Supplemental Figure Legends

Supplemental Figure S1. Equivalent binding of anti-CTLA-4 isotypes to cell surface CTLA-4.

Anti-CTLA-4 isotypes were tested for binding to cells constitutively expressing CTLA-4 (58a'b' mCTLA-4-CD3ζ).

Supplemental Figure S2. Serum concentrations of anti-CTLA-4 isotypes in C57BL/6 mice.

For details see Supplemental Table S2.

Supplemental Figure S3. Representative FACS plots for peripheral regulatory T cells.

Supplemental Figure S4. A. Representative FACS plots for intratumoral regulatory T cells. B. Kinetics of intratumoral Treg reduction following anti-CTLA-4 treatment. MC38 tumor bearing C57BL/6 mice (d7 after implantation) were treated with a single dose of 200 ug of isotype control mIgG1, anti-CTLA-4 9D9-IgGD265A or anti-CTLA-4 9D9-IgG2a, and tumors were isolated on d1, d2 or d5 after treatment. Tumors and spleen were harvested, manually dissociated into single cell suspensions, and stained for flow cytometry as in Figure 3.

Supplemental Figure S5. Isotype-dependent recruitment of MDSCs and IL-1α production.

2x10^6 MC38 colon tumor cells were implanted subcutaneously into C57BL/6 mice. At day 7 post-implantation, tumor bearing mice were randomized and received 3 doses of antibody by intraperitoneal injection (10 mg/kg) every 3 days. On day 15 post-implantation, tumors were harvested, manually dissociated into single cell suspensions, and numbers of MDSCs (CD11b^+Gr-1^+) among CD45^+ cells (A), as well as levels of interleukin 1-alpha (IL-1α) (B), were assessed. Data are representative of (A) two independent experiments with ≥ 3 mice per
group or (B) three independent experiments with \( \geq 5 \) mice/group/experiment (\( **P < 0.01 \), \( ***P < 0.001 \)).