

Supplemental Figure 1. 4-1BB-mediated induction of IDO and NOS on antigenpresenting cells is dependent on IFN- γ signaling, but not TGF- β . C57BL/6 mice were injected i.p. with 0.3 µg SEA. Anti-4-1BB mAb or rat IgG was administered i.p. (200 µg per injection) on PI days 0 and 2. And some mice were additionally treated with 500 µg anti-IFN- γ mAb or anti-TGF- β mAb on PI days -1, 4, and 7. CD11c⁺ DCs (A) or CD11b⁺ macrophages (B) were purified from each group of mice on PI day 10. Complementary DNA was synthesized from the RNA of the purified DCs and macrophages, and transcript levels of IDO and iNOS were confirmed by PCR.



Supplemental Figure 2. The expansion of CD11c⁺CD8⁺ T cells. Wild or IFN- γ KO C57BL/6 mice were injected i.p. with 0.3 µg SEA. Anti-4-1BB mAb or rat IgG was administered i.p. (200 µg per injection) on PI days 0 and 2 (A, B). The mice were additionally treated with 500 µg anti-IFN- γ on PI days -1, 4, and 7 (A). And ILN cells were prepared from each group of mice on PI day 10 and stained with anti-CD8 β and anti-CD11c mAb. All samples were analyzed by FACScan.



Supplemental Figure 3. The effects of IFN- γ neutralization and IDO blockade *in vitro*. C57BL/6 mice were injected i.p. with 0.3 µg SEA. Anti-4-1BB mAb or rat IgG was administered i.p. (200 µg per injection) on PI days 0 and 2. Cells were isolated from ILNs on PI day 10. And cells were restimulated with 0.1 µg/ml SEA in the presence or absence of anti-IFN- γ mAb (10 or 50 µg/ml) or 1-MT (200 µM) for for 3 days, and pulsed with [³H]-thymidine (1 µCi/well) during the last 12 h, harvested onto glass fiber filter mats, and analyzed for [³H]-thymidine incorporation by liquid scintillation counting. The plotted data are means ± s.d.



Supplemental Figure 4. The analysis of cell populations. C57BL/6 mice were injected i.p. with 0.3 μ g SEA. Anti-4-1BB mAb or rat IgG was administered i.p. (200 μ g per injection) on PI days 0 and 2. On PI day 10, ILN cells were stained with fluorescent Abs specific for CD8 β , CD11c, 1-A^b, H-2K^b, and CD3 (A). Single-cell suspensions were prepared from spleens and ILNs of anti-4-1BB mAb-treated mice, and then populations, including CD11c⁺CD8⁺ T cells and CD11c⁻CD8⁺ T cells were purified by immunomagnetically deleting CD4⁺, F4/80⁺, CD40⁺, and B220⁺ cell populations by incubation with the relevant antibodies. The purified T cell populations and depleting cell population (CD4⁺, F4/80⁺, CD40⁺, and B220⁺ cells) were also stained with fluorescent Abs specific for CD8 β , CD11c, 1-A^b, H-2K^b, and CD3 (B). All samples were analyzed by FACScan. The data shown are the percentage of positive cells in the gated each cell population.