Oleate but not stearate induces the regulatory phenotype of myeloid suppressor cells

Running title: Oleate effect on myeloid suppressor cells

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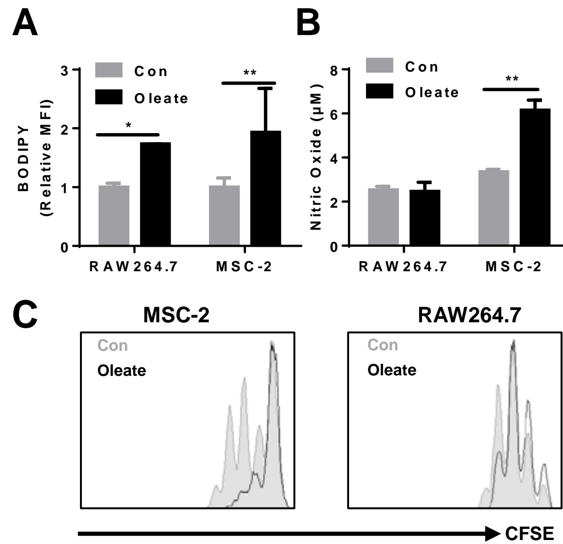

Figure S1. Sodium oleate-induced inhibitory properties are restricted to MSC-2 cells. MSC-2 or RAW264.7 cells were cultured for 24 h in the presence of BSA or sodium oleate and lipid droplets formation was determined by BODIPY staining via flow cytometry (**A**). Both cell lines were incubated as before and were then subsequently stimulated with IFNγ (10 µg/ml) for 8 h and NO production was measured by Griess reaction (**B**). Either RAW264.7 or MSC-2 cells were co-cultured with $CD4^{+}$ T cells in the ratios as indicated in the presence or absence of sodium oleate. Cell proliferation was evaluated by CFSE staining after 72 h (**C**). Shown are representative staining as well as mean±SD from two to four independent experiments. * $p<0.05$; ** $p<0.01$.

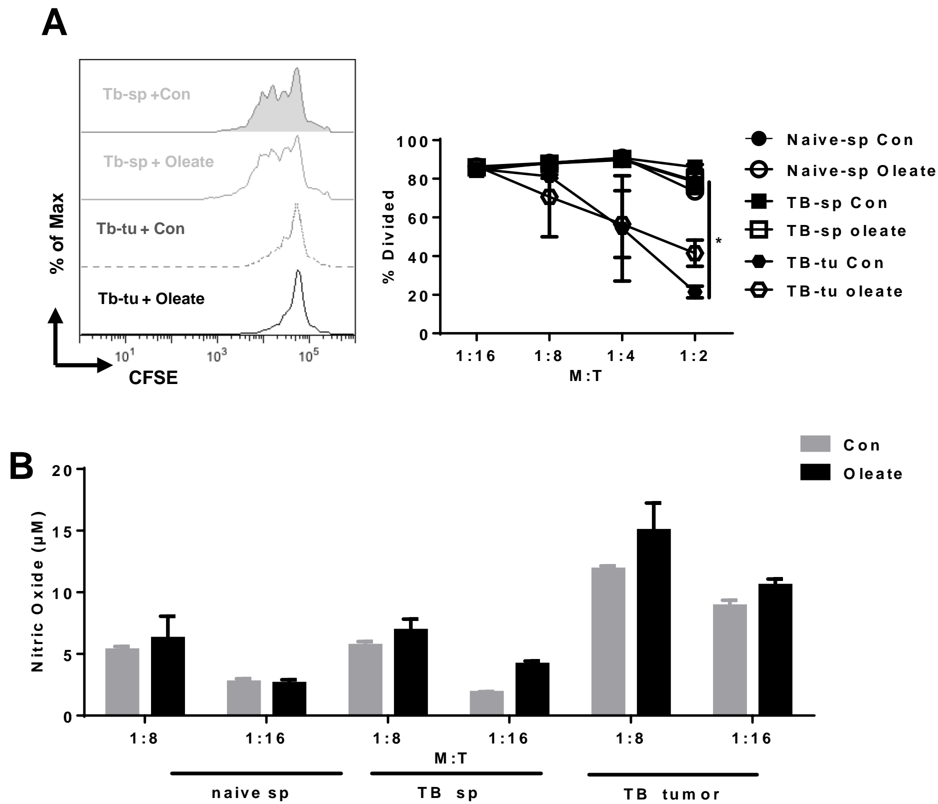

Figure S2. Sodium oleate treatment of $CD11b^+$ cells isolated from tumor bearing mice. $CD11b^+$ cells were isolated from spleen and tumor tissue from tumor bearing and healthy wildtype mice respectively. Cells were treated with BSA or sodium oleate (0.2 mM) for 24 h and co-cultured with $CD4^+$ T cells in the ratios as indicated. Cell proliferation was evaluated by CFSE staining after 72 h (**A**). Supernatants of the co-culture system served for NO quantification via Griess reaction (**B**). Shown is the mean ± SD from two to four independent experiments. * $p<0.05$.

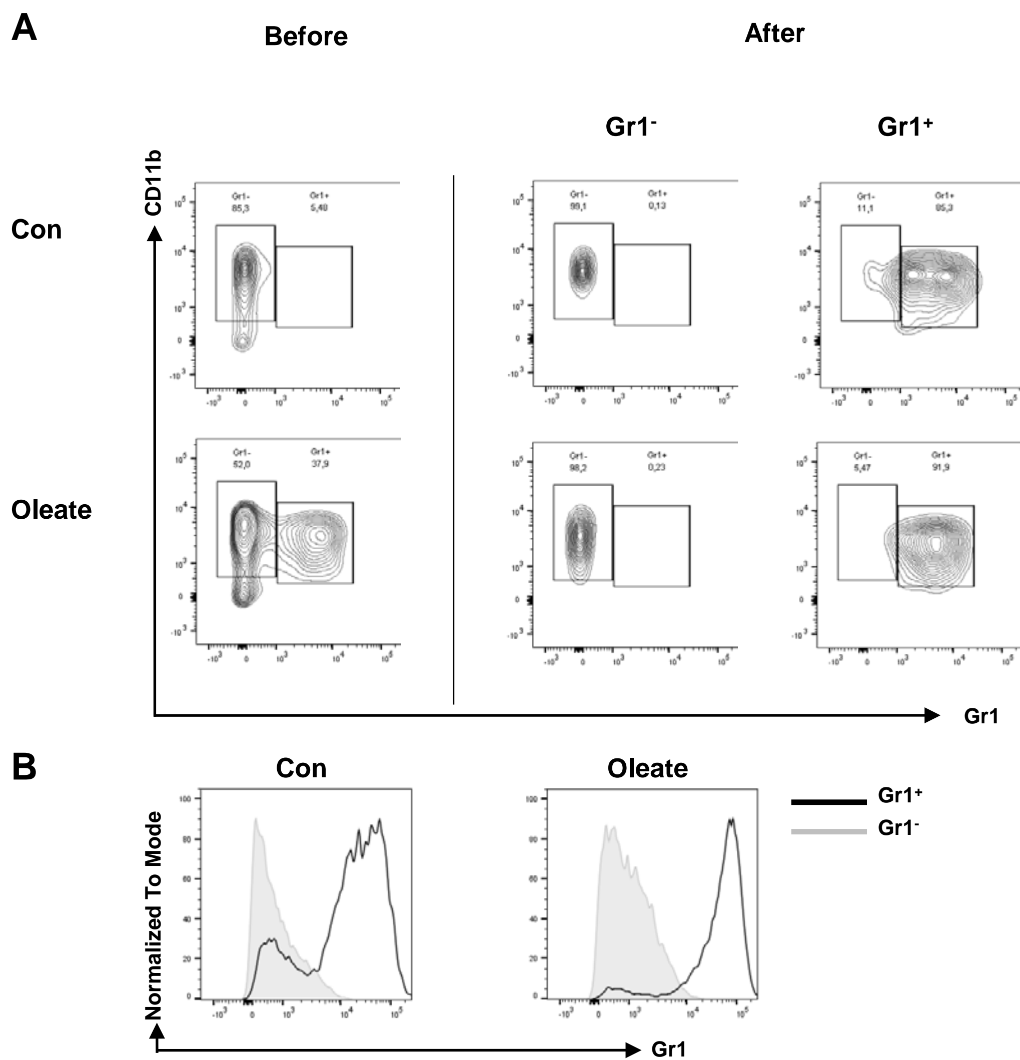

Figure S3. Gr1 sorting via flow cytometry and magnetic beads. Bone marrow cells were polarized in the presence of 40 ng/ml GM-CSF and treated with the indicated compounds for 7 days. $Gr1^{+}$ and $Gr1^{-}$ populations were purified via Flow cytometry (**A**) and magnetic beads (**B**).

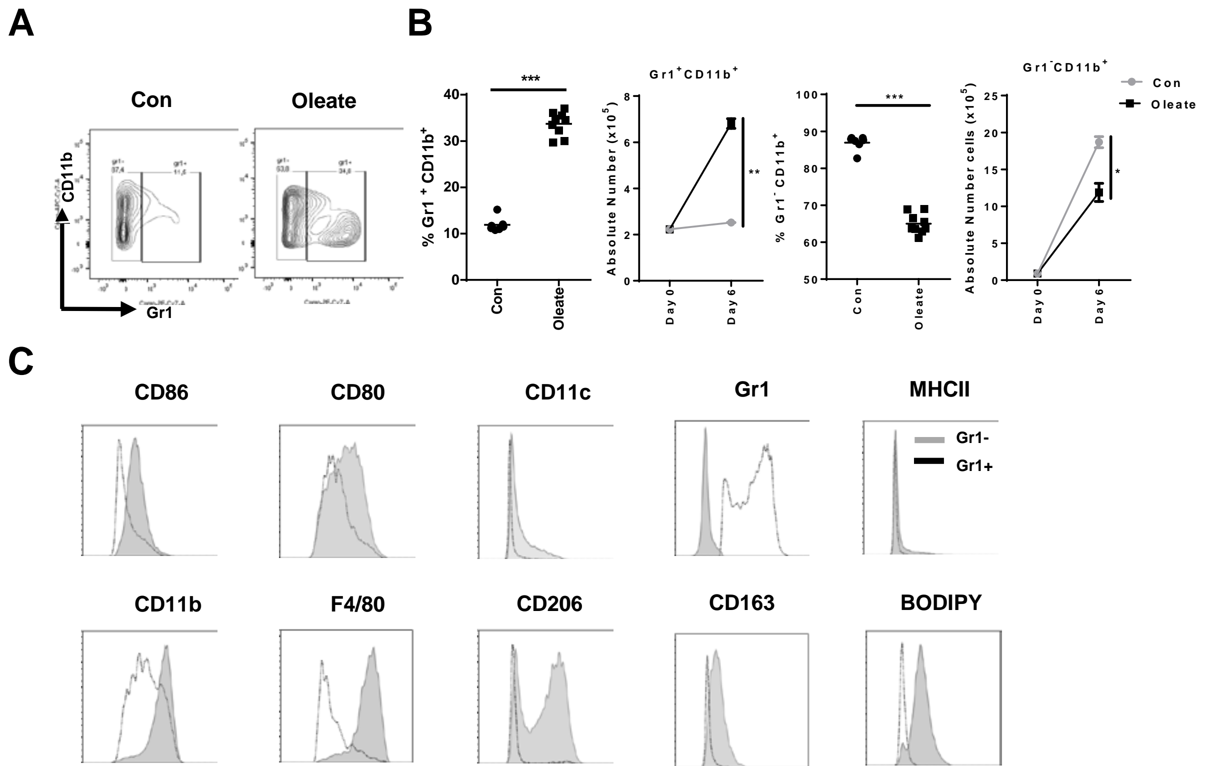

Figure S4. Sodium oleate induced accumulation of Gr1$^+$ myeloid cells in vitro. Bone marrow cells were polarized in the presence of 40 ng/ml GM-CSF and treated with the indicated compounds for 7 days. Flow cytometry was performed to detect the percentage and absolute number of Gr1$^+$ cells (A, B). Gr1$^-$ and Gr1$^+$ subsets gated from CD11b$^+$ populations was stained by indicated surface marker and acquired via FACs canto (C). Shown is the mean±SD from two to four independent experiments. **, $p \leq 0.01$;***, $p \leq 0.001$.

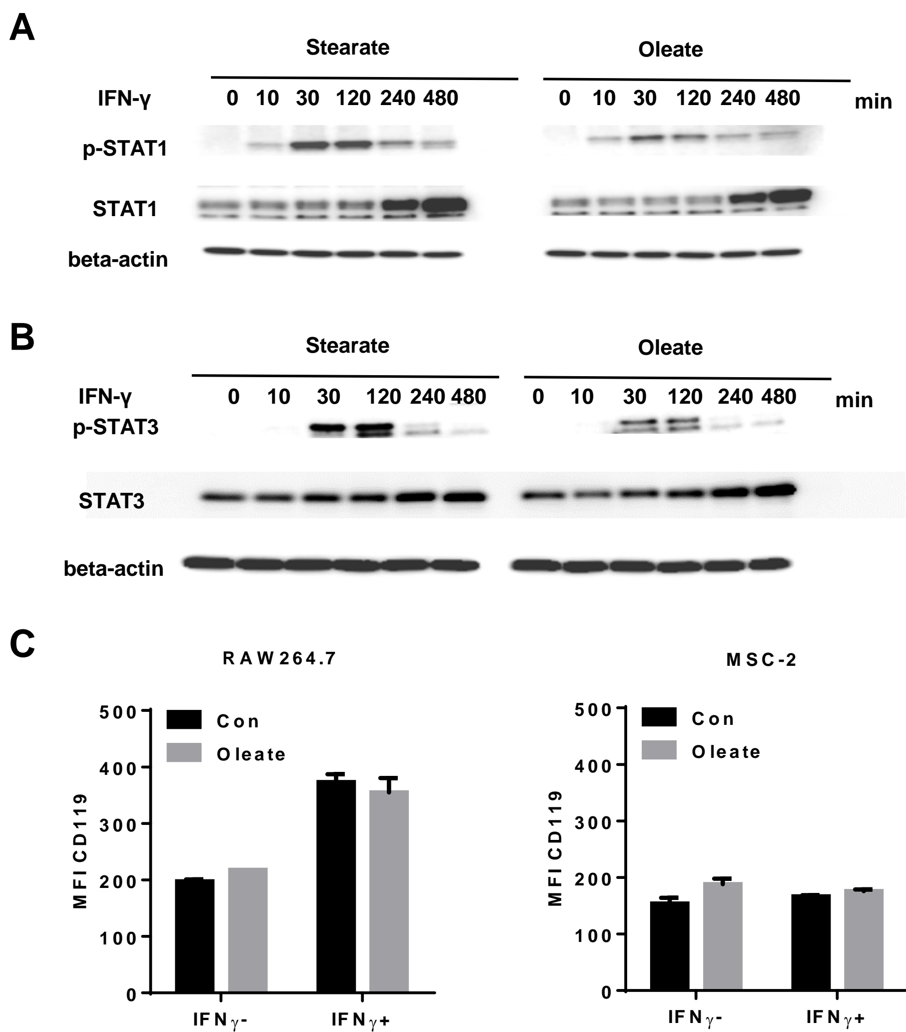

Figure S5. The effect of oleate is independent of CD119 expression and the phosphorylation of STAT1 or STAT3. MSC-2 cells was treated with FFAs for 24 hours, followed by an IFNγ stimulation (1 ng/ml) for indicated time. Cells were lysed and western blot was performed for STAT1, pSTAT1, STAT3 and pSTAT3 (A, B). Both RAW 264.7 and MSC-2 cell line were stimulated overnight in the presence of 10ng/ml IFNγ. The expression of CD119 was detected via flow cytometry (C).