supplementary Table 1**. Comparison of serum iron parameters in LFD and HFD mice.

| Parameters (units) | LFD (± SEM) | HFD (± SEM) |
|---|---|---|
| Iron (μg/dL) | 110.0 (10.9) | 117.6 (10.5) |
| Transferrin (mg/dL) | 65.8 (5.0) | 87.6 (1.9)** |
| Ferritin (ng/ml) | 214.4 (10.9) | 247.1 (7.4)* |
| Transferrin Saturation (%) | 49.8 (4.3) | 36.3 (3.8)* |
| TIBC | 233.5 (11.9) | 316.0 (16.9)** |

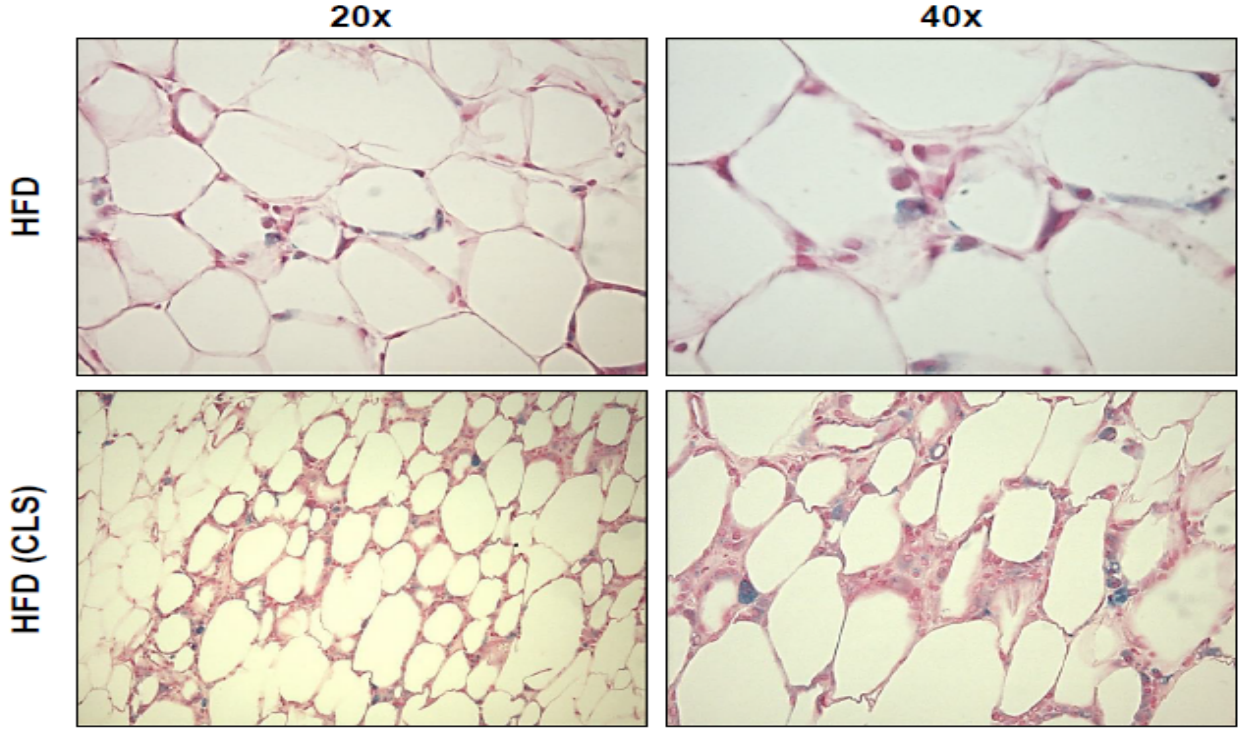
TIBC = total iron binding capacity, n=8/gp, *$P$<0.05, ** $P$<0.01

**Supplementary Figure 1.** Elemental concentrations in $MFe^{hi}$ and $MFe^{lo}$ cells. $MFe^{hi}$ and $MFe^{lo}$ cells were isolated from male, chow diet-fed, C57BL/6 mice. Cells were prepared for ICP-MS as described in the Methods section. Quantification of iron, cobalt, nickel, copper, zinc, magnesium, calcium, and manganese was performed. Data are presented as the mean ± SEM (n=6/group, *P<0.05).



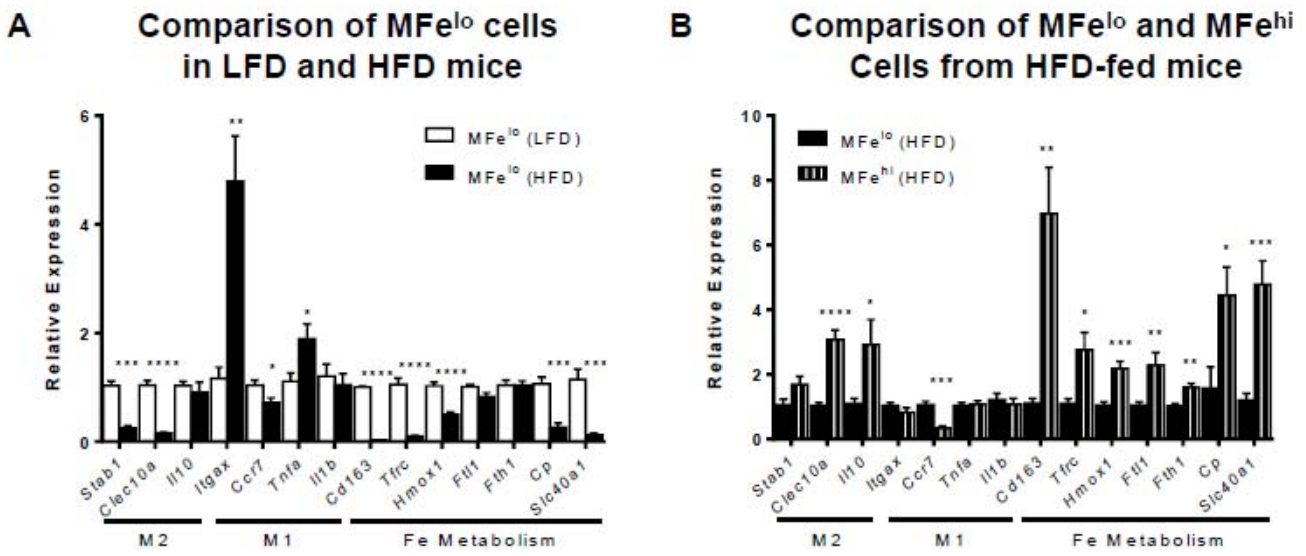
SUPPLEMENTARY DATA

**Supplementary Figure 2.** Localization of $MFe^{hi}$ ATMs in epididymal fat pads of HFD mice. Mice were perfused with Perls' prussian blue staining solution, and sections were counterstained with nuclear fast red. $MFe^{hi}$ ATMs are stained blue. Sections from HFD mice are displayed in the top row and areas of AT from HFD mice containing crown-like structures (CLS) are shown in the bottom row (left column = 20x; right column = 40x magnification).



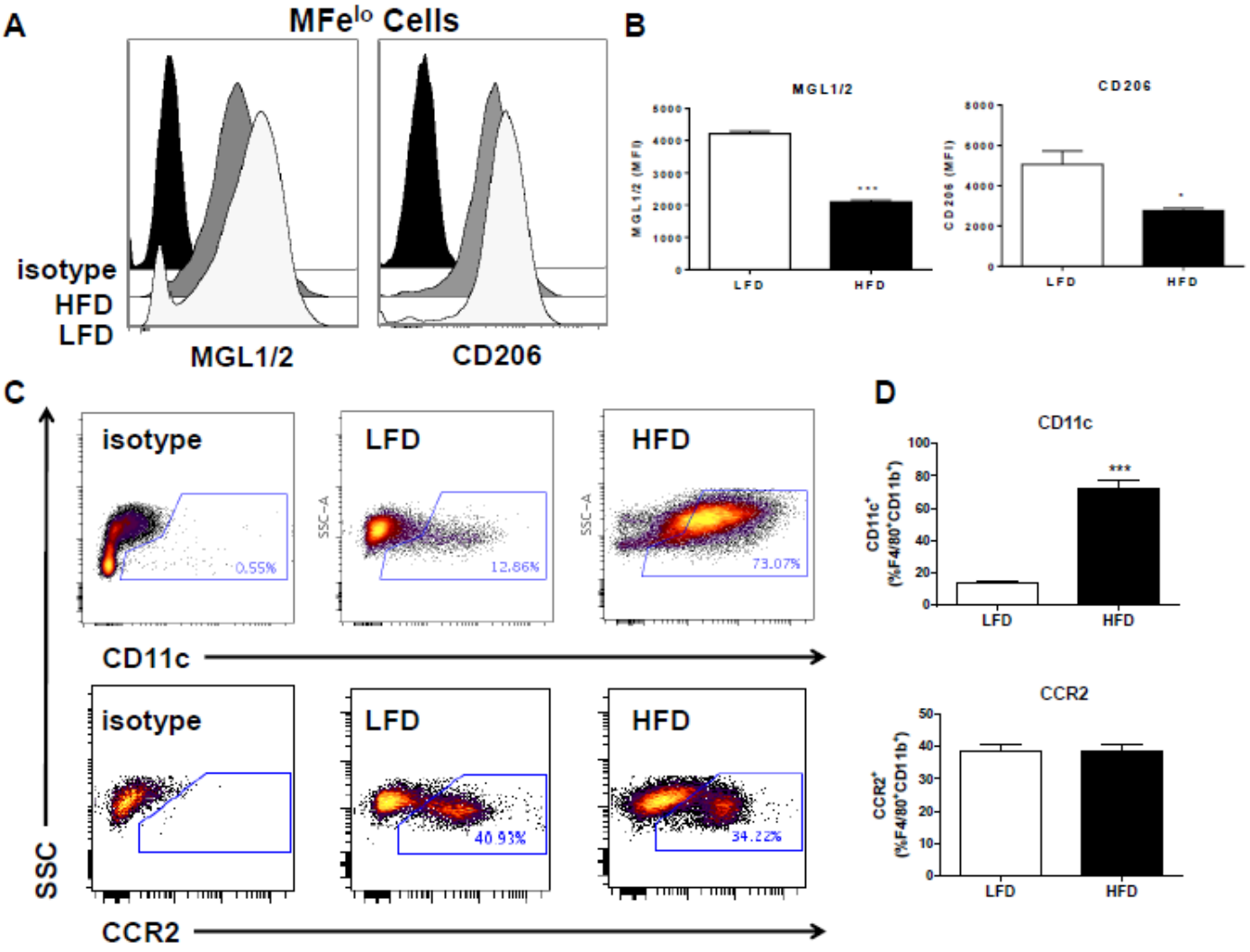
SUPPLEMENTARY DATA

**Supplementary Figure 3.** $MFe^{lo}$ and $MFe^{hi}$ ATM gene expression. (A) Obesity reduces M2 marker and iron metabolism gene expression, and increases M1 marker gene expression by $MFe^{lo}$ ATMs. $MFe^{lo}$ ATMs were isolated from LFD and HFD mice and analyzed by real time rtPCR for M2/M1 and iron metabolism gene expression ($n$ = 6-7/group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$). (B) $MFe^{hi}$ ATMs from HFD mice are M2 polarized and display increased iron metabolism gene expression relative to $MFe^{lo}$ ATMs. Gene expression in $MFe^{lo}$ and $MFe^{hi}$ ATMs isolated from the epididymal fat pads of HFD mice ($n$ = 4-7/group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$).



SUPPLEMENTARY DATA

**Supplementary Figure 4.** FACS analysis of MFe$^{lo}$ cells. M2 marker expression by viable F4/80$^{hi}$CD11b$^{hi}$, MFe$^{lo}$ ATMs was measured by flow cytometry. Isotype controls, HFD MFe$^{lo}$ ATMs, and LFD MFe$^{lo}$ ATMs, are represented by black, gray, and white histograms, respectively. (**B**) Quantification and comparison of MGL1/2 (left panel) and CD206 (right panel) expression by LFD and HFD MFe$^{lo}$ ATMs ($n$ = 3/group for each marker, *$P$ < 0.05, ***P=0.001). (**C**) Obesity increases the proportion of MFe$^{lo}$ ATMs expressing the M1 marker, CD11c (top row) but not CCR2 (bottom row). M1 marker expression by viable F4/80$^{hi}$CD11b$^{hi}$, MFe$^{hi}$ ATMs was measured by flow cytometry. Representative FACS plots of isotype controls, LFD MFe$^{lo}$ ATMs, and HFD MFe$^{lo}$ ATMs, are shown in the left, middle, and right columns, respectively. (**D**) Quantification and comparison of the number of CD11c$^{+}$ (top panel) and CCR2$^{+}$ (bottom panel) MFe$^{lo}$ ATMs as a percent of total viable F4/80$^{hi}$CD11b$^{hi}$, MFe$^{lo}$ ATMs in LFD and HFD mice ($n$ = 2-3/group for each marker, ***$P$ < 0.001).



SUPPLEMENTARY DATA

**Supplementary Figure 5.** Dietary effects on ATM iron content and splenic iron concentrations. (A) Comparison of $MFe^{lo}$ and $MFe^{hi}$ ATM iron content from NCD, LFD, and HFD mice. $MFe^{lo}$ and $MFe^{hi}$ ATMs were isolated via sequential magnetic and FACS sorting, and cellular iron content was quantified by ICP-MS ($n$ = 4-6/group, $P < 0.05$ for groups not connected by same letter). (B) Visual comparison of splenic iron content between LFD and HFD mice. Mice were perfused with Perls' prussian blue staining solution, and tissues were removed 1 hr post perfusion. (C) Comparison of splenic iron concentrations between LFD and HFD mice. Splenic iron content was quantified via atomic absorption spectrometry and expressed relative to total protein.