

Supplementary information, Figure S9 Evaluation of blocking activities of commonly used anti-mouse Ctla-4 mAbs 9H10 and 9D9. (A, B) 9H10 does not block B7-CTLA-4 interaction if B7-1 (A) and B7-2 (B) are coated onto plates. Biotinylated mouse Ctla-4-Fc fusion protein were incubated with B7-coated plates in the presence of given concentration of control IgG or anti-mouse Ctla-4 mAb 9D9 and 9H10. Data shown are means of duplicated wells and are representative of two independent experiments. (C, D) 9D9 and 9H10 exhibit differential binding ability to soluble (C) and plate bound Ctla-4-Fc (D). MPC-11(mouse IgG2b) and Hamster IgG (Ham IgG) are isotype-matched control Ig proteins. Data shown are means of duplicated wells and are representative of at least two independent experiments. (E, F) Differential effect of anti-mouse Ctla-4 mAbs 9D9 and 9H10 on upregulating the levels of B7-1 (E) and B7-2 (F) on splenic CD11c^{high} DCs from WT (*Ctla4^{m/m}*) mice. At 24 hours after treatment with 500 μg

antibodies, mice were sacrificed and splenocytes were harvested for flow staining immediately. IgG group indicates mice receiving 500 μ g of MPC-11 and 500 μ g of Ham IgG. The data (Mean ± S.E.M.) are summarized from 6 independent mice per group in two independent experiments involving 3 mice per group each. Statistical significance in e and f was determined using Student's t test. *P<0.05, **P<0.01, ***P<0.001. n.s., not significant.