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Figure legends

Figure 1. A role for IL-21 in CD4^{+} T cell-mediated GVHD. **(A)** Survival of recipients of wild type and IL-21R$^{-/-}$ CD4^{+} T cells. C57BL/6-DBA2-F1 mice were irradiated with 11 Gy and received 5 x 10^{6} IL-21R$^{-/-}$ BM with 5 x 10^{6} wild type or IL-21R$^{-/-}$ CD4^{+} T cells. Shown are combined data from two independent experiments. A total of 13 recipients each for wild type and IL-21R$^{-/-}$ CD4^{+} T cells were analyzed. P values were calculated by Logrank test. **(B)** Body weight after bone marrow transplantation. Asterisks denote statistical significance ($p<0.05$) by the Student's t-test.

Figure 2. Pathological analysis of recipients. H&E staining of liver, small intestine, and skin are shown (Original magnification was x 400). In recipients of wild type CD4^{+} T cells, cell infiltration around portal vein (P), around bile duct (B), and into interstitial region in small intestine are evident. Arrowheads in small intestine indicate apoptotic bodies near the surface of crypts. These changes were barely seen in recipients of IL-21R$^{-/-}$ CD4^{+} T cells. Skin did not show any significant difference between recipients of wild type and IL-21R$^{-/-}$ CD4^{+} T cells. Shown is a representative result of 6 mice analyzed in each group. Only one recipient of IL-21R$^{-/-}$ CD4^{+} T cell showed apoptotic bodies in the lumens of intestine and infiltration around the bile duct and portal vein, as was observed in the recipients of wild type CD4^{+} T cells.

Figure 3. Cytokine production by bulk splenocytes before and after CD4^{+} T cell-transplantation. At day 14 and 21 after transplantation, splenocytes (5 x 10^{5}) were taken and stimulated with anti-CD3/CD28 antibodies for 18 hours. Concentrations of cytokines in the supernatants were determined by ELISA. In total 12-13 recipients of wild type CD4^{+} T cells and 10 recipients of IL-21R$^{-/-}$ CD4^{+} T cells were analyzed. Prior to transplantation, 5 wild type and 8 IL-21R$^{-/-}$ mice were analyzed. P values were calculated by the Student's t-test. Asterisks denote statistical significance ($p<0.05$). At day 14-21 after transplantation, the proportion of donor cells in the spleen was >95% in our settings.

Figure 4. Cytokine production by splenic CD4^{+} T cells before and after

transplantation. (**A**) Absolute number of donor H-2K^d-negative CD4$^+$ T cells in the spleen. The number of donor CD4$^+$ T cells was determined by multiplying the number of splenocytes by the percentage of H-2K^d-negative CD4$^+$ T cells. Each dot depicts the number of donor CD4$^+$ T cells in a mouse. Lines in the middle of dots indicate the average. Total mice analyzed were 15 recipients of wild type CD4$^+$ T cells and 12 recipients of IL-21R$^{-/-}$ CD4$^+$ T cells. (**B**) Intracellular staining of splenocytes after anti-CD3/CD28 stimulation. Splenocytes (1 x 10^6) were stimulated with anti-CD3/CD28 antibodies for 5-6 hours and stained with either anti-IFN-γ or anti-TNF-α antibody in combination with anti-CD4 antibody. A total of three recipients in each group were analyzed and a representative result is shown. (**C**) Cytokine production by CD4$^+$ T cells *in vitro*. At day 14 or 21 after transplantation, splenic CD4$^+$ T cells (5 x 10^5) were purified and stimulated with anti-CD3/CD28 antibodies for 18 hours. Concentrations of cytokines in the supernatants were determined by ELISA. Twelve mice were analyzed in each group after transplantation. Total mice analyzed before transplantation were 5-6 wild type and 8-9 IL-21R$^{-/-}$ mice. Asterisks denote statistical significance ($p<0.05$).

Figure 5. IL-17$^{-/-}$ CD4$^+$ T cells induced lethal GVHD. Survival of recipients of wild type (WT), IL-21R$^{-/-}$ (IL-21RKO), or IL-17$^{-/-}$ (IL-17KO) CD4$^+$ T cells. Lethally irradiated (11 Gy) C57BL/6-DBA2-F1 mice were transplanted with 5 x 10^6 IL-21RKO-BM and 5 x 10^6 WT (filled square), IL-21R KO (filled triangle), or IL-17 KO (filled circle) CD4$^+$ T cells. The data represent the combined result of two independent experiments.

Figure 6. Increase of splenic Treg cells. (**A**) Up-regulation of serum TGF-β1. Serum TGF-β1 concentrations at the indicated day after transplantation were determined by ELISA per the manufacturer's instruction. Three samples from recipients of wild type CD4$^+$ T cells and four samples from recipients of IL-21R$^{-/-}$ CD4$^+$ T cells at day 6, 23 samples from recipients of wild type CD4$^+$ T cells and 25 samples from recipients of IL-21R$^{-/-}$ CD4$^+$ T cells at day 14, and 8 samples from recipients of wild type CD4$^+$ T cells and 7 samples from recipients of IL-21R$^{-/-}$ CD4$^+$ T cells at day 21 were analyzed. Asterisks denote statistical

significance ($p<0.05$). (**B**) The percentage and absolute number of splenic Foxp3$^+$CD4$^+$ regulatory T cells at day 14 after transplantation. The left panel shows a representative flow cytometric result from 8-9 similar samples. The right panel indicates the number of all samples; the averages are indicated by the horizontal bars.

Figure 7. An impaired CD4 allo-reaction is not dependent on CD25$^+$CD4$^+$ T cells. CD4 allo-reaction in vitro was impaired after transplantation, and this impairment was not restored by CD25$^+$ T cell depletion. (**A**) At day 14 after transplantation, 1 x 10^5 sorter-purified splenic CD4$^+$ or CD25-negative CD4$^+$ T cells (>98% purity) were cultured with 4 x 10^5 irradiated allogeneic C57BL/6-DBA2-F1 splenocytes for 4 days. The cells were pulsed with 1 μCi of [^{3}H]-thymidine for the last 24 hours. Relative thymidine uptake to the value of wild type CD4$^+$ T cells is depicted. (**B**) Culture was the same as in (**A**), but IFN-γ concentrations in the supernatants were determined by ELISA. (**C**) Sorter-purified splenic CD4$^+$ or CD25-negative CD4$^+$ cells from non-transplanted mice were cultured with irradiated allogeneic C57BL/6-DBA2-F1 splenocytes. Relative thymidine uptake to the number of wild type CD4$^+$ T cells is depicted. Asterisks denote statistical significance ($p<0.05$).

Figure 8. The ameliorated GVHD induced by IL-21R$^{-/-}$ CD4$^+$ T cells is not dependent on CD25$^+$CD4$^+$ T cells. The ameliorated GVHD induced by IL-21R$^{-/-}$ CD4$^+$ T cells was not exacerbated by depletion of CD25$^+$CD4$^+$ T cells. Body weight (**A**) and survival (**B**) of recipients are shown. Comparison between recipients of wild type CD4$^+$ T cells and IL-21R$^{-/-}$ CD4$^+$ T cells, and further comparison with and without anti-CD25 antibody treatment were performed. Control antibody used was non-specific rat IgG. (**C**) Splenic CD25$^+$CD4$^+$ T cells and splenic Foxp3$^+$CD4$^+$ T cells at day 14 after transplantation with or without anti-CD25 antibody treatment were analyzed by flow cytometry (upper panels). The lower panels indicate the mean of percent reduction of CD25$^+$CD4$^+$ and Foxp3$^+$CD4$^+$ cells from three similar results. (**D**) Foxp3 mRNA level in CD25-negative CD4$^+$ T cells at day 21 after transplantation. Cell-sorter purified CD25-negative CD4$^+$ T cells were subjected to mRNA purification,

reverse-transcriptase-treatment, and Taqman® quantitative PCR. Relative value to β-actin was denoted.

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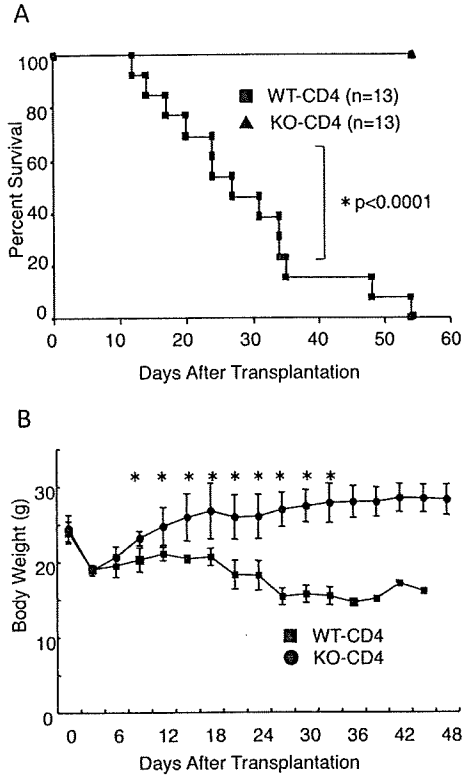

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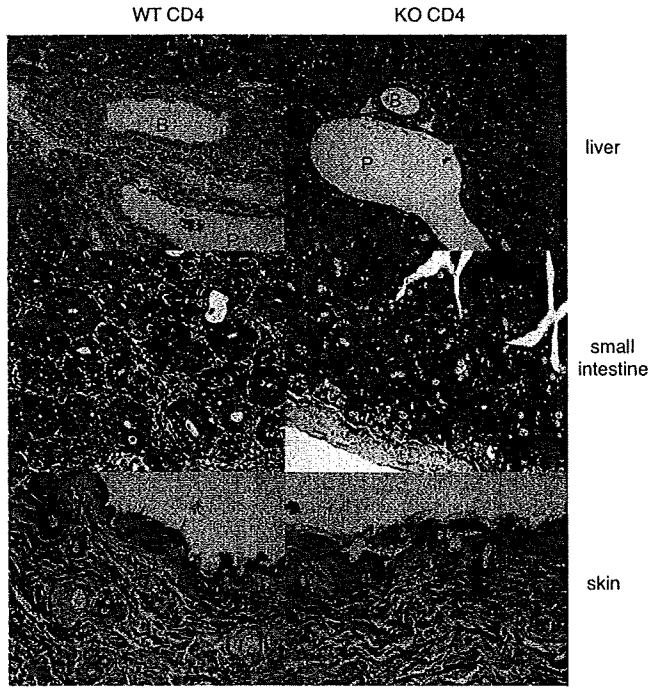

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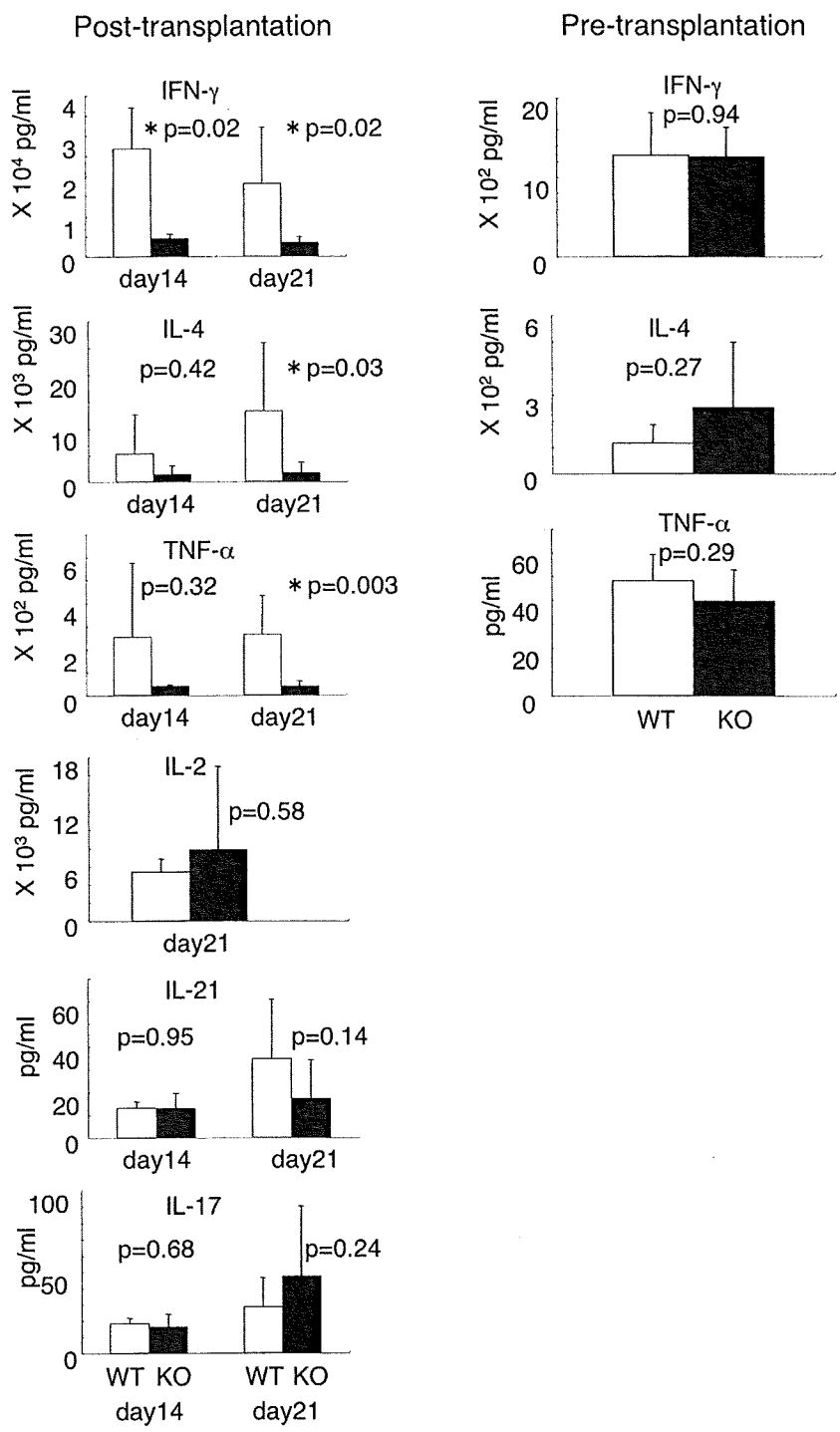

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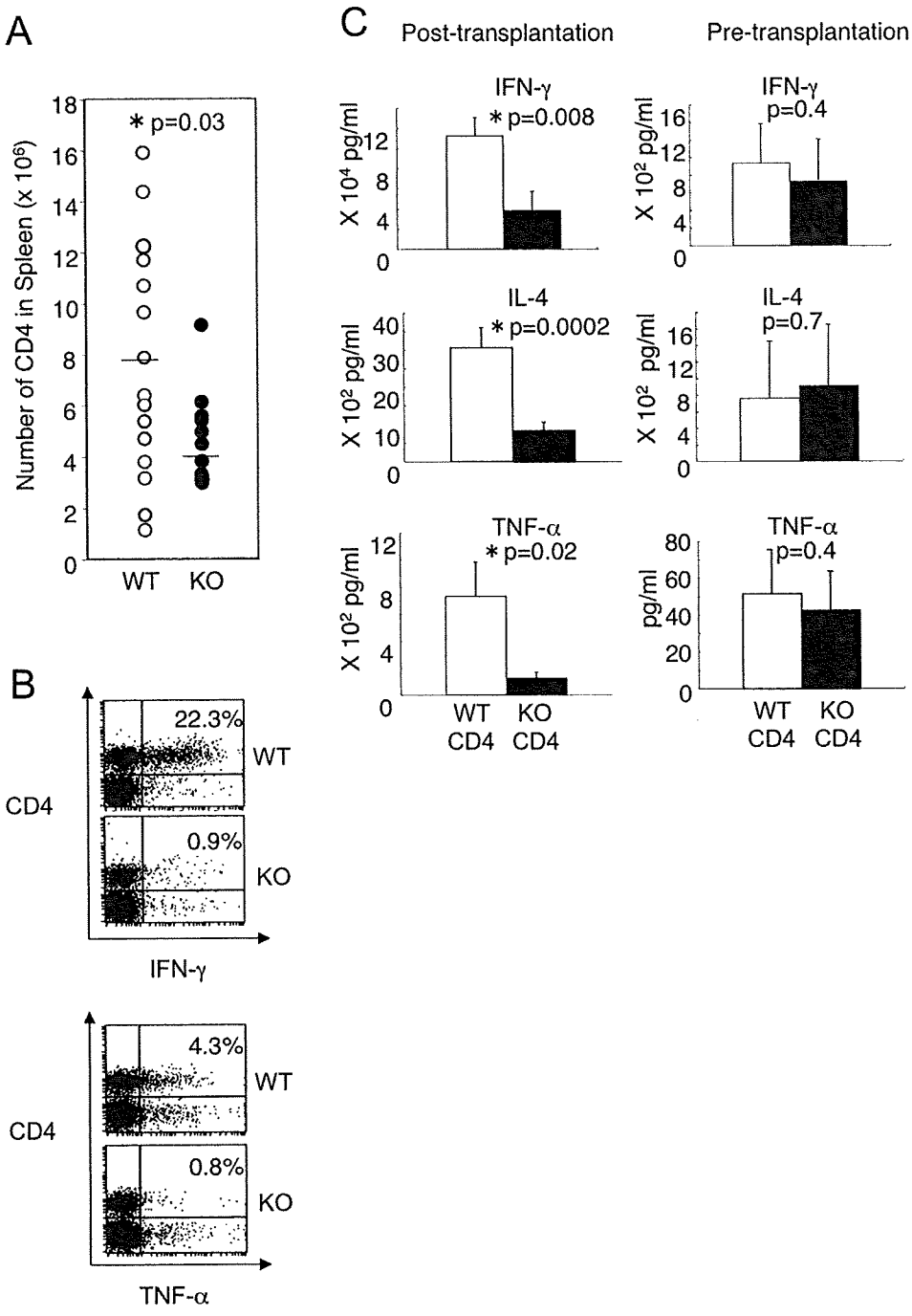

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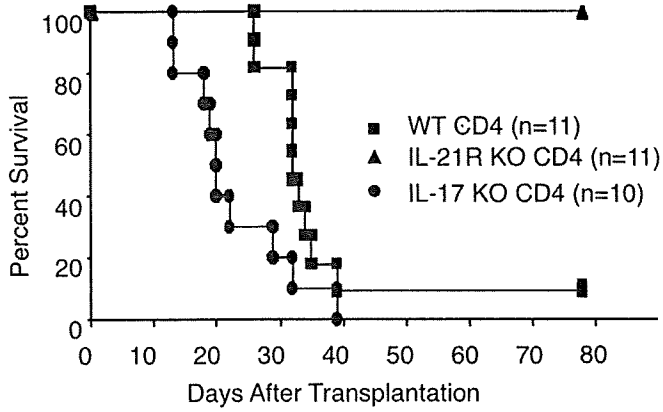

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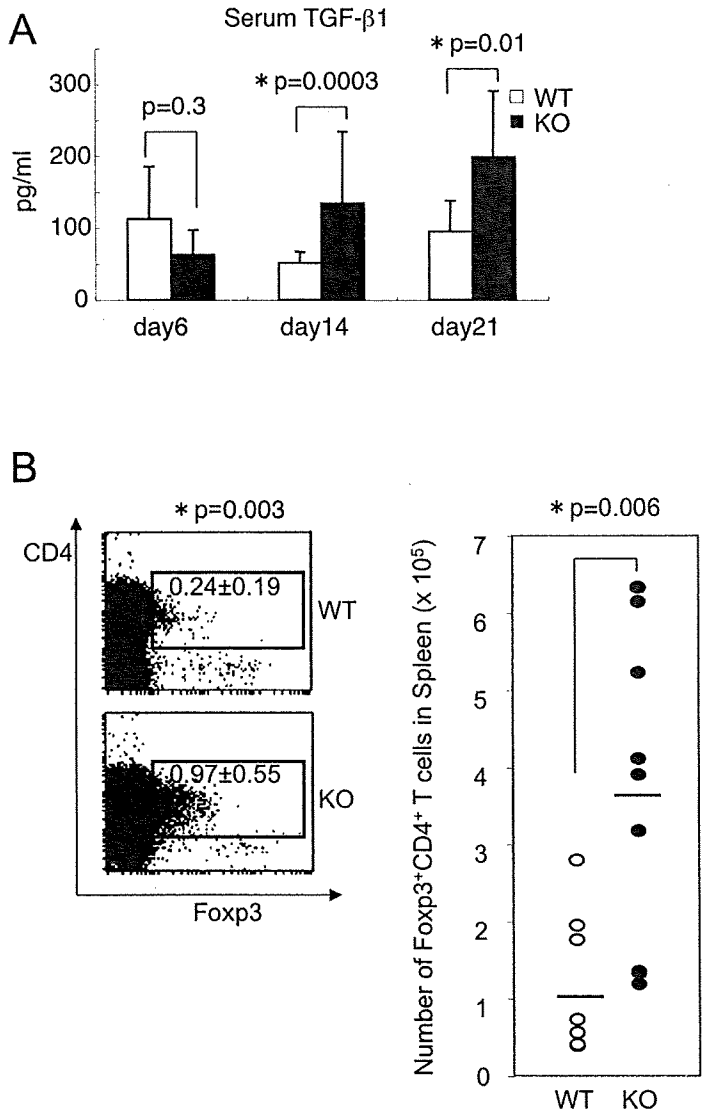

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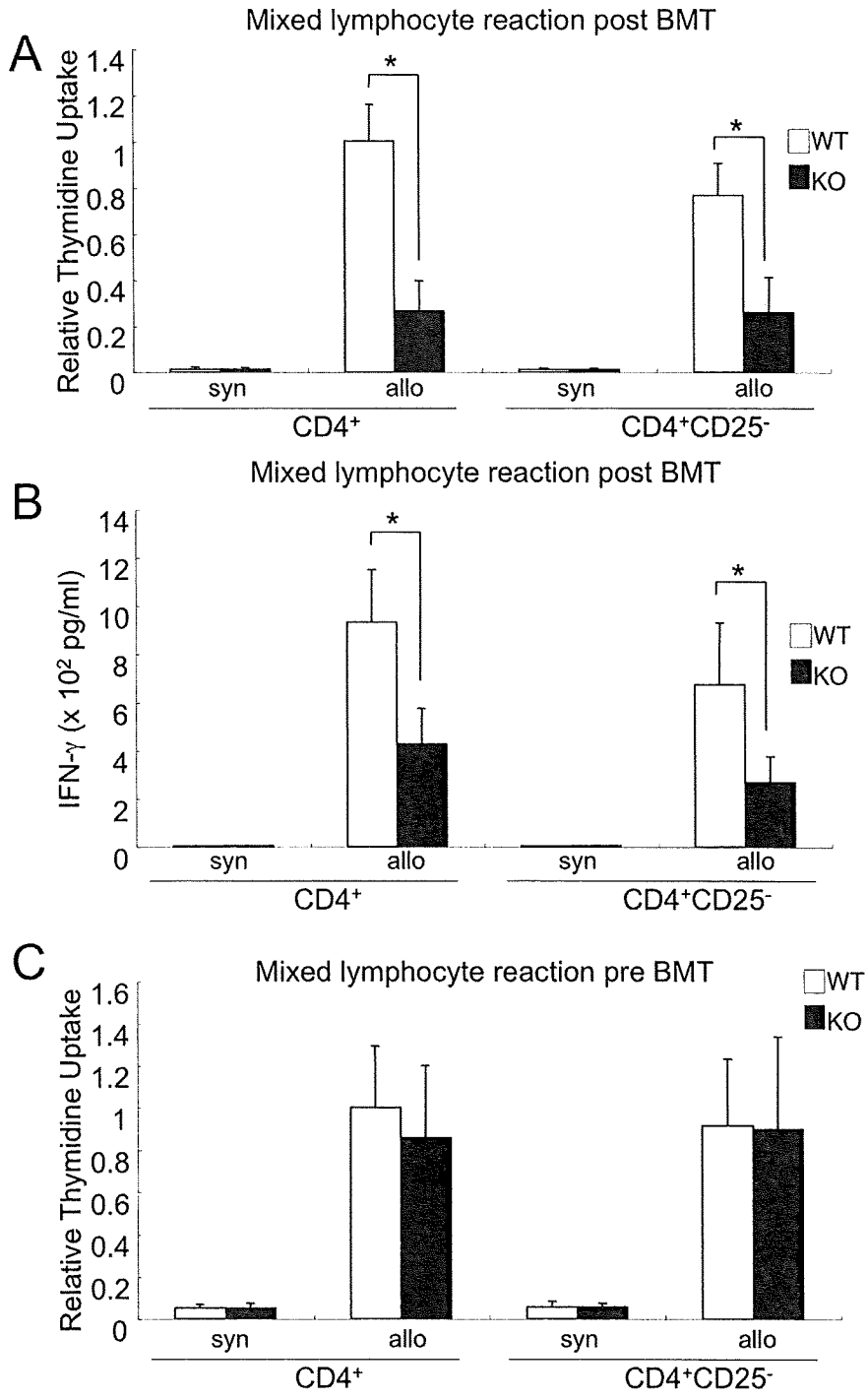

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