

Figure S1 A2780 cells induce phenotypic differentiation of CD8⁺ T cells in a transwell coculture system. **(a)** Expression of CD25, GITR, CTLA-4, Foxp3, and CD28 mRNA in CD8⁺ T cells cultured with or without A2780 was analyzed by quantitative real-time PCR. **(b)** CD8⁺ T cells in the transwell coculture system with A2780 expressed higher levels of CTLA-4 and Foxp3 molecules and lower levels of CD28 than that in CD8⁺ T cells cultured alone. The expression of CD25 and GITR was low, and there was no significant difference between CD8⁺ T cells cultured with and without A2780. **(c)** Representative dot plots of CD8 and Foxp3, CD28, and CTLA-4 staining of CD8⁺ T cells cultured with or without A2780. * $P < 0.05$, ** $P < 0.01$.

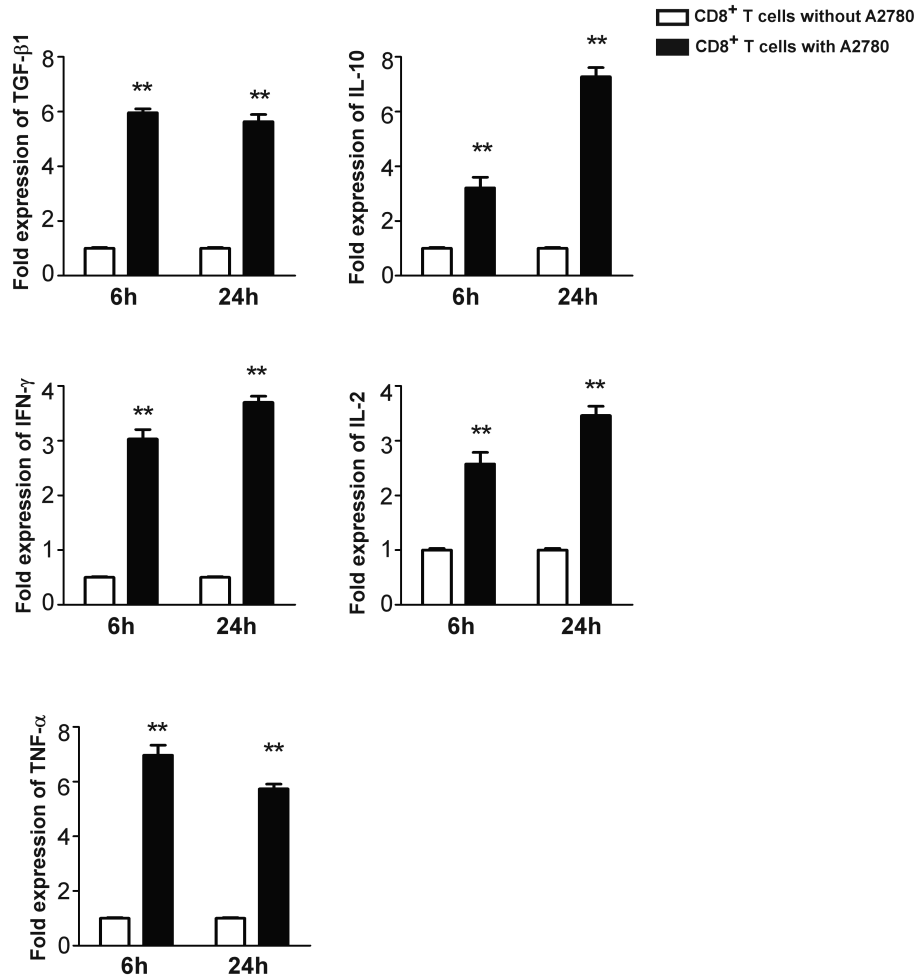


Figure S2 Cytokine expression profile of in vitro-differentiated CD8⁺ Treg cells. Real-time RT-PCR analysis of TGF-β1, IL-10, IFN-γ, IL-2, and TNF-α mRNA expression in CD8⁺ T cells cultured with or without A2780. Total RNA was isolated from CD8⁺ T cells stimulated with anti-CD3/CD28 mAbs for 6 or 24 h. ** $P < 0.01$.

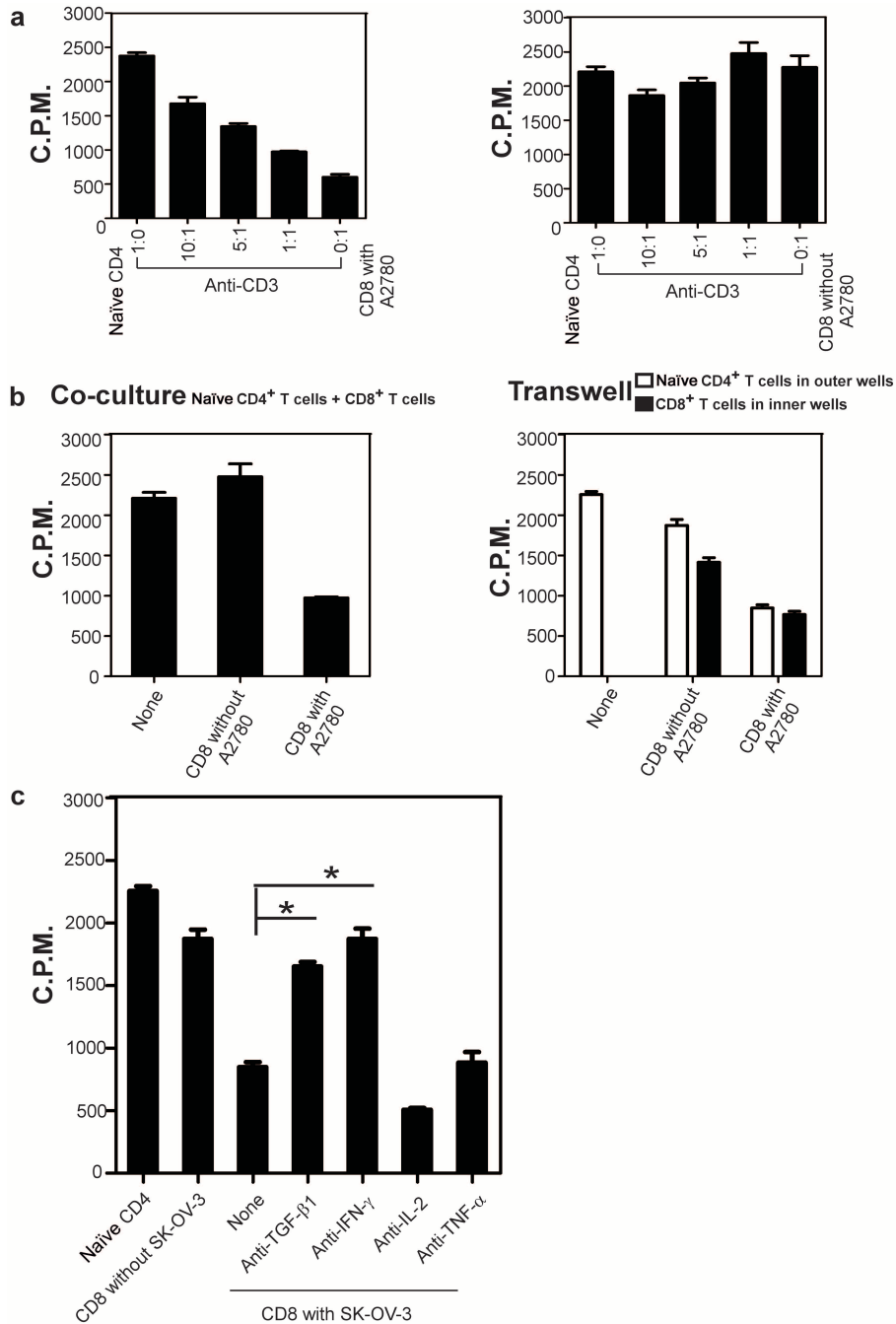


Figure S3 In vitro differentiated CD8⁺ T cells suppressed the proliferation of naive CD4⁺ T cells. **(a)** CD8⁺ T cells cultured with A2780 suppressed naive CD4⁺ T cell proliferation in a dose-dependent manner with stimulation by anti-CD3 mAb. Conversely, CD8⁺ T cells cultured alone did not have the suppressive activity. The proliferative response was determined by [³H] thymidine uptake. **(b)** Soluble factors were partially required for CD8⁺ T cells cultured with A2780 to exert their suppressive effects. In the transwell system, equal numbers of naive CD4⁺ T cells were cultured in the outer wells, and CD8⁺ T cells cultured with/without A2780 were cultured in the inner wells. **(c)** The suppressor effect of CD8⁺ T cells cultured with A2780 was in part blocked when the assay was performed in the presence of an anti-TGF-β1, anti-IFN-γ, anti-IL-2, anti-TNF-α mAb. Data were presented as the mean ± SEM of three independent experiments. * *P* < 0.05.

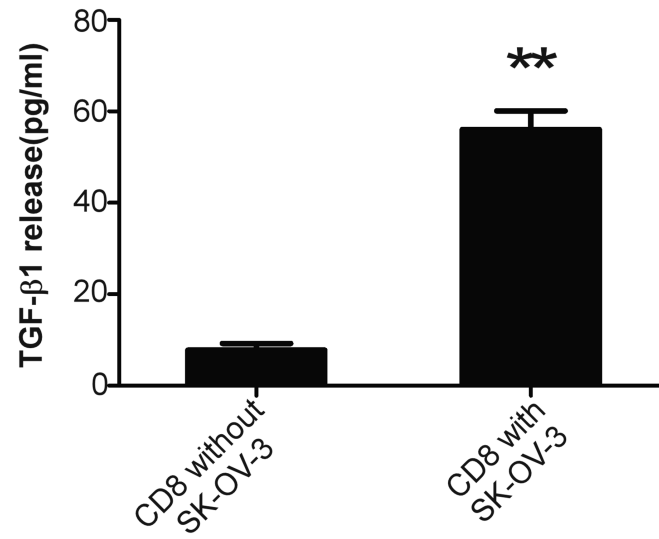


Figure S4 Expression of TGF-β1 in CD8⁺ T cells and SK-OV-3 transwell system. Production of TGF-β1 in medium of transwell system that CD8⁺ T cells cultured with/without SK-OV-3 was detected by ELISA, ** $P < 0.01$.