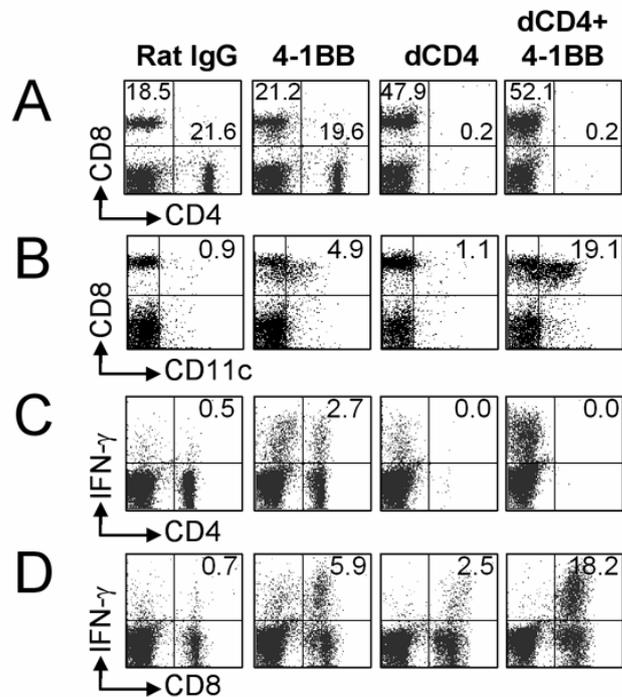
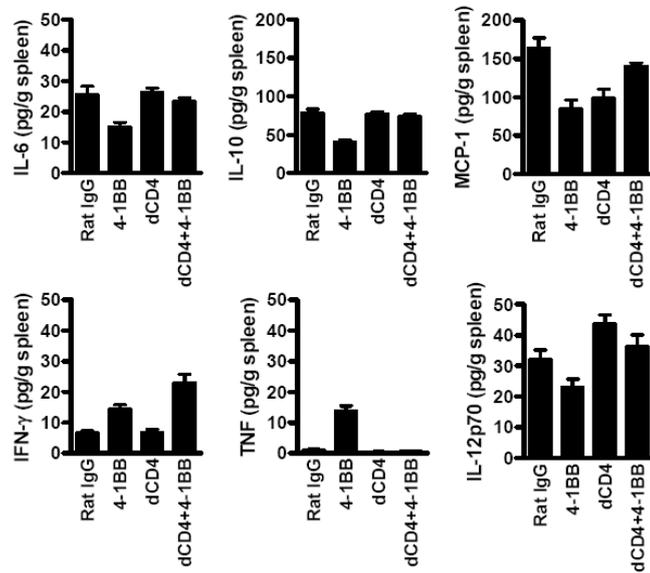


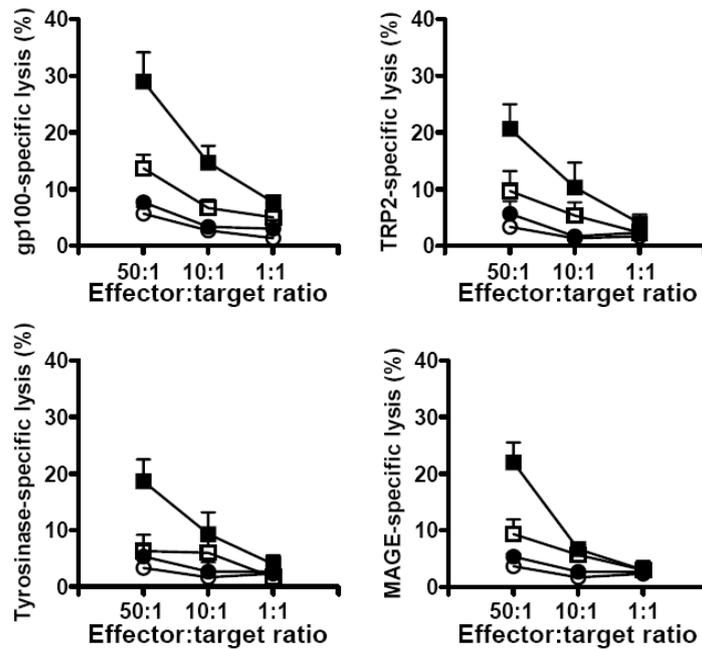
Supplementary Figure 1. Anti-tumor effects of the combined therapy with anti-4-1BB and anti-CD4 mAb. C57BL/6 mice were challenged subcutaneously on the back with 4×10^5 B16-F10 melanoma cells. Simultaneously with the tumor cell injection, the mice were injected i.p. with $100\mu\text{g}$ anti-4-1BB mAb (3E1) and/or $400\mu\text{g}$ anti-CD4 mAb (GK1.5) 5 times every 5 days. To illustrate the tumor size, photographs of the cohort of mice in each group taken 25 days after tumor injection are shown in A-D.



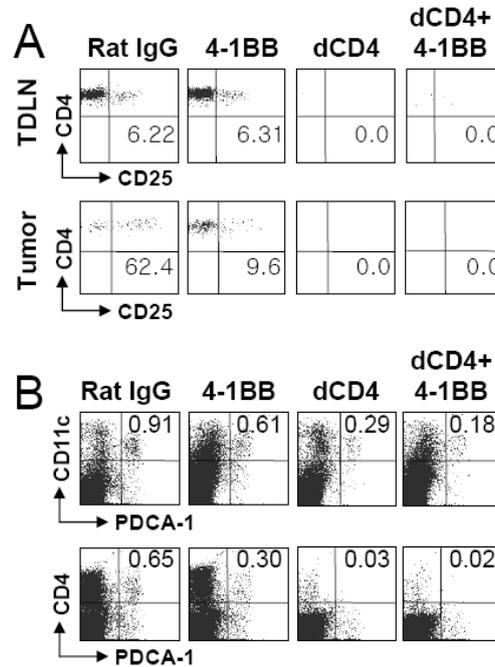
Supplementary Figure 2. Combination therapy with anti-4-1BB and anti-CD4 enhances CD8⁺ T cell response. Tumor-challenged mice were treated with antibodies as indicated. TDLN cells were stained with anti-CD4-PE plus anti-CD8 β -FITC or anti-CD8 β -FITC plus anti-CD11c-PE on PI day 15 (A and B). TDLN cells were also stimulated *in vitro* with PMA and ionomycin in the presence of monensin for 4 h. The percentages of IFN- γ -producing CD4⁺ and CD8⁺ T cells were determined by FACS (C and D).



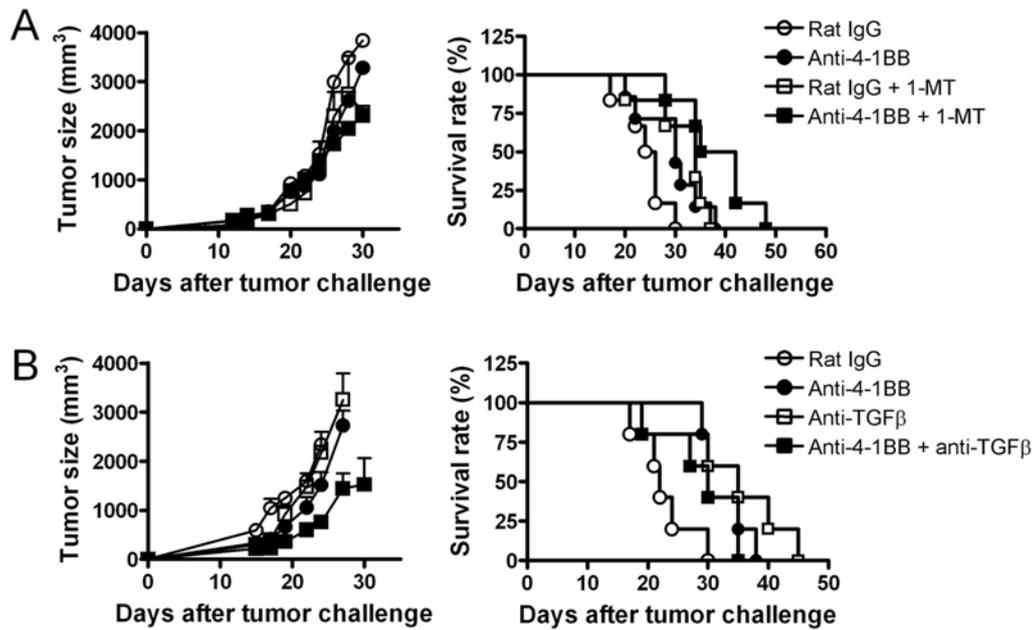
Supplementary Figure 3. Cytokine production by the combination of anti-4-1BB and anti-CD4 mAbs in spleen. C57BL/6 mice were challenged subcutaneously with B16-F10 melanoma cells and treated with antibodies as described above. Spleen was collected from the each groups of mice 15 days after tumor challenge and homogenized. The debris was spun down and the supernatant was collected and subjected to cytometric bead array kit (CBA, BD Bioscience). Data shown were cytokine concentration per 1g spleen.



Supplementary Figure 4. Enhancement of CTL activities by the combination of anti-4-1BB and anti-CD4 mAbs. C57BL/6 mice were challenged subcutaneously with B16-F10 melanoma cells and treated with antibodies as described above. TDLN cells were prepared from each group of mice on PI day 15 and used as effector cells. EL4 cells were labeled with ^{51}Cr with or without melanoma-specific peptides (gp100, TRP2, tyrosinase, and MAGE-A1) for 1hr at 37°C and used as target cells. The target and effector cells were mixed in ratios of 1:50, 1:10, and 1:1, and incubated for 4 hrs at 37°C , after which ^{51}Cr release was measured.



Supplementary Figure 5. Anti-CD4 mAb removes CD4⁺CD25⁺ regulatory T cells and plasmacytoid dendritic cells (pDCs). C57BL/6 mice were challenged subcutaneously with B16-F10 melanoma cells and treated with antibodies as described above. (A) Lymphocytes were prepared from TDLNs and tumor tissue on PI day 15 and stained with FITC-conjugated anti-CD4, PE-conjugated anti-CD25, and PE-Cy5-conjugated anti-CD3 mAbs. CD3⁺CD4⁺ T cells were gated to analyze the CD4⁺CD25⁺ regulatory T cells. (B) For pDCs, single suspensions of collagen-digested TDLNs were stained with FITC-conjugated anti-CD11c or anti-CD4 mAb along with PE-conjugated PDCA-1. All samples were subsequently analyzed by FACScan (BD Bioscience).



Supplementary Figure 6. Combination therapy of anti-4-1BB with 1-methyltryptophan (1-MT) or anti-TGF β mAb. C57BL/6 mice were challenged subcutaneously on the back with 4×10^5 B16-F10 melanoma cells. The mice were injected i.p. with $100\mu\text{g}$ anti-4-1BB (3E1) or rat IgG, 5 times every 5 days. Some of antibody-treated mice were given 3mg/ml of 1-MT (Sigma-Aldrich) in their drinking water for a period of 16 days starting one day before the tumor challenge (A) or i.p. injected with $400\mu\text{g}$ anti-TGF β mAb (1D11), 5 times every 5 days (B). Mice were monitored every day, and tumor volume and survival were measured as described above.