Supplementary Materials and Methods

Mixed Lymphocyte Reaction (MLR)

MLR assays were performed as previously described[1]. Briefly, purified T cells from the spleen of the recipient mice at 10 days after transplantation were used as responder cells and pre-labeled with 5 μM CFSE (Invitrogen, USA). Spleen cells obtained from naive BALB/c mice were used as stimulator cells and pretreated with mitomycin C. The proliferation was analyzed by flow cytometry.

T Cell Differentiation

Naïve $CD4^+$ T cells were purified from spleens by using a naïve CD4+ T cell Isolation Kit (Biolegend, USA). The naïve $CD4^+$ T cells were cultured for three days under Th1/Th17 differentiation conditions with various concentrations of 2-D-gal. For Th1 cells polarization, naïve $CD4^+$ T cells were cultured with plate-bound anti-CD3, soluble anti-CD28 (1μg/ml，eBioscience，USA), anti-IL-4 (10 μg/ml, Biolegend, USA), IL-2 (50 U/ml, PeproTech, USA), and IL-12 (1 ng/ml, PeproTech, USA). For Th17 cells polarization, naïve $CD4^+$ T cells were cultured with plate-bound anti-CD3, soluble anti-CD28 (1μg/ml，eBioscience,USA), anti-IFN-γ(10 μg/ml, eBioscience, USA), anti-IL-4 (10 μg/ml, eBioscience, USA), IL-6 (20 ng/ml, PeproTech, USA), TGF-β (10 ng/ml, PeproTech, USA) , and IL-23(10 ng/ml, PeproTech, USA).

Immunoprecipitation

Purified T cells were incubated with anti-CD3/CD28 antibodies(1 ug/ml)

in the absence or presence of 2-D-gal(1.2mM) for 24h and then lysed with 500 μL RIPA buffer containing with protease inhibitor and phosphatase inhibitor. Cell extracts were incubated with antibodies at 4 ◦C overnight. Protein A/G agarose (Santa Cruz Biotechnology, USA) was added and incubated at 4 ◦C for 4–6 h. After washing three times in lysis buffer, the pull downed samples were boiled for 5 min. The eluted proteins were separated by SDS-PAGE, followed by Western blot and lectin blot analysis.

Supplementary Figures

Figure S1

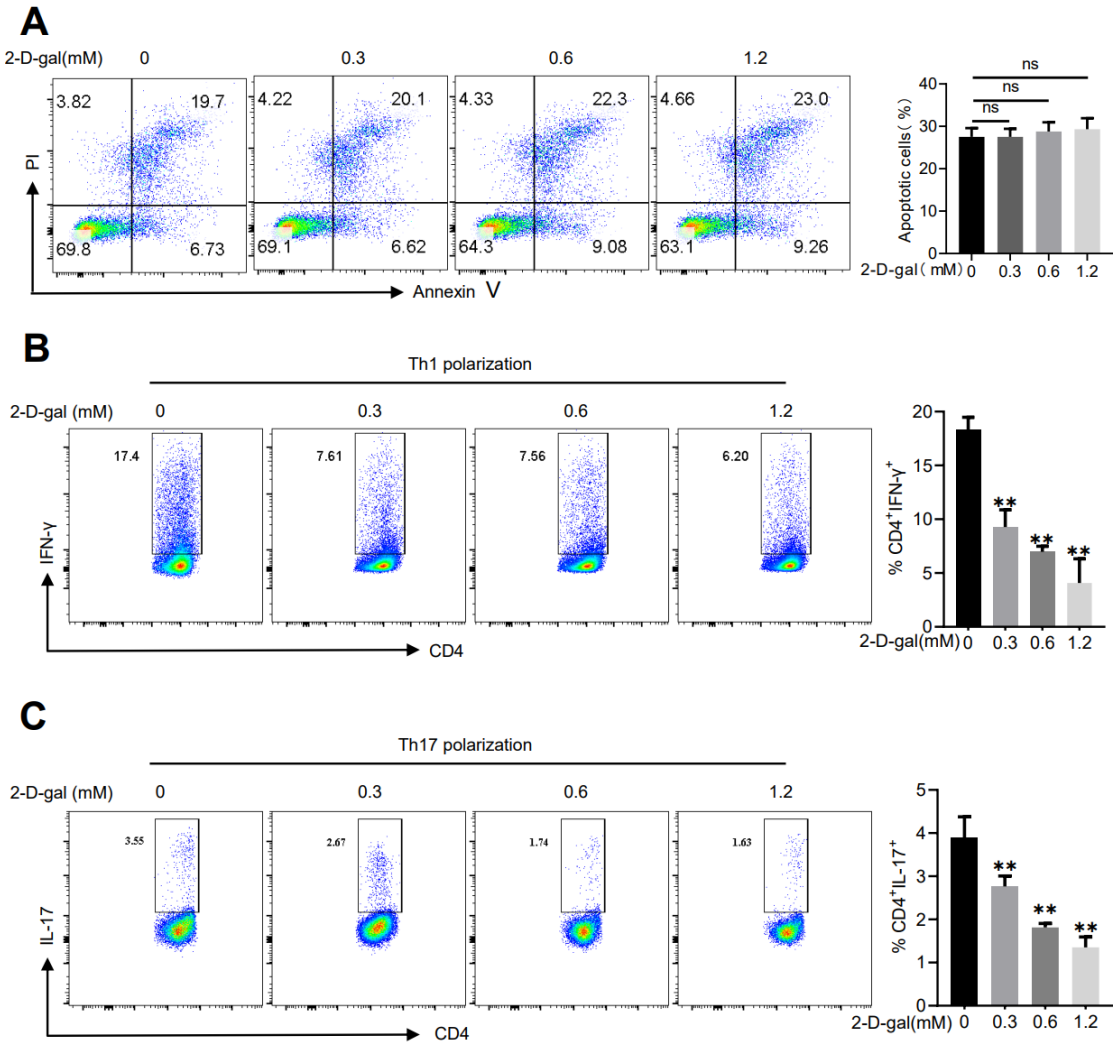

Figure S1. 2-D-gal in vitro experiments. (A) After 2-D-gal treatment, T cells apoptosis was analyzed by flow cytometry. (B) The frequency of Th1 cells were assessed using flow cytometry. (C) The frequency of Th17 cells were assessed using flow cytometry. Data represent the mean ± SEM of three independent experiments. ns, no significant, *p<0.05, **p<0.01.

Figure S2

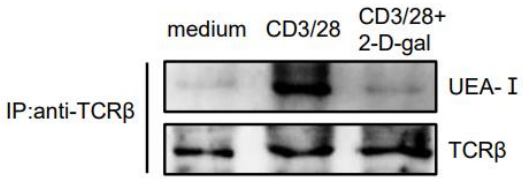

Figure S2. Fucosylation on TCR. The purified T cells were stimulated with anti-CD3/28 antibodies in the presence or absence of 2-D-gal (1.2 mM) for 48 hours. After the immunoprecipitation, the fucosylation level on TCR was assessed by western blotting. The data represent three independent experiments with similar results.

Figure S3

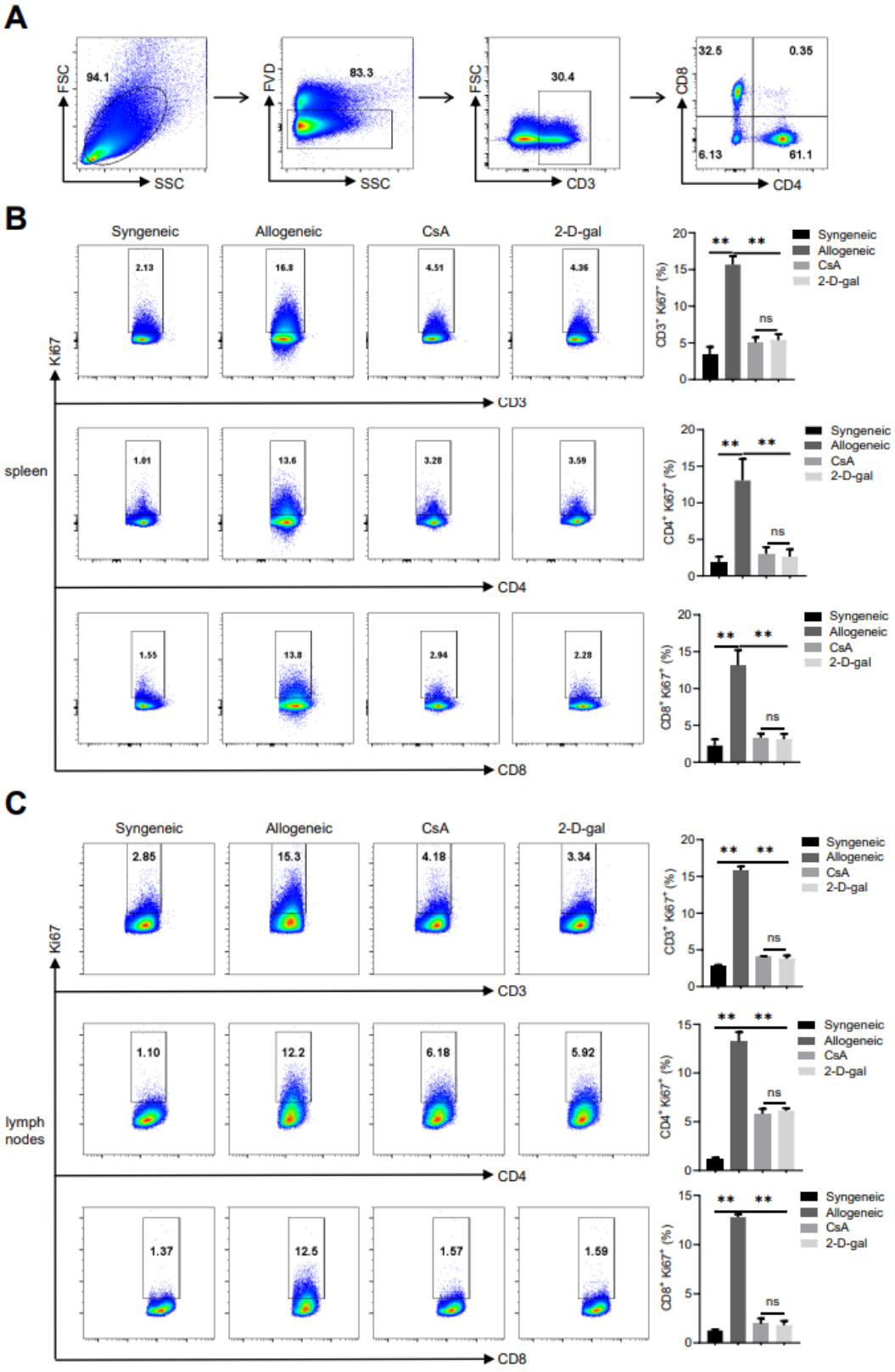

Figure S3. The frequency of Ki67⁺ on T cells in vivo. (A) The gating strategy of Ki67+ T cells. (B) Spleens were collected, and single-

cell suspensions of splenocytes were obtained. Total cell count was determined, and the frequencies of $CD3^{+}Ki67+$, $CD4^{+}Ki67^{+}$, and $CD8^{+}Ki67^{+}$, T cells were assessed using flow cytometry. (C) Lymph nodes were collected, and single-cell suspensions of cells were obtained. Total cell count was determined, and the frequencies of $CD3^{+}$ $Ki67^{+}$, $CD4^{+}$ $Ki67^{+}$, and $CD8^{+}$ $Ki67^{+}$, T cells were assessed using flow cytometry. Data represent the mean ± SEM of three independent experiments. ns, no significant, *$p<0.05$, **$p<0.01$.

Figure S4

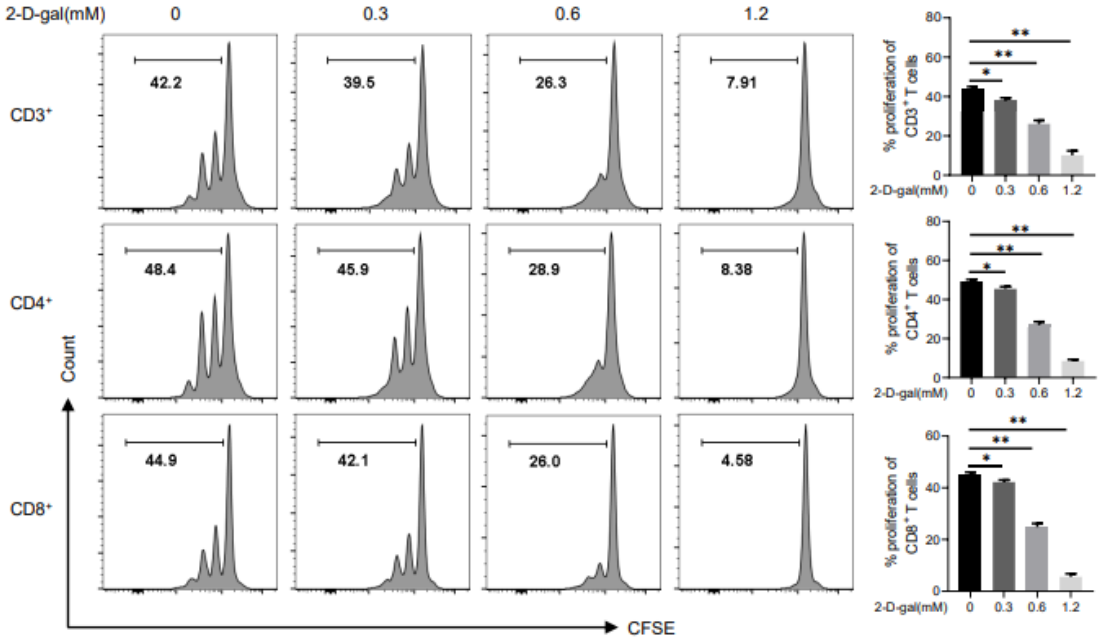

Figure S4. MLR assay of 2-D-gal. T cells were isolated from the spleen of the recipient mice at 10 days after skin transplantation and used as responder cells. Purified T cells from the spleen of the recipient mice at 10 days after transplantation were used as responder cells and pre-labeled with 5 μM CFSE (Invitrogen). Spleen cells obtained from naive BALB/c mice were used as stimulator cells and pretreated with mitomycin C. The proliferation was analyzed by flow cytometry. Data represent the mean ± SEM of three independent experiments. ns, no significant, *p<0.05, **p<0.01.

References:

1. Gao C, Jiang J, Ma P, et al. Arsenic Trioxide Induces T Cell Apoptosis and Prolongs Islet Allograft Survival in Mice. *Transplantation.* 2015;99(9): 1796-1806.