**Supplemental Figure 1: FAO is increased in immunosuppressive T-MDSCs.** (A) Average mean fluorescence intensity (MFI- bar graph) of 2NBDG in nMCs, splenic MDSCs, T-MDSCs, and non-myeloid fraction of 3LL tumors. (B-D) Average MFI (bar graph) of Mitotracker (B), Mitosox (C), and DCFDA (D) in the indicated subsets. (E) Arginase I western blot band intensities normalized to actin. (F) Cytokines were measured in lysates from sorted nMCs, splenic MDSCs, and T-MDSCs using a Bioplex immunoassay. \**P*<0.05; \*\* *P*<0.01; \*\*\**P*<0.001.

**Supplemental Figure 2: Etomoxir lowers CPT1 enzymatic activity but does not alter the generation of MDSC subsets, induce apoptosis, or affect proliferation in BM-MDSCs.** BM-MDSCs were generated in the presence or absence of etomoxir (100 μM). (A) CPT1 activity was measured in cell lysates. (B) Cells were stained for total BM-MDSCs (CD11b+ Gr1+), G-MDSCs (CD11b+ Ly6G+ Ly6CInt), and M-MDSCs (CD11b+ Ly6Glo Ly6Chi). (C) The percentage of Annexin V+ apoptotic cells was determined by flow cytometry. Positive control was BM-MDSCs treated overnight with staurosporine (1μM). (D) The percentage of proliferating cells was tested by the dilution of CFSE fluorescence. (E) Arginase I western blot band intensities normalized to actin.

**Supplemental Figure 3: Etomoxir decreases CPT-1 enzymatic activity *in vivo*, decreases Treg accumulation in spleens.** 3LL Tumors from C57BL/6 mice treated daily with or without etomoxir were harvested on day 21, and tumors and spleens were harvested. (A) T-MDSCs were sorted, and CPT1 activity was measured in cell lysates. (B) Arginase I western blot band intensities (from Fig 3F) normalized to actin. (C) CD4+ FOXP3+ Tregs were quantified by flow cytometry in spleens of control and etomoxir-treated mice.

**Supplemental Figure 4: FAO inhibitors do not affect the growth of tumor cells, cancer stem cells (spheres) or the expression of cancer stem-cell markers.** (A) Clonogenic assays of 3LL and MCA-38 tumor cells in tissue culture media containing 100 μM etomoxir or 100 μM ranolazine. (B) Cancer stem-cell spheres from 3LL or MCA-38 tumors were grown in the presence or absence of etomoxir (100M) as described in Material and Methods. (C) Stem-cell markers Sox2, Nanog and Oct3/4 were tested by qPCR in cells forming the cancer stem-cell spheres.

**Supplemental Figure 5: FAO inhibitors do not affect the activation and function of T cells.** (A) CD4 and CD8 T-cell depletion *in vivo*. 3LL tumor-bearing mice were treated with depleting antibodies for CD4 or CD8. Control received a corresponding dose of IgG isotype antibodies. The depletion of CD4+ or CD8+ T cells was tested by flow cytometry on day 10 of the experiment. (B-C) CFSE-labeled CD3+ T cells were stimulated with anti-CD3/CD28 in the presence or absence of 100 μM etomoxir for 3 days. The proliferation (B) and IFNγ production (B) were assessed in etomoxir-treated cells, compared with control. (D) OT-1 cells were activated with 1 μg/ml SIINFEKL with or without 100 μM etomoxir for 3 days. OT-1 activated T cells were co-cultured overnight at a 1:1 ratio with SIINFEKL-loaded EL-4 cells labeled with high CFSE (1 μM) and control peptide-loaded EL-4 cells labeled with low CFSE (0.1 μM). The percentage of remaining CFSE-labeled cells was determined by flow cytometry. Left panel = mixture of target EL4 cells with unstimulated OT-1 T cells, middle panel = activated OT-1 cells, and right panel = activated OT-1 cells + ET. The expression of granzyme B and perforin was determined in OT-1 cells activated in the presence or absence of etomoxir.

**Supplemental Figure 6. The combined use of Etomoxir and CTX *in vivo* inhibits all immunosuppressive activity in T-MDSCs.** C57BL/6 mice bearing s.c. 3LL tumors were treated with saline (controls), etomoxir (50 mg/Kg starting 1 day after tumor injection), etomoxir plus a single injection of 200 mg/Kg CTX on day 7. Tumors were harvested on day 21, made into a single-cell suspension and T-MDSCs isolated, counted, and tested for immunosuppressive function by co-culture with T cells stimulated with anti-CD3 + anti-CD28.