Supplemental Fig. 1

Role of TGF-β signaling on cell surface expression of Fas by $CD4^+CD25^-$ T cells and $CD4^+CD25^+$ Tregs

(A) $CD4^+CD25^-$ T cells isolated from the spleen of C57BL/6 mice were stimulated with plate-bound anti-CD3/anti-CD28 antibodies in the presence or absence of TGF-β in media supplemented with IL-2. Cells harvested at day 3 were stained for Fas expression and analyzed by flow cytometry. Solid line represents data from cells stimulated in the absence of exogenous TGF-β. Dotted line shows data from cells stimulated in the presence of exogenous TGF-β. (B) $CD4^+CD25^+$ Tregs isolated from the spleen of C57BL/6 mice were stimulated with plate-bound anti-CD3/anti-CD28 antibodies in the presence or absence of SB431542 (TGF-β super-family type I receptor kinase inhibitor) in media supplemented with IL-2. Cells were harvested after 2 days and stained for Fas expression and analyzed by flow cytometry. Solid line represents data from cells stimulated in the presence of DMSO. Dotted line shows data from cells stimulated in the presence of SB431542. The data are representative of three independent experiments.

Supplemental Fig. 2

Effect of TGF-β and IL-6 on PICA by $CD4^+CD25^-$ T cells from BALB/c mice

$CD4^+CD25^-$ T cells isolated from the spleen of BALB/c mice were stimulated with plate-bound anti-CD3/anti-CD28 antibodies or plate-bound anti-CD3 and soluble anti-CD28 antibodies in the presence or absence of TGF-β or TGF-β plus IL-6 in media supplemented with IL-2. (A) Cells harvested at day 3 were re-stimulated with PMA plus

Ionomycin for 4 hours in the presence of monensin and stained for IL-9 and IL-17, then analyzed by flow cytometry. (B) Culture supernatant was collected at day 3. IL-4, IL-17, and IFN-γ production were determined by ELISA. ** $p<0.01$, * $p<0.05$.

Supplemental Fig. 3

Effect of TGF-β and IL-6 on IFN-γ expression by $CD4^+CD25^-$ T cells

$CD4^+CD25^-$ T cells isolated from the spleen of C57BL/6 mice were stimulated with either plate-bound anti-CD3/anti-CD28 antibodies or plate-bound anti-CD3 and soluble anti-CD28 antibodies in the presence or absence of TGF-β or TGF-β plus IL-6 in media supplemented with IL-2. (A) Culture supernatant was collected at day 3. IFN-γ production was determined by ELISA. (B) Cells harvested at day 3 were re-stimulated with PMA plus Ionomycin for 4 hours in the presence of monensin and stained for IFN-γ, then analyzed by flow cytometry to determine the frequency of IFN-γ^+ cells. Total number of IFN-γ^+ cells was determined based on the total live cell number and the frequency of IFN-γ^+ cells. The data are representative of three independent experiments. ** $p<0.01$, * $p<0.05$.

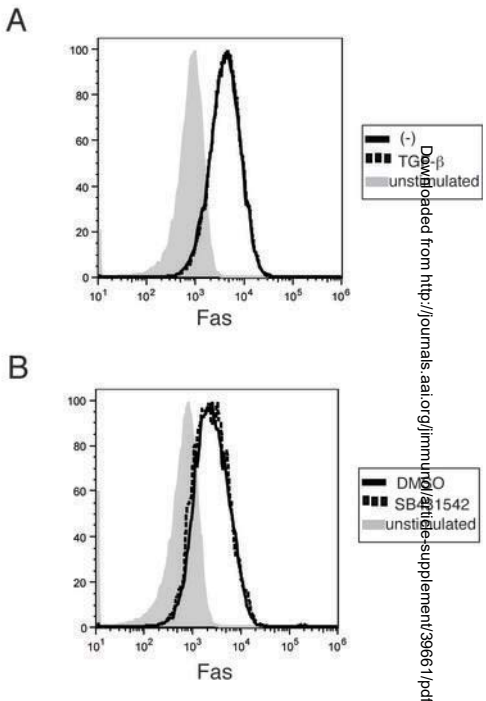
Supplemental Fig. 4

Effect of IL-6 on total live cell number

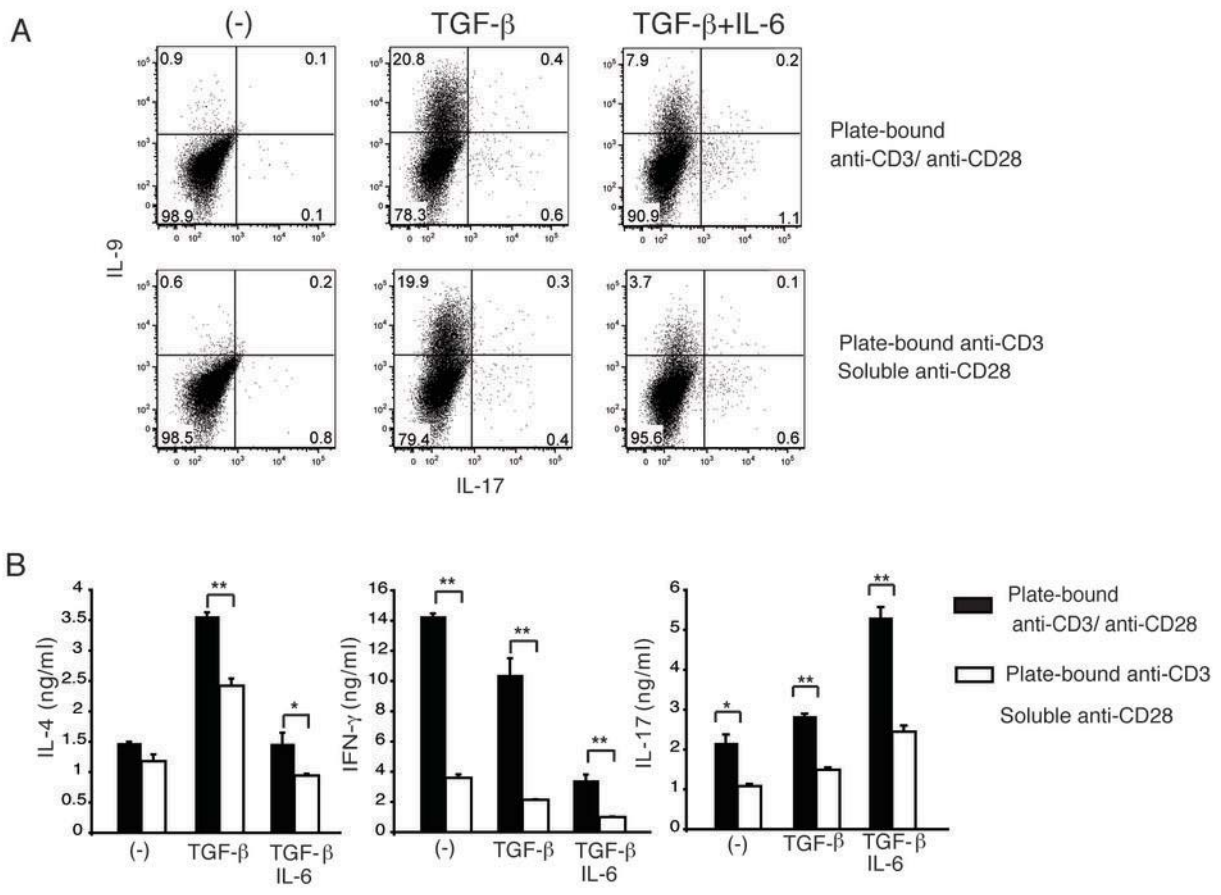
$CD4^+CD25^-$ T cells isolated from the spleen of C57BL/6 mice were stimulated with either plate-bound anti-CD3/anti-CD28 antibodies or plate-bound anti-CD3 and soluble anti-CD28 antibodies in the presence or absence of TGF-β or TGF-β plus IL-6 in media

supplemented with IL-2. Total live cell numbers were determined by Trypan Blue exclusion test.

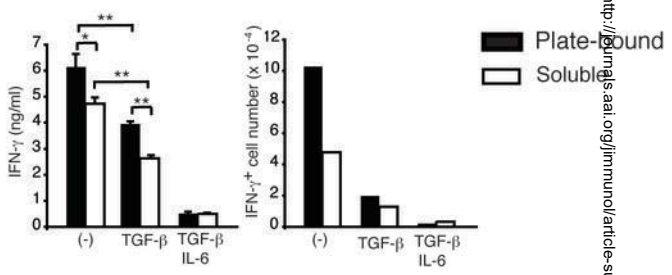
Supplemental Fig. 1



Supplemental Fig. 2



Supplemental Fig. 3



Supplemental Figure 4