Supplementary data

Supplementary Figure S1. Characterization of MDSCs expanded during the EAE progression. C57BL/6 mice were immunized with MOG_{35-55}/CFA. Two weeks post immunization, the phenotype of splenic MDSCs was analyzed using FACS. **A**. Ly6C and Ly6G expression on $CD11b^+$ cells was examined. **B**. The morphology of $CD11b^+Gr\text{-}1^+$ cells was examined using standard Giemsa staining and microscope. **C**. Expression of CD11c, F4/80, MHC II and CD80 on $CD11b^+Gr\text{-}1^+$ cells. **D**. FACS analysis of the expression of CD115, CCR2, CD62L and Ly6C. **E-G**. MDSCs from EAE mice suppress T cell activation by CD3/CD28 engagement or antigen stimulation. **E**. CFSE labeled splenocytes from naïve C57BL6 mice were treated with anti-CD3/CD28 antibodies (1 μg/mL) in the absence or presence of MDSCs for 3 days. T cell proliferation was analyzed using CFSE dilution assay (***left***). Cell viability was determined by FACS after staining with 7-AAD (***right***). Filled grey: T cells without stimulation; red: T cells with stimulation; black, T cells with stimulation in the presence of MDSCs (1:1). **F.** IFN-γ production from CD4 cells was analyzed by intracellular staining assays. **G**. For antigen-specific activation, BMDCs loaded with MOG_{35-55} peptide (1 μg/mL) were co-cultured with MOG_{35-55}-specific $CD4^+$ T cells derived from the 2D2 transgenic mice (1:100) in the absence or presence of MDSCs. $CD4^+$ T cell proliferation was determined using ^3H thymidine incorporation assay. *, $p < 0.05$

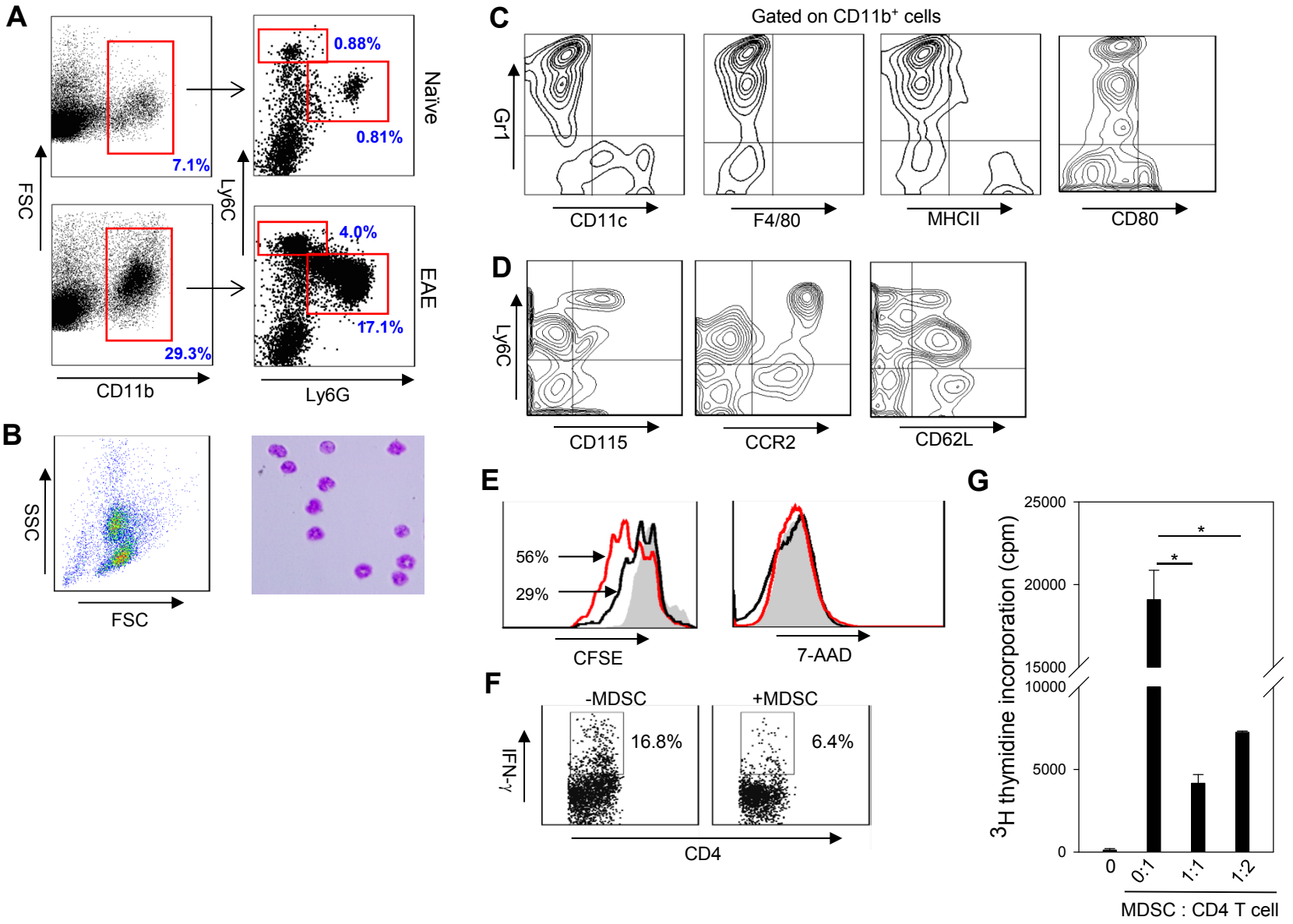
Supplementary Figure S2. Effects of MDSCs from different sources on Th17 differentiation. **A.** Similar effect of MDSCs isolated using MACS beads or FACS sorting on Th17 cell polarization. Naïve $CD4^+$ T cells were induced for Th17 cell differentiation in the presence of MDSCs prepared using different methods. The frequency of Th17 cells and IL-17 levels were analyzed by flow cytometry and ELISA, respectively. N.S. Not significant. **B**. MDSCs derived from tumor-bearing mice are highly efficient in promoting Th17 differentiation. Naïve $CD4^+CD25^-CD62L^+$ T cells (5×10^5 per well in 24-well plates) were co-cultured with or without MDSCs from B16 tumor bearing mice under Th17-polarizing conditions for 3 days. Cells were washed and examined for IL-17A or IFN-γ expression by intracellular staining assays and FACS. Data represent two independent experiments. **C-E**. MDSCs promote Th17 cell expansion. Naïve $CD4^+$ T cells were induced for Th17 cell differentiation in the presence of MDSCs for 3 days. $CD4^+$ T cells were then sorted, CFSE-labeled, and seeded in 24-well plates pre-coated 1 μg/ml anti-CD3 Abs (5×10^5 cells per well). Cells were cultured in the presence or absence of MDSCs (1:1) for additional 72 hours. Cell proliferation was analyzed using CFSE dilution assays (**C**). Cells were subjected to intracellular IL-17A staining and FACS analysis, and the number of Th17 cells are calculated (**D**). The levels of IL-17A in the supernatants were assessed using ELISA assays (**E**). Data represent two independent experiments.

Supplementary Figure S3. Arginase1, not NOS2, is also involved in MDSC-enhanced Th17 differentiation. MDSCs were co-cultured with naïve $CD4^+CD25^-CD62L^+$ T cells (5×10^5 per well in 24-well plates) cells at 1:1 ratio under Th17 polarizing conditions in the presence of NOS2 inhibitor, L-N^6-(1-iminoethyl)-lysine (L-NIL, 500 μM), IL-17A expression was examined using intracellular staining and ELISA assays (**A**). Alternatively, arginase1 inhibitor, nor-NOHA (300 μM) was used in the co-culture (**B**). Intracellular IL-17A expression was examined using

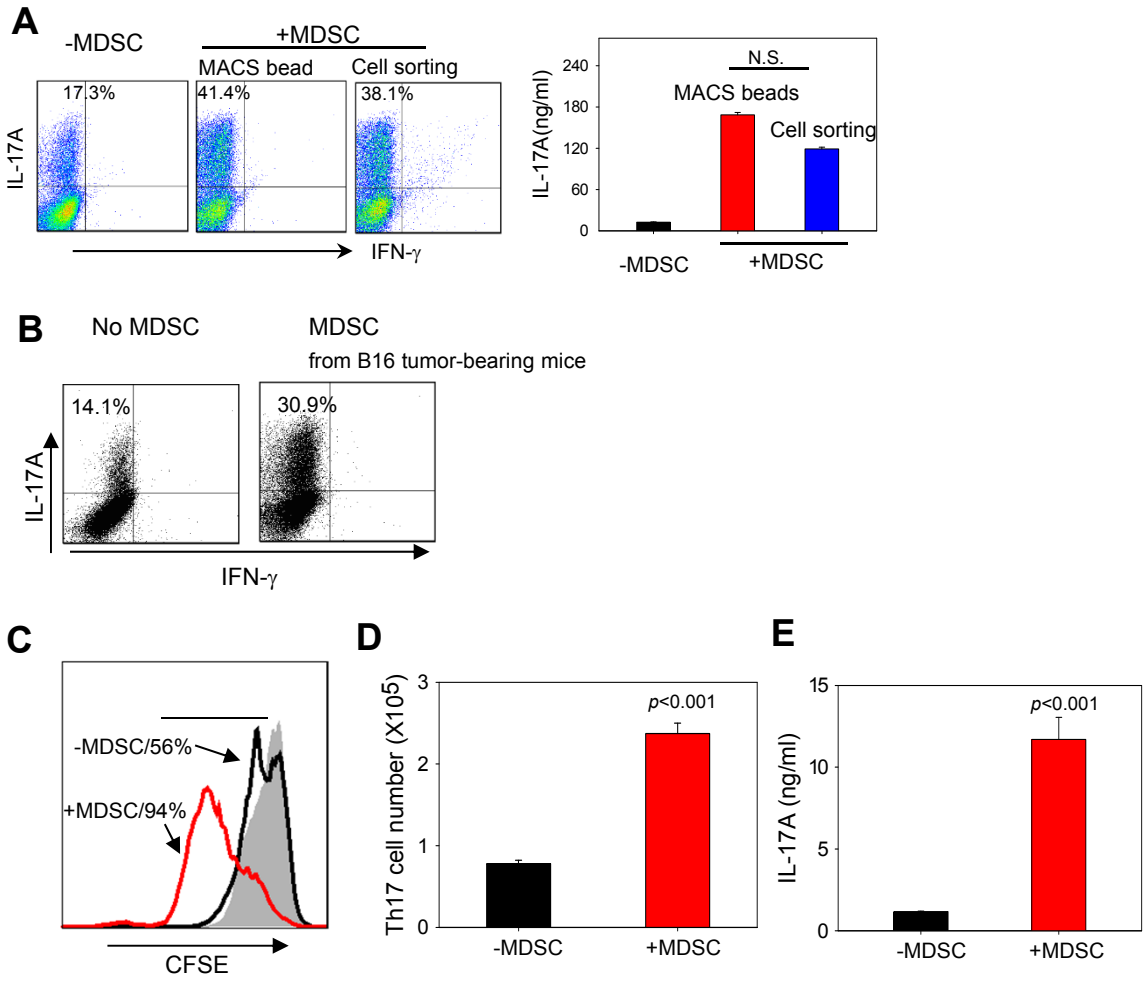
FACS analysis. Additionally, IL-1β production from MDSCs was also assessed using intracellular staining assays.

Supplementary Figure S4. Effects of GEM treatment on immune cell subsets and EAE disease progression. A-B. GEM treatment does not alter the overall levels of T, B, NK cells and DCs. Mice (n=3) were immunized with MOG_{35-55}/CFA, followed by administration of pertussis toxin. GEM (100 mg/kg) was injected 11 days after EAE induction. At the indicated time points, cells prepared from the peripheral blood (**A**), spleen and lymph nodes (**B**) were analyzed by flow cytometry for potential influence of GEM-mediated MDSC depletion on various immune cell subsets, including CD4 T cells ($CD3^+CD4^+$), CD8 T cells($CD3^+CD8^+$), NK cells($CD3^-NK1.1^+$), B cells ($B220^+$), cDCs ($CD11c^+B220^-$), and pDCs ($CD11c^+B220^+$). Data represent two independent experiments. **C. Effect of different doses of GEM on EAE incidence and progression.** C57BL/6 mice (n=5) were immunized with MOG_{35-55}/CFA, followed by intraperitoneal administration of pertussis toxin on days 0 and 2. Mice received GEM treatment at the doses of 10 mg/kg or 50 mg/kg as indicated on days 4, 8, 12, 16. EAE incidence (**C**) and mean clinical scores (**D**) were recorded. **E.** MDSCs in the peripheral blood were analyzed 2 days after the last GEM treatment using FACS. Data represent two independent experiments.

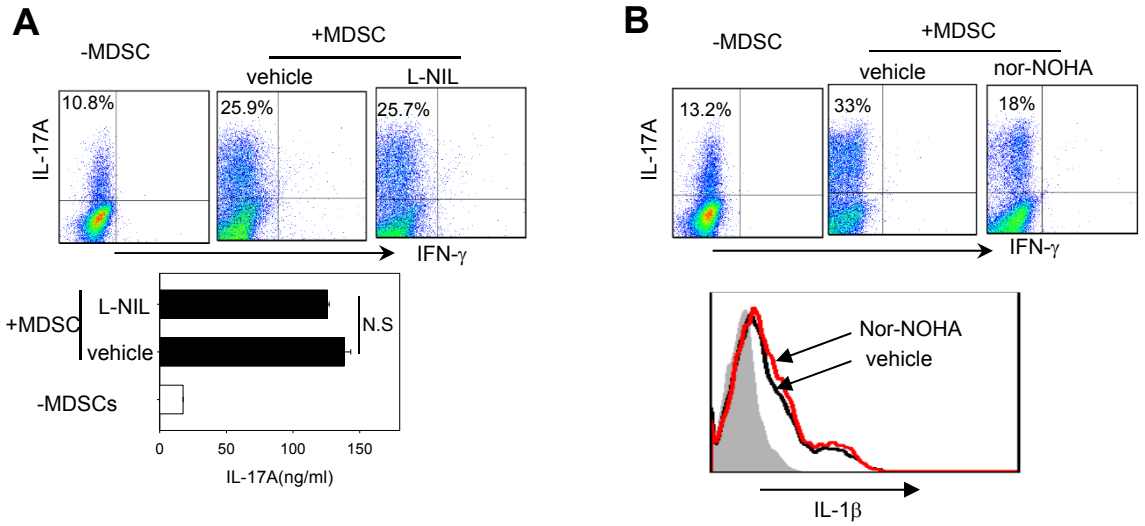
Supplementary Figure S1



Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4