**Supplementary Figure S1. Anti-4-1BB and anti-PD-1 synergized to suppress B16F10 tumor growth.**

C57BL/6 mice were inoculated s.c. (day 0) with 2 x 106 B16F10. After tumors were established, mice were randomized into groups of 10 animals per group with an average tumor volume of ~120 mm3 (day 8). The anti-4-1BB and anti-PD-1 mAbs were dosed i.p. at 1 mg/kg and 10 mg/kg respectively in a volume of 200 L, on days 8 and 13 (indicated by arrows). Tumor size was measured 2-3 times a week. Mean ± SEM of each treatment group is shown in (A) and individual tumor growth over time is reported in (B). \*\*\*\* p < 0.0001 when comparing groups as indicated by the horizontal lines.

**Supplementary Figure S2. The flow cytometric gating strategy to analyze immune cells.**

Spleens from B16F10-bearing mice after antibody treatment were harvested and disaggregated into a single cell suspension. After immunostaining, cells were analyzed by flow cytometry for the expression of CD3, CD4, CD8, NK1.1, PD-1 and 4-1BB. Representative plots of isotype- or anti-4-1BB-treated samples are shown in (A) and (B) respectively.

**Supplementary Figure S3. Elevated expression of genes associated with anti-tumor immune response in MC38 colon carcinomas.**

At the end of the efficacy study as described in Figure 1D, MC38 tumor samples were collected and total RNA isolated. Gene expression from each treatment group was calculated using real-time PCR analysis with GAPDH and Actin as the endogenous controls. Column dot plots are shown for the genes of CD3, CD8, IFN-, Eomes, PD-1, PD-L1, 4-1BB, CD4, and FoxP3. Each symbol represents an individual animal within the same treatment group. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 and \*\*\*\* p < 0.0001 when comparing groups as indicated by the horizontal lines.

**Supplementary Figure S4. Accumulation of CD8+ T cell and memory phenotype differentiation in MC38 model.**

At the end of the efficacy study as described in Figure 1D, spleens from MC38-bearing mice were harvested and processed to single cell suspensions for immunostaining. Stained immune cells were analyzed by flow cytometry for the expression of CD3, CD4, CD8, CD44, and CD62L. The ratio of CD8+/CD4+ T cells is shown in (A). The percentages of effector memory (CD44+ CD62L-) of CD8+ or CD4+ are shown in (B) and (C), respectively. Each symbol represents an individual animal within the same treatment group. \*\* p < 0.01, \*\*\* p < 0.001 and \*\*\*\* p < 0.001 when comparing groups as indicated by the horizontal lines.

**Supplementary Figure S5. Serum liver enzyme levels and hematology alterations in response to 4-1BB activation therapy in B16F10 tumor-bearing mice.**

C57BL/6 mice were inoculated s.c. (day 0) with 1 x 106 B16F10. Mice were randomized into groups of 5 animals per group with an average tumor volume of ~100 mm3 (day 9) and received a single s.c. administration of treatments. Whole blood samples were collected 10 days after treatment and were subjected for the counts of platelet, lymphocyte and neutrophil and the levels of alanine aminotransferase (ALT). \* p < 0.05 vs. isotype.