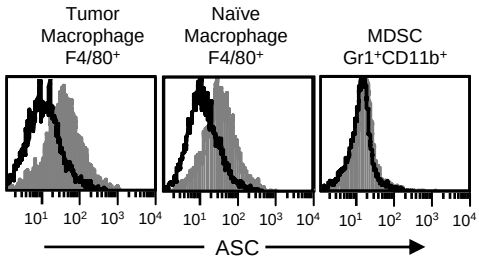
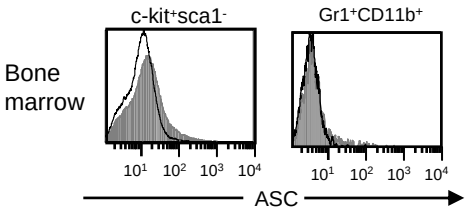
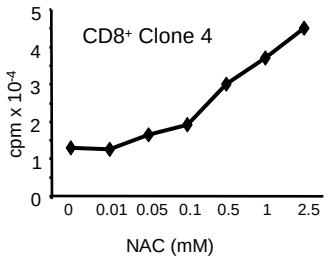
Supplemental Figure 1



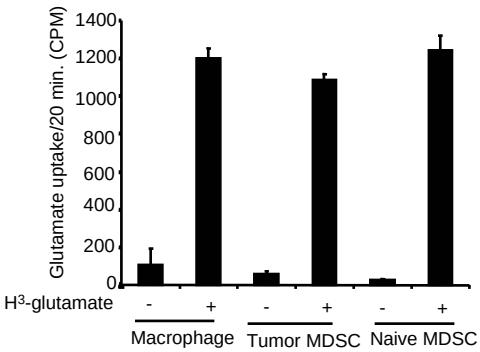
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Table 1.

	Percent Cells	
	Without NAC	NAC
$CD11b^{+}Gr1^{+}$	88	87.5
$F4/80^{+}Gr1^{-}$	0.28	0.15
$CD11c^{+}Gr1^{-}$	0.05	0.04
$CD80^{+}Gr1^{-}$	0.33	0.26
$A^{d+}Gr1^{-}$	0.04	0.05

Supplemental figure 1. Absence of the ASC neutral amino acid transporter is limited to MDSC and is not shared with other myeloid cells in tumor-bearing mice. Blood MDSC (>94% $Gr1^+CD11b^+$ cells) and peritoneal macrophages from tumor-free (>91% $F4/80^+$) or 4T1 tumor-bearing (>74% $F4/80^+$) BALB/c mice were stained for F4/80 and ASC, and the gated $F4/80^+$ population analyzed for ASC expression. MCF for control Ab is 20, 21, and 19; MCF for ASC Ab is 60, 50, and 26 for tumor macrophages, naive macrophages, and MDSC, respectively.

Supplemental figure 2. Myeloid progenitor cells in the bone marrow express low levels of the ASC transporter. Bone marrow cells were harvested as described (1) from tumor-free mice and stained for Gr1, CD11b, c-kit, Sca-1, and ASC. Gated $Gr1^+CD11b^+$ and $c\text{-}kit^+Sca\text{-}1^-$ cells (myeloid progenitor cells; (2)) were analyzed for ASC.

Supplemental figure 3. Transgenic $CD8^+$ Clone 4 T cells were co-cultured with cognate peptide in the presence of 0-2.5 mM NAC. T cell activation was measured by tritiated thymidine uptake. Data are representative of two independent experiments.

Supplemental figure 4. Thioglycolate-induced peritoneal macrophages (>89% $F4/80^+$), MDSC from naive mice (>86% $Gr1^+CD11b^+$), and MDSC from mice with 4T1 tumors (> 90% $Gr1^+CD11b^+$) were cultured in sodium-free buffer for 20 min, followed by a 20 min incubation in buffer supplemented with ^{3}H-glutamate. Glutamate uptake was terminated by washing with cold buffer and cells were lysed and the lysates were counted for in tritiated glutamate. Data are from one of two independent experiments.

Supplemental Table 1. NAC does not cause MDSC differentiation. Leukocytes from the blood of BALB/c mice with 4T1 tumors (>91% $Gr1^+CD11b^+$ cells) were cultured in serum-free HL-1 medium with or without 0.5 mM NAC. Cells were harvested after 16 hours, stained with mAbs to Gr1, CD11b, F4/80, CD11c, CD80, and $I\text{-}A^d$ and analyzed by flow cytometry. By day 3 of culture, 97.5% of the cells were dead as measured by trypan blue exclusion.

References

1. Sinha P, Clements VK, Fulton AM, et al. Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. Cancer Res 2007;67:4507-13.
2. Akashi K, Traver D, Miyamoto T, et al. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature 2000;404:193-7.