Supplementary materials and methods

Flow cytometry

Splenocytes obtained by mechanical disruption were stained with CD45, CD4, CD8a, TCRβ, CD62L, CD44, CD11b, CD11c, Ly6C, Ly6G, CD11c, F4/80, MHC class I, MHC class II, CD80, CD86, CD40, IL-4Rα, and CSF-1R (CD115) (eBioscience or Biolegend). Cells were blocked with mouse Fc block (eBioscience) before surface staining with indicated antibodies. Dead cells were excluded by Aqua Live/dead (Thermofisher) staining. Isotype controls were used to determine positive staining.

In vitro T cell suppression assay

Sorted $Apc^{Min/+}$ MDSCs or BM-MDSC subsets were co-cultured with CFSE labeled CD4 or CD8 T cells from WT mice for 72 hours at T cell to MDSC ratios of 1 to 1, 1 to 2, 1 to 4, and 1 to 10 in 96 well plates coated with αCD3 and αCD28 antibodies (eBioscience) at 1μg/ml. WT CD4 or CD8 T cells were isolated from total splenocytes using CD4 or CD8 isolation kits (Biolegend) or EasySep positive biotin kit (Stemcell). Proliferation of CFSE labeled T cells was measured by flow cytometry and suppression was determined from reduced proliferation of activated T cells co-cultured with MDSCs compared to activated T cells cultured without MDSCs.

Supplementary references

1. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. J Immunol 2008;181(8):5791-802.

2. Thevenot PT, Sierra RA, Raber PL, Al-Khami AA, Trillo-Tinoco J, Zarreii P, et al. The stress-response sensor chop regulates the function and accumulation of myeloid-derived suppressor cells in tumors. Immunity 2014;41(3):389-401.