Myeloid Cells
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Supplementary information

AS01 adjuvant is a trained immunity inducer with potent antitumor activity

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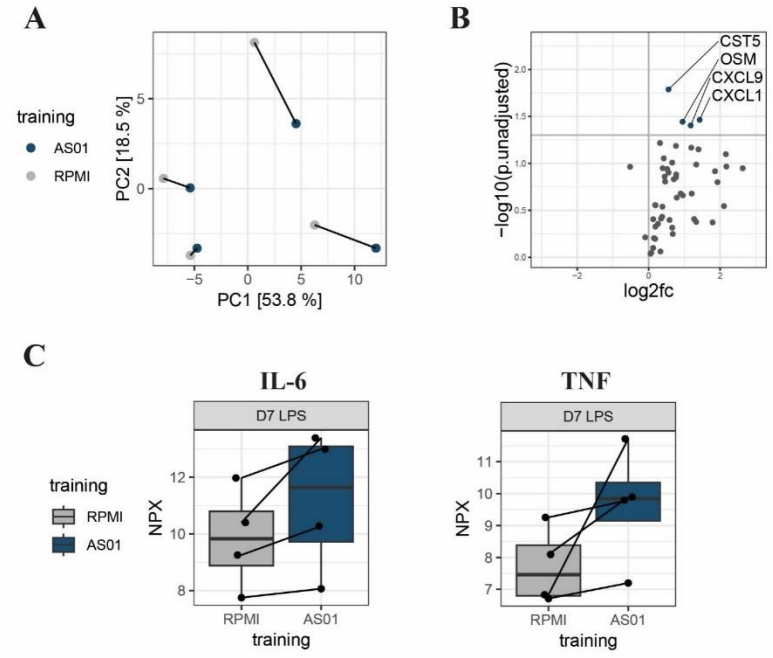

Figure S1. AS01-trained macrophages show a trend toward a more potent inflammatory protein response. Monocytes were trained with 40 ng/mL AS01 or left untrained and restimulated with 10 ng/mL LPS 5 days later. Proteomic data was obtained with the Olink Target 96 Inflammation panel. (A) Principal component analysis (PCA) plot of the curated protein NPX values; (B) Volcano plot showing the non-adjusted p values for the $\log_2$ fold-changes between AS01-trained macrophages and RPMI-treated ones after LPS restimulation. *p* values obtained with a paired t student's test; (C) IL-6 and TNF raw NPX values in untrained and AS01-treated cells. n = 4, data obtained from two independent experiments.

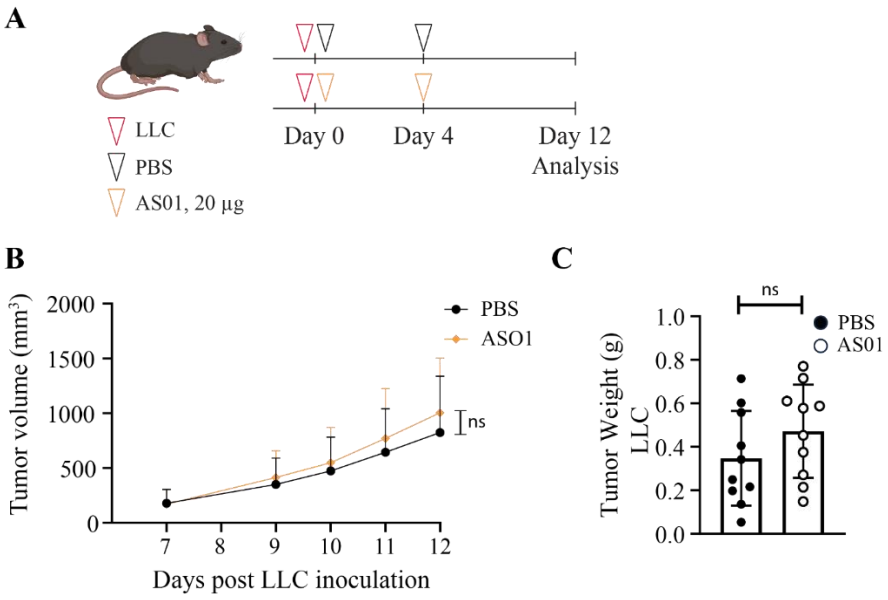

Figure S2. AS01 treatment does not suppress LLC tumor growth. C57BL/6 mice were injected with LLC tumor cells and then treated with intramuscular PBS or AS01. (A) Experimental design for the LLC model; (B) Mice received either two doses of PBS or two doses of 20 µg AS01 at days 0 and 4 after LLC injection. Tumor volumes were monitored for 12 days after tumor inoculation (n = 10 mice for the PBS group, n = 10 for the AS01 group); (C) Tumor weights determined on the day of the sacrifice (day 12 after inoculation). Data is shown as mean ± SD, statistical tests performed are two-way ANOVA (B) and unpaired t-test (C).

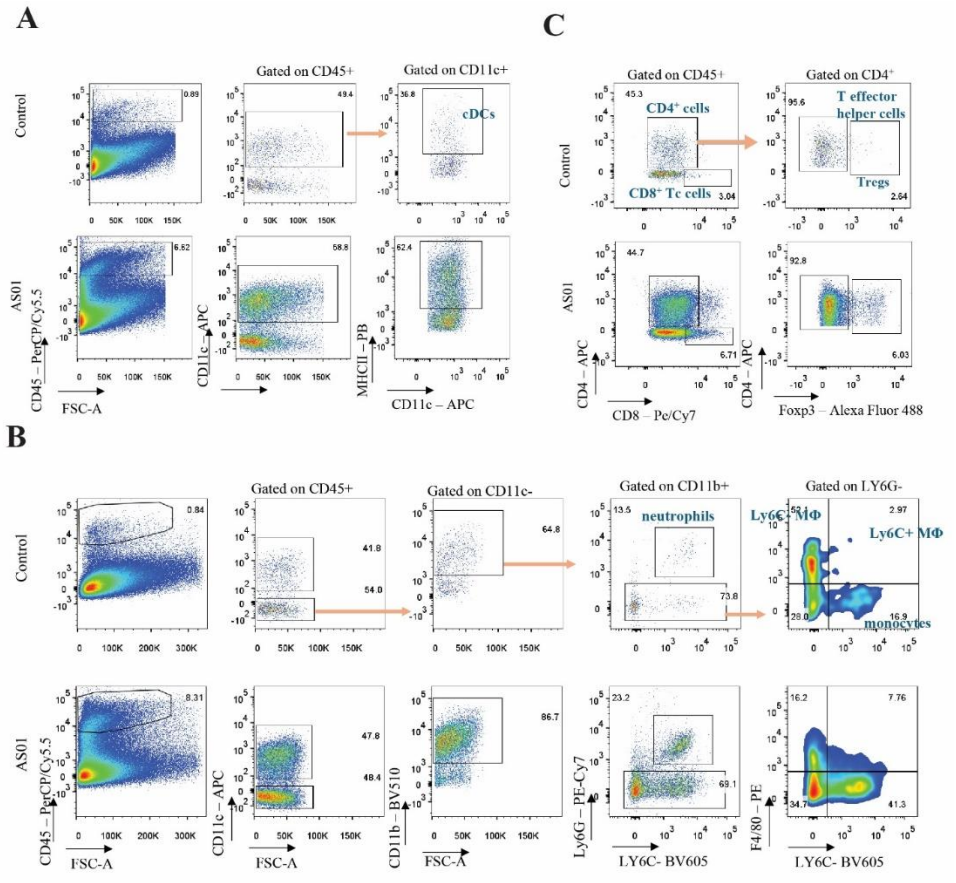

Figure S3. Gating strategy of myeloid and T cells in the B16-F10 tumor microenvironment. B16-F10 tumors of mice treated with PBS or AS01 (two doses of 20 µg) were excised and analyzed by flow cytometry. Gating strategy of intratumoral (A) cDCs ($CD45^+$ $CD11c^+$ $MHCII^+$); (B) Neutrophils ($CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6G^+$), $Ly6C^-$ or $Ly6C^+$ macrophages (MΦ, $CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6G^-$ $Ly6C^{-/+}$ $F4/80^+$) and monocytes ($CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6G^-$ $Ly6C^+$ $F4/80^-$); (C) T helper cells ($CD45^+CD4^+$) and cytotoxic T cells ($CD45^+$ $CD8^+$) are shown as percentages of $CD45^+$ cells. $CD4^+$ effector T helper cells ($CD45^+$ $CD4^+$ $Foxp3^-$) and Tregs ($CD45^+$ $CD4^+$ $Foxp3^+$) shown as percentages of $CD45^+$ $CD4^+$ cells. Numbers denote percentages of gated population.

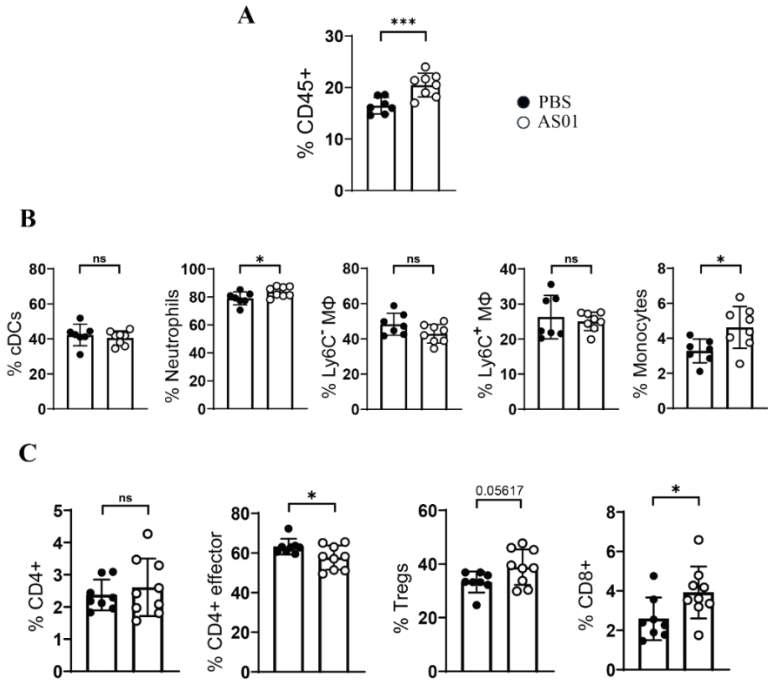

Figure S4. MB49 tumors of mice treated with PBS or AS01 (two doses of 20 µg) were excised and analyzed by flow cytometry. Percentages for (A), (B) and (C) are shown for the parent populations. (A) $CD45^+$ leukocytes as a percentage of live cells within the tumors (n = 7-8); (B) Myeloid lineage changes in tumor infiltrating cells. cDCs ($CD45^+$ $CD11c^+$ $MHCII^+$) as percentage of $CD45^+$ $CD11^+$ cells. Neutrophils ($CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6G^+$) as percentage of $CD45^+$ $CD11c^-$ $CD11b^+$ cells. $Ly6C^-$ or $Ly6C^+$ Macrophages (MΦ, $CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6G^-$ $Ly6C^{-/+}$ $F4/80^+$) and monocytes ($CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6G^-$ $Ly6C^+$ $F4/80^-$) as percentages of $CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6G^-$ cells (n=7-8); (C) Tumor-infiltrating lymphoid cell changes. T helper cells ($CD45^+CD4^+$) and cytotoxic T cells ($CD45^+$ $CD8^+$) are shown as percentages of $CD45^+$ cells. $CD4^+$ effector T helper cells ($CD45^+$ $CD4^+$ $Foxp3^-$) and Tregs ($CD45^+$ $CD4^+$ $Foxp3^+$) shown as percentages of $CD45^+$ $CD4^+$ cells (n = 8-9). n = biologically independent mouse samples. Data is shown as mean ± SD, p values obtained by unpaired t tests, *$p \leq 0.05$ and **$p \leq 0.01$.

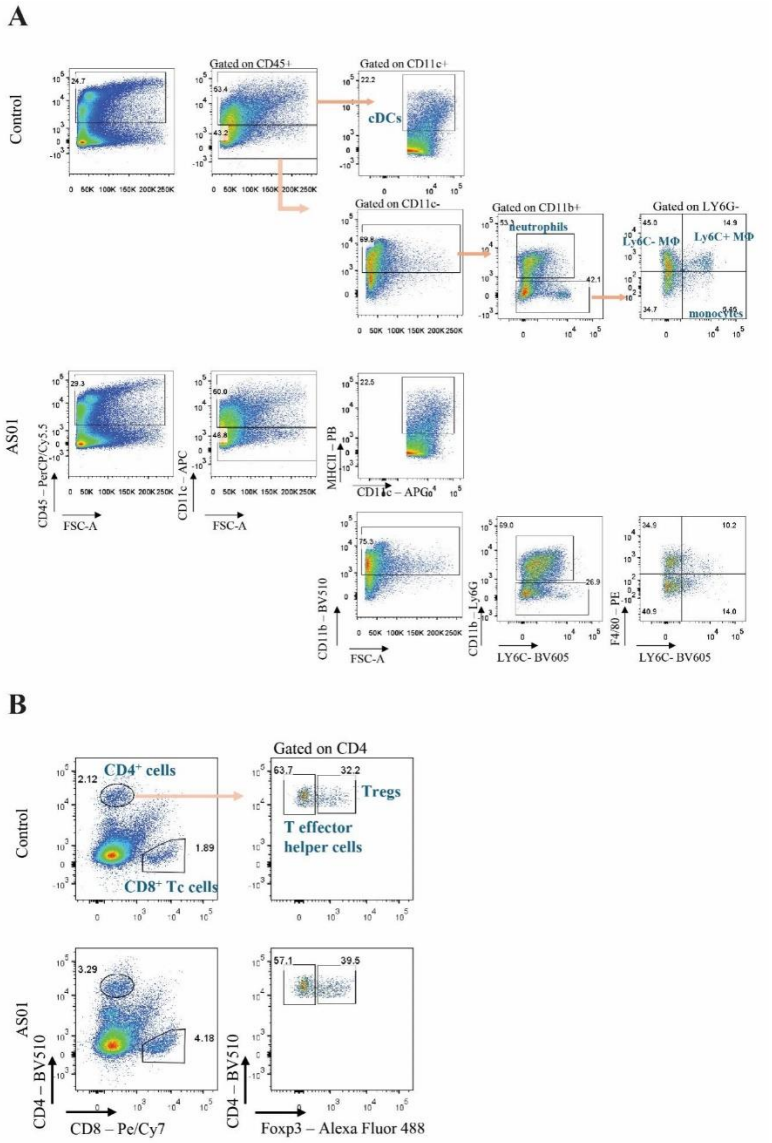

Figure S5. Gating strategy for identification of tumour-infiltrating myeloid and T cells in MB49-bearing mice. MB49 bladder tumors of mice treated with PBS or AS01 (two doses of 20 µg) were excised and analyzed by flow cytometry. Gating strategy of intratumoral (A) cDCs ($CD45^+$ $CD11c^+$ $MHCII^+$), Neutrophils ($CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6G^+$), $Ly6C^-$ or $Ly6C^+$ Macrophages (MΦ, $CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6G^-$ $Ly6C^{-/+}$ $F4/80^+$) and monocytes ($CD45^+$ $CD11c^-$ $CD11b^+$ $Ly6G^-$ $Ly6C^+$ $F4/80^-$); (B) T helper cells ($CD45^+CD4^+$) and cytotoxic T cells ($CD45^+$ $CD8^+$) are shown as percentages of $CD45^+$ cells. $CD4^+$ effector T helper cells ($CD45^+$ $CD4^+$ $Foxp3^-$) and Tregs ($CD45^+$ $CD4^+$ $Foxp3^+$) shown as percentages of $CD45^+$ $CD4^+$ cells. Numbers denote percentages of gated population.