Supplemental Figure Legends

Supplemental Figure 1. Loss of CTLA-4 results in heightened CD28-mediated T cell responses in vitro. Naïve $CD4^+$ T cells from WT or CTLA-$4^{-/-}$/B7-$1^{-/-}$/B7-$2^{-/-}$ mice were cultured in the presence of irradiated T cell-depleted C57BL/6 splenocytes and anti-CD3 (2 μg/ml) with rhIgG1-Fc (12.5 μg/ml) or hCTLA-4-Ig (12.5 μg/ml) for 48 h. The production of IL-17, IL-4, and IFN-γ was determined by ELISA. The values represent mean±SEM.

Supplemental Figure 2. Blocking CTLA-4-B7 interaction does not promote Th1 and Th2 cell differentiation. Naïve $CD4^+$ T cells from $CD28^{-/-}$ mice were cultured under Th1 or Th2 differentiation conditions in the absence or presence of 12.5 μg/ml hCTLA-4-Ig. IFN-γ- and IL-4-producing cells were assessed by intracellular staining. Numbers indicate the percentage of cells in gated $CD4^+$ T cells, and are representative of three independent experiments.

Supplemental Figure 3. CTLA-4-B7 interaction suppresses the expression of master Th17 transcription factors ROR-γt and ROR-α and inhibits IL-17A and IL-17F production. A. q-PCR analysis of mRNA for transcription factors ROR-γt (RORc) and ROR-α (RORa) of the Th17 cells differentiated in Figure 1B and 1C. Total RNA was prepared from ($2x10^5$) differentiated Th17 cells with indicated treatment using an RNeasy mini kit (Qiagen, Valencia, CA). Complementary DNA was synthesized with a high-capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA). Quantitative real-time reverse-transcriptase polymerase

chain reaction (qRT–PCR) was performed using the Applied Biosystems 7300 apparatus with Power SYBR® Green PCR Master Mix(Applied Biosystems) according to manufacturer's suggestions. The following primer sequences were used: forward primer 5-CAAGTCATCTGGGATCCACTAC-3' and reverse primer 5'-TGCAGGAGTAGGCCACATTACA-3' for RORc; forward primer 5'-TCTCCCTGCGCTCTCCGCAC-3' and reverse primer 5'-TCCAC AGATCTTGCATGGA-3' for RORa, and forward primer 5'-GGA GAA ACC TGC CAA GTA TG-3' and reverse primer 5'- GGA GTT GCT GTT GAA GTC G-3' for GAPDH. Relative expression of genes of interest was expressed as the comparative concentration of the gene product to the GAPDH product as calculated by Delta Delta Ct. Gene levels were relative to $CD28^{-/-}$ T cells with rhIgG1-Fc, which was set as 1. Significance was determined with an unpaired Student t test. * indicates P value <0.05, compared to $CD28^{-/-}$T cells; # indicates that P value <0.05 compared to WT T cells. Results are representative of three independent experiments. **B**. Naïve $CD4^+$ T cells from $CD28^{-/-}$ mice were activated with anti-CD3 and irradiated T-depleted splenocytes as feeders under Th17-polarized conditions as in Figure 1B. The differentiated $CD4^+$ T cells were restimulated with anti-CD3 (1 μg/ml) for 24 h, and production of IL-17, IL-17F, and IFN-γ in the supernatants was determined by ELISA. The values represent mean±SEM.

Supplemental Figure 4. CTLA-4 blockade does not potentiate Th17 cell proliferation. Purified naïve $CD4^+$ T cells from $CD28^{-/-}$mice were labeled with CFSE

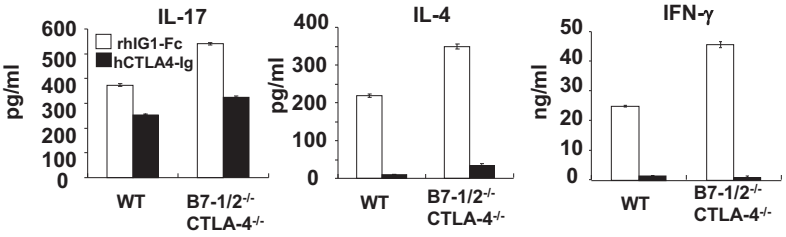
using CellTrace™ CFSE Cell Proliferation Kit (Invitrogen; San Diego, CA), then differentiated into Th17 cells as described in materials and methods. After 72 h, the CFSE level of differentiated Th17 cells were analyzed by flow cytometry. Numbers in the quadrant indicate the percentage of cells in the gated $CD4^+$ T cells, and the bracketed numbers indicate percentage of $CFSE^{low}$ $IL\text{-}17^+$ cells in the total $IL\text{-}17^+$ $CD4^+$ T cells.

Supplemental Figure 5. Blocking CTLA4-B7 interaction facilitates Th17 differentiation in vivo. The splenocytes obtained in Figure 2A were stimulated with anti-CD3 (1 μg/ml) and the supernatants were subjected to IL-17 ELISA. The data shown are representative of two independent experiments with a group of 4 mice.

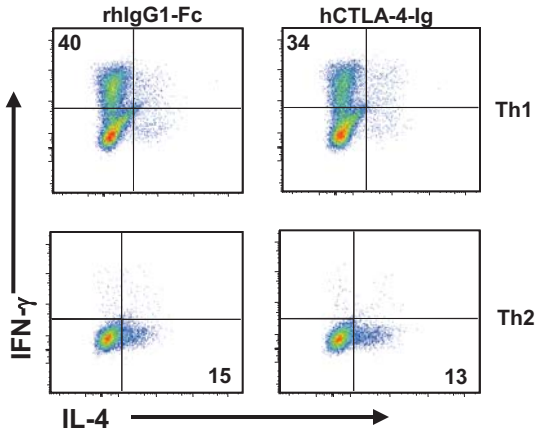
Supplemental Figure 6. Blocking CTLA-4-B7 interaction in vivo during EAM induction facilitates Th17 responses and T cell recruitment in the heart. A. Draining lymph node cells from MYHC-α-immunized $CD28^{-/-}$ mice with the indicated treatment were surface-stained with anti-CD4 and intracellularly stained with PE-anti-IL-17, and analyzed by flow cytometry. **B.** For in vivo BrdU labeling, MYHC-α-immunized $CD28^{-/-}$ mice treated with rhIgG1-Fc or hCTLA-4-Ig were i.p.injected with 1.0 mg BrdU (Sigma-Aldrich) every 12 h for three days starting from day 19 of initial immunization. In vivo $CD4^+$ T cell proliferation was determined by surface-staining with anti-CD4 in combination with intracellular staining of BrdU using BrdU staining kit (BD Biosciences) and the percentages of BrdU-stained cells

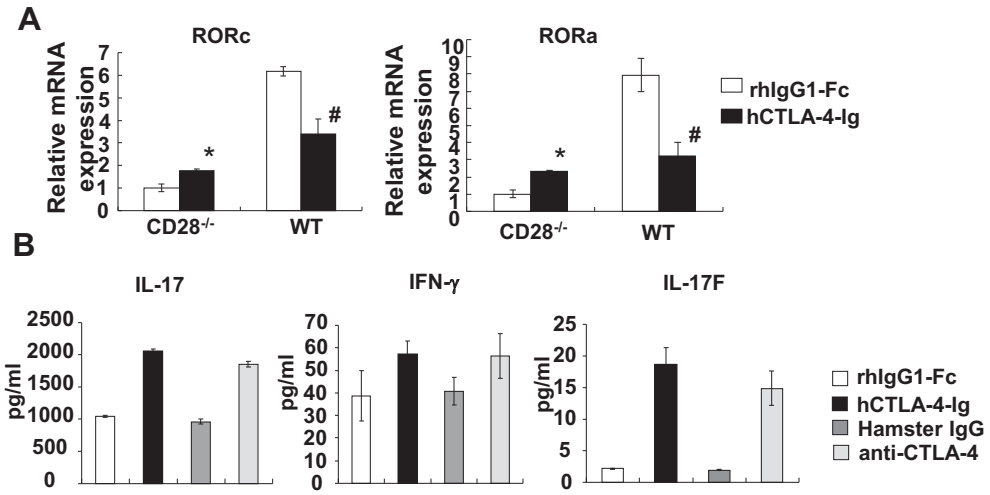
within gated $CD4^+$ T cells in the heart are shown.

Ying, et al., Supplemental Figure 1



Ying, et al., Supplemental Figure 2

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pg/ml
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pg/ml
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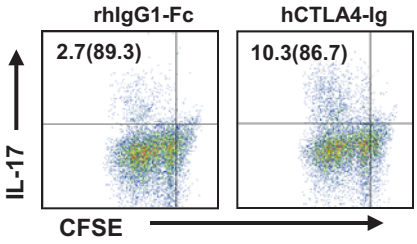
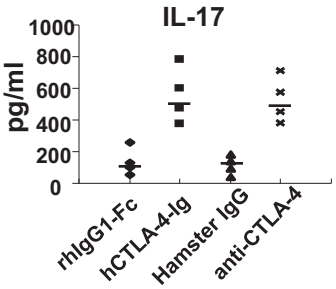
anti-CTLA-4

Ying, et al., Supplemental Figure 4

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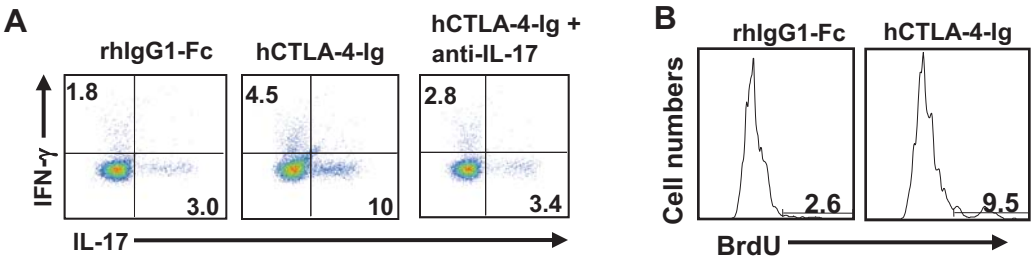
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Cell numbers
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9.5
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