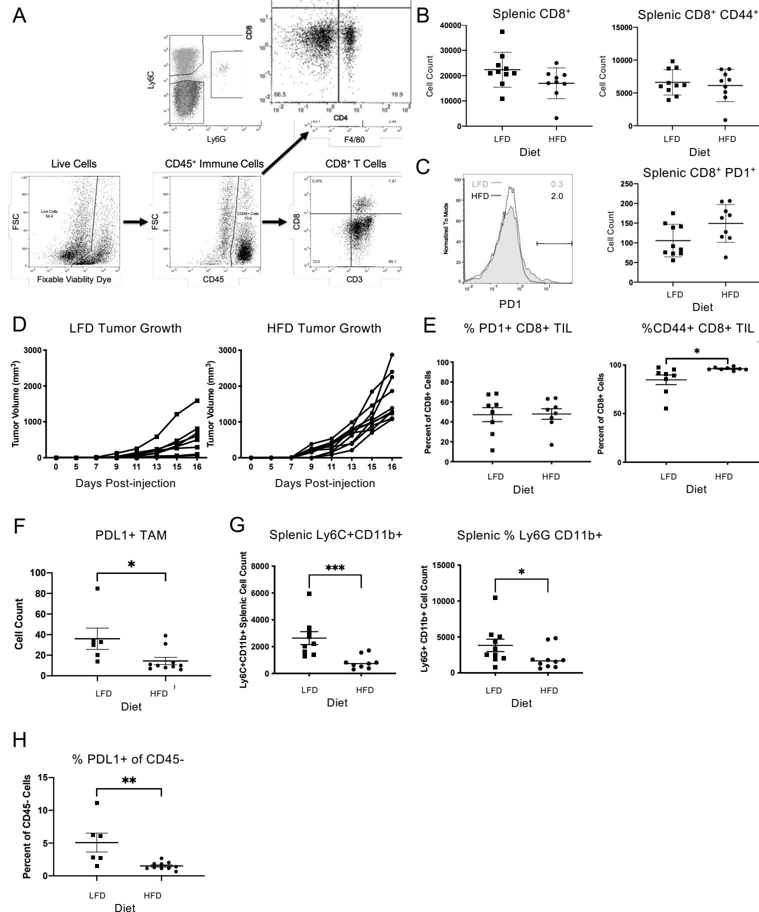
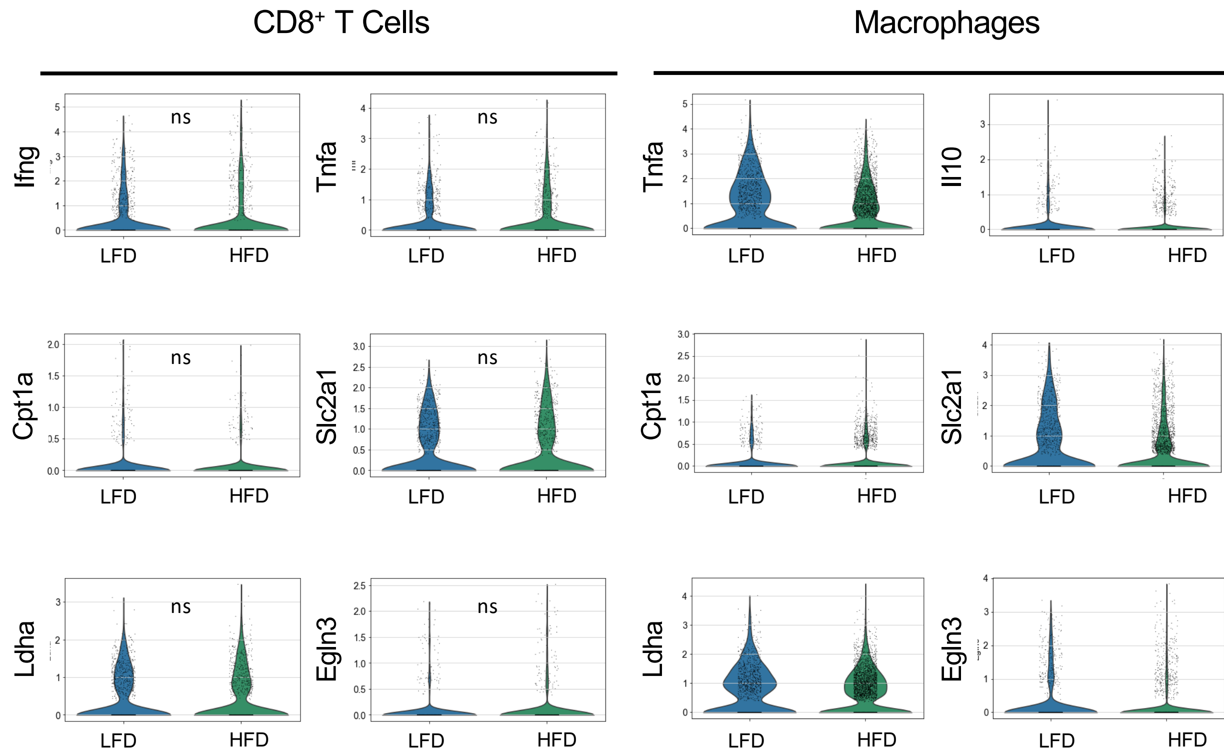
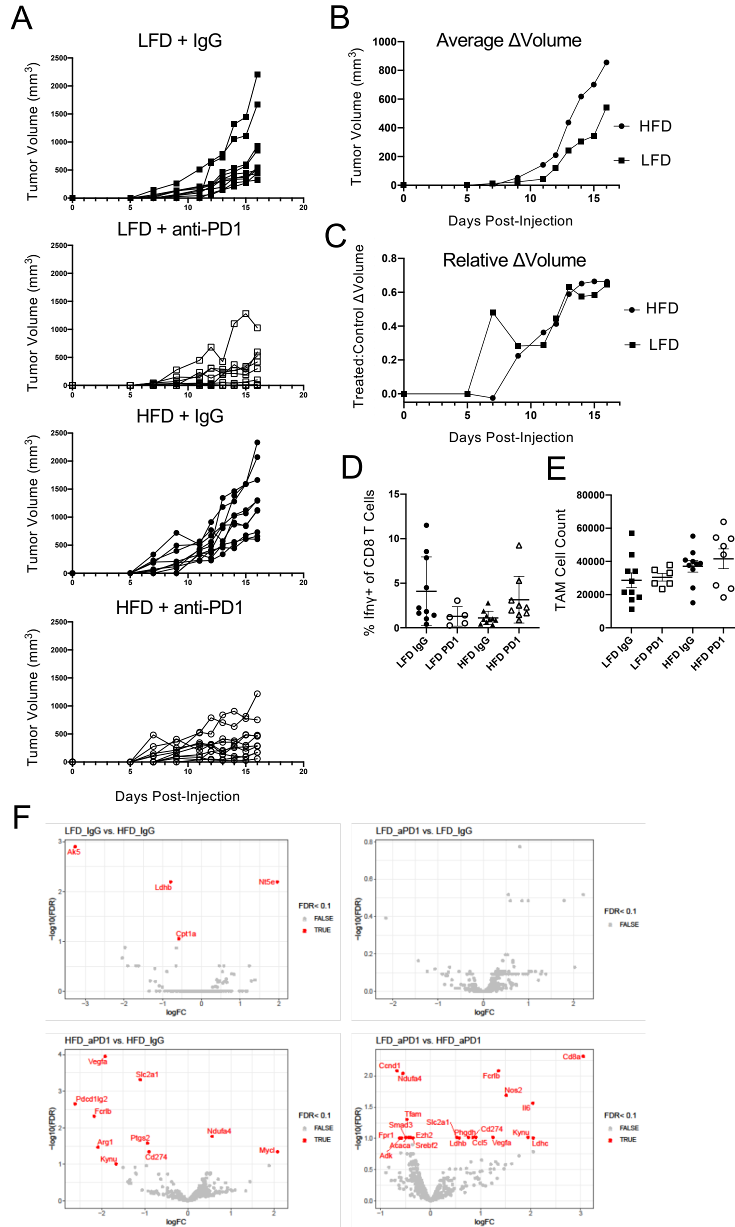


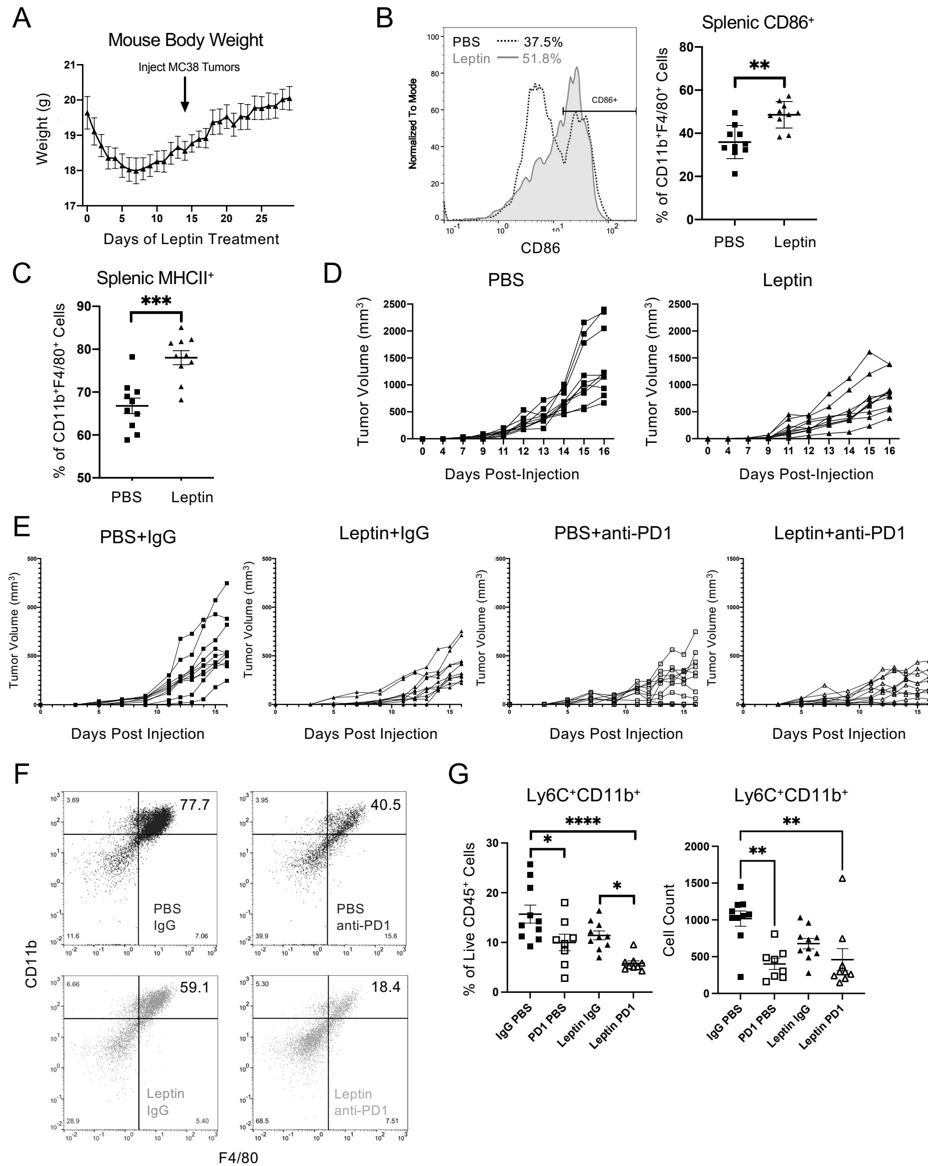
Supplementary Figure 1. Immune phenotyping of obesity and MC38 tumors. A. To identify immune cell populations, a thorough flow cytometry gating strategy was used. First, live cells were selected as those that stained negatively with the fixable viability dye. Then, immune live cells were selected as those positive for $CD45^{+}$. Last, different immune cells were identified by selecting macrophages as CD11b and F4/80 double positive cells and cytotoxic cells as $CD8^{+}$ and $CD3^{+}$ double positive cells **B-C.** Five-week-old C57BL/6 male mice were maintained on a control standard chow diet (n=8) or 60kcal high-fat diet (n=8) for 12 weeks and were weighed weekly. Two-way ANOVA with Tukey post-hoc test *p* values used. Spleens from mice on their respective diet for 12 weeks were processed into single cell suspensions and were analyzed by flow cytometry for cytotoxic T cells. Representative flow plots and frequency from low-fat diet (LFD) (n=10) and high-fat diet (HFD) (n=9) mouse spleens. **B.** Cell counts of $CD8^{+}$ splenic T cells and of $CD44^{+}$ $CD8^{+}$ splenic T cells. **C.** Representative MFI expression and absolute cell count of $PD1^{+}$ $CD8^{+}$ splenic T cells. **D.** C57BL/6 male mice on a control standard chow diet (n=10) or 60kcal high-fat diet (n=10) for 12 weeks were injected with 105 MC38-CEA1 cells in the right flank. Individual tumor growth over time from respective LFD and HFD treatment groups. **E.** Tumors from mice with MC38-CEA1 tumors were collected 16 days post-injection and were processed single cell suspensions, before analyzing by flow cytometry. Frequency of $PD1^{+}$ $CD8^{+}$ TILs and $CD44^{+}$ $CD8^{+}$ TILs. **F.** Absolute cell count of $PDL1^{+}$ TAMs **G.** Cell count of $Ly6C^{+}CD11b^{+}$ monocytic and $Ly6G^{+}CD11b^{+}$ granulocytic myeloid derived suppressor cells from the spleens of tumor bearing LFD mice (n=10) and HFD mice (n=10). **H.** Frequency of $PDL1^{+}$ CD45- cells (non-immune cells) following diet treatment. Data are shown as mean± S.E.M., with all individual points shown. Two-tailed Mann Whitney test *p* values shown. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.



Supplementary Figure 2. Single cell RNA sequencing of immune cell populations in MC38 lean and obese tumors. C57BL/6 male mice on a control standard chow diet (n=10) or 45kcal high-fat diet (n=10) for 25 weeks were injected with 2 x 10^5 MC38-CEA1 cells in the right flank. Tumors were processed on Day 14 post injection and live $CD45^+$ cells were isolated and submitted for scRNA sequencing. Singe Cell Gene Expression violin plots of $CD8^+$ or TAM immune subsets designated using SingleR program. Lack of statistical significance is indicated by “ns” while the other plots were at least $p<0.05$ significant following corrections.



Supplementary Figure 3. Anti-PD-1 and MC38 tumor growth and immune phenotyping. C57BL/6 male mice on a control LFD (n=10) or 60kcal HFD (n=10) for 12 weeks were injected with 2.5 x 10^5 MC38-CEA1 cells in the right flank. On day 5 post tumor-injection, mice were injected with either 200 μg IgG control antibody or αPD-1 antibody, and the injections continued every two days until tumors were collected on day 16 post-injection. **A.** Individual Tumor growth over time for respective treatment groups. **B.** The average tumor volume of αPD-1 antibody treated mice was subtracted from the average tumor volume of control IgG treated mice. The HFD mice had a larger change in tumor volume compared to the LFD mice. **C.** The proportion of αPD-1 treated tumor volume to control IgG treated tumor volume is analyzed as the tumors grow. **D.** Frequency of IFNγ$^+$ CD8$^+$ in the TIL population. **E.** Cell count of CD11b$^+$ F4/80$^+$ Tumor-Associated Macrophages. **F.** Volcano plots of Nanostring gene expression from isolated tumor associated macrophages. Data in this figure are all depicted as mean± S.E.M., with all individual points shown. Ordinary one-way ANOVA test *p* values shown. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.



Supplementary Figure 4. Tumor growth and immunophenotyping of Leptin treatment of MC38 tumors. Five-week-old C57BL/6 male mice on a control standard chow diet were injected with either leptin (1 μg/g body weight) or PBS control twice a day for two weeks before subcutaneous injections with 105 MC38-CEA1 cells were given in the right flank. Leptin injections continued throughout tumor growth. **A.** Daily body weights over time following leptin and MC38 tumor injection Spleens from mice with MC38-CEA1 tumors were collected 16 days post-injection and were processed single cell suspensions, before analyzing by flow cytometry. **B.** Representative MFI histogram and corresponding frequency of $CD86^+$ Splenic macrophages following chronic leptin treatments. **C.** Frequency of $MHCII^+$ splenic macrophages following leptin treatments. **D.** Average tumor growth over time during co-treatments of leptin and anti-PD1. **E.** Individual tumor growth over time during co-treatments of leptin and anti-PD1. **F.** Representative flow cytometry gates of $CD11b^+$ $F4/80^+$ Tumor associated macrophages. **G.** Frequency and absolute cell count of monocytic MDSCs from tumors following leptin and anti-PD-1 co-treatment. of Two-tailed Mann Whitney test *p* values shown. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$, $****p < 0.0001$.