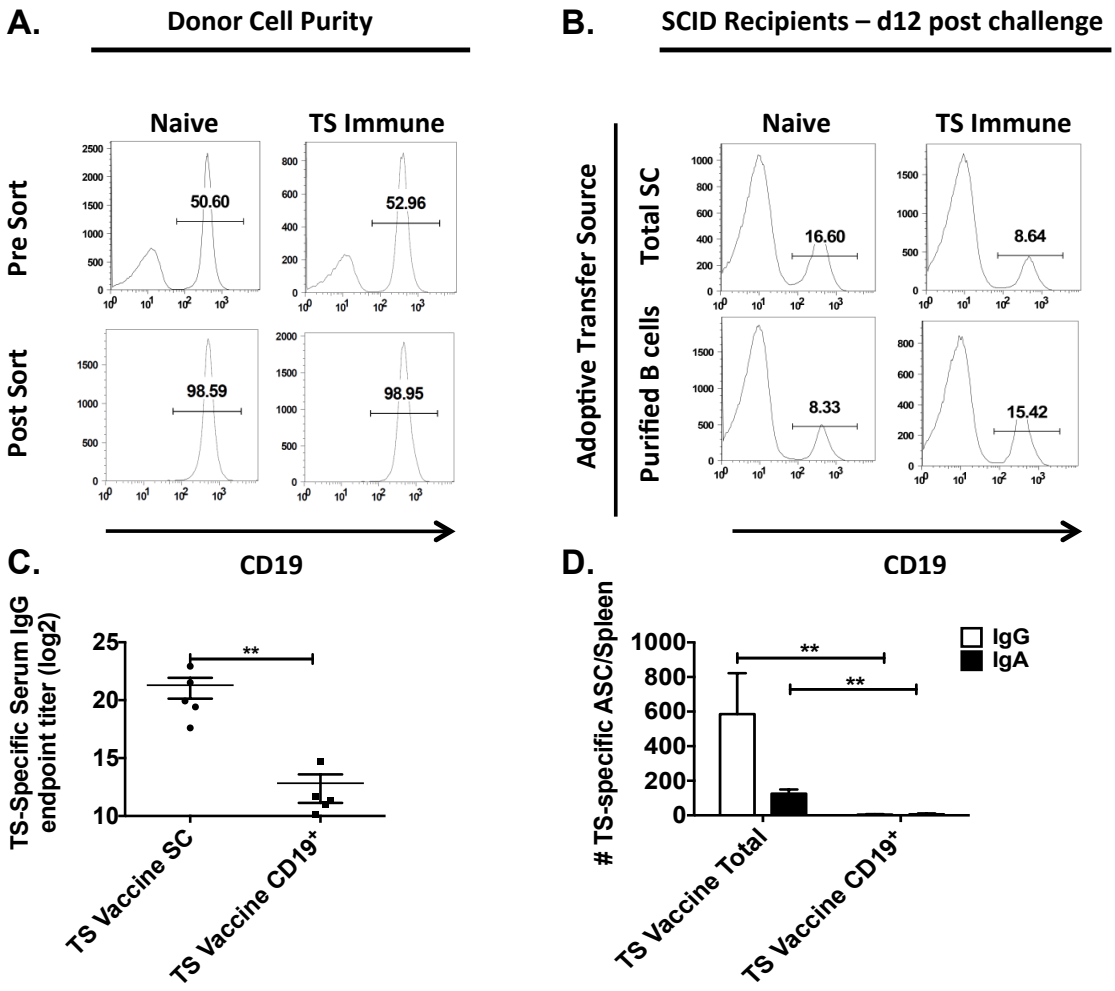
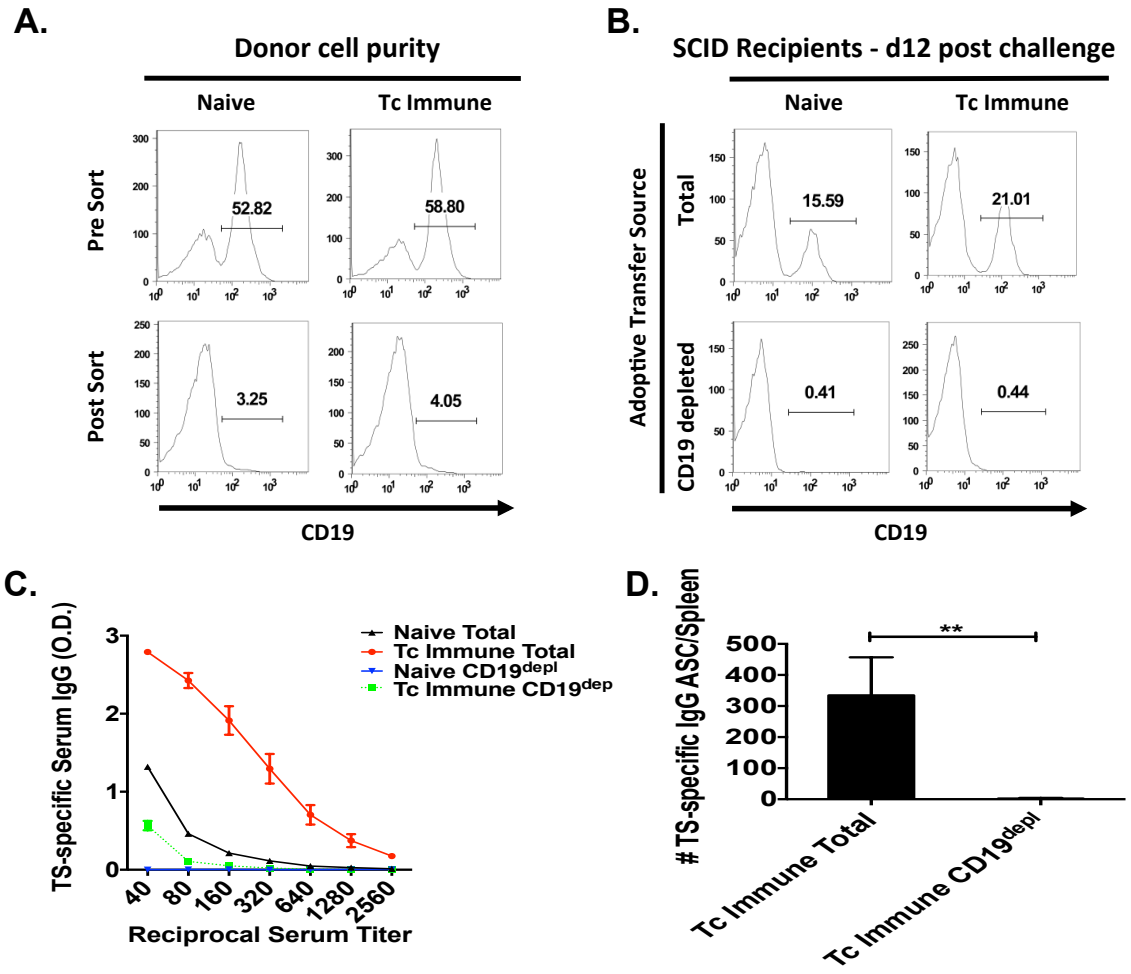
Supplemental Figure 1



Supplemental Figure 1: B cells need T cell help to become fully activated. Ten to twenty Tc immune and naïve BALB/c mice were sacrificed, splenocytes pooled together from each group and either used for total SC transfer or $CD19^+$ T cells were purified from SC using $CD19^+$ microbeads (Miltenyi Biotech). **(A)** The percentages of $CD19^+$ B cells were assessed pre- and post-sort. Cells were stained with anti-CD8 FITC, anti-CD3 PE, anti-CD4 PerCP and anti-CD19 PE-Cy7 and analyzed with a LSR II Flow cytometer and FlowJo software. One mouse spleen equivalent of total or purified B cells from Tc immune or naïve BALB/c mice were transferred i.v. into naïve SCID mice. One day later, these

mice were orally challenged with *T. cruzi*. Twelve days later, recipient mice were sacrificed and assessed for **(B)** the percentage of $CD19^+$ B cells in the spleen, **(C)** levels of TS-specific serum IgG via Elisa and the **(D)** absolute number of TS-specific IgG and IgA antibody secreting cells (ASC) per spleen. N=5 mice/group. ** $p < 0.01$, Mann-Whitney U test.

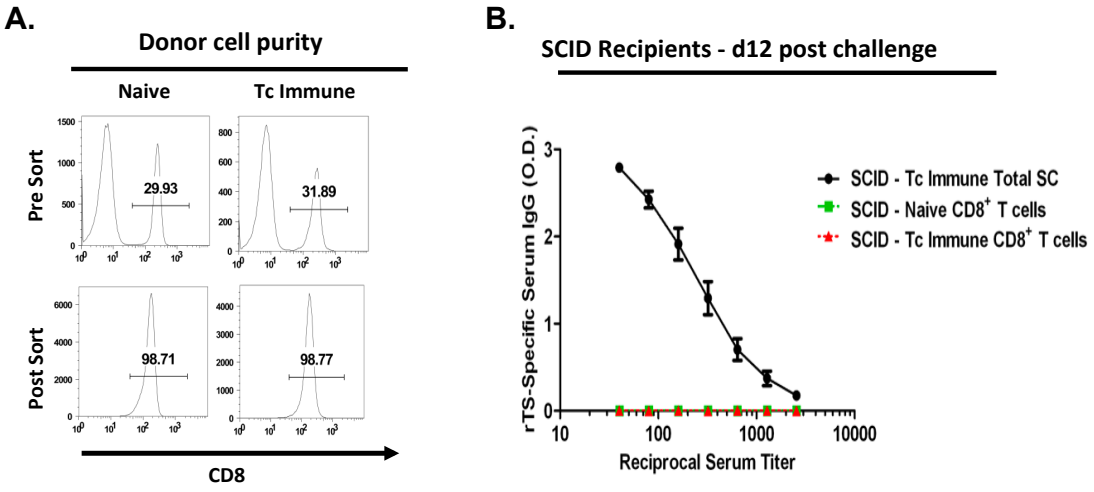
Supplemental Figure 2



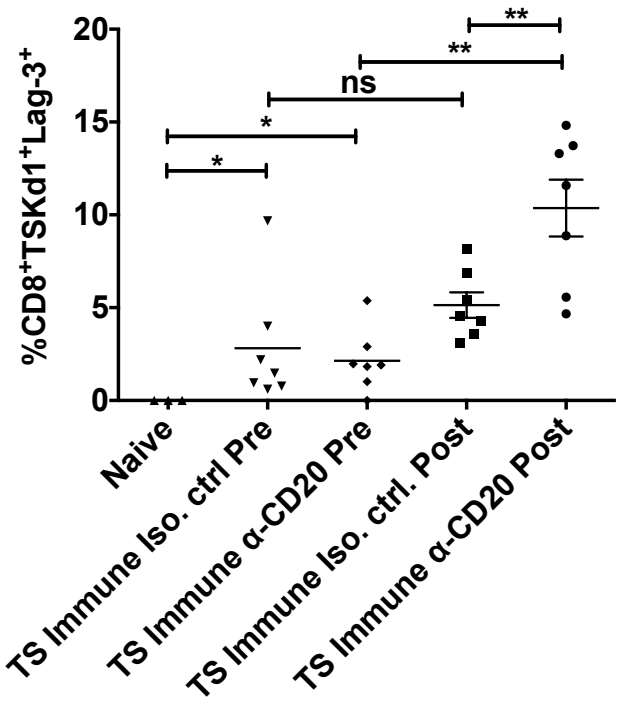
Supplemental Figure 2: Highly depleted CD19$^+$ T cells used for adoptive transfer studies. Five to ten Tc immune and naïve BALB/c mice were sacrified, splenocytes pooled together from each group and CD19$^+$ B cells depleted using CD19$^+$ microbeads (Miltenyi Biotech). **(A)** The percentages of CD19$^+$ B cells were assessed pre-sort and post-sort. Cells were stained with anti-CD8 FITC, anti-CD3 PE, anti-CD4 PerCP and anti-CD19 PE-Cy7 and analyzed with a LSR II Flow cytometer and FlowJo software. One mouse SC+dLN equivalent of total or CD19$^+$ depleted SC+dLN cells from Tc immune and naïve BALB/c mice were

transferred i.v. into naïve SCID mice. One day later, these mice were orally challenged with *T. cruzi*. Recipient SCID mice were sacrificed 12 days later and assessed for **(B)** $CD19^+$ B cells in the spleen via flow cytometry, **(C)** *T. cruzi*-specific serum IgG via ELISA, and **(D)** *T. cruzi*-specific IgG antibody secreting cells (ASC) via ELISPOT. n=5 mice/group. * $p < 0.01$, Mann-Whitney U test. Data are representative of two independent experiments with similar results. Error bars represent SEM.

Supplemental Figure 3



Supplemental Figure 3: Highly purified CD8$^+$ T cells used for adoptive transfer studies. Five to ten Tc immune and naïve BALB/c mice were sacrified, splenocytes and gastric LN cells pooled together from each group and CD8$^+$ T cells purified using CD8$^+$ microbeads (Miltenyi Biotech). **(A)** The percentages of CD8$^+$ T cells were assessed pre-sort and post-sort. Cells were stained with anti-CD8 FITC, anti-CD3 PE, anti-CD4 PerCP and anti-CD19 PE-Cy7 and analyzed with a LSR II Flow cytometer and FlowJo software. One mouse spleen/gastric LN equivalent of purified CD8$^+$ T cells (> 98% CD8$^+$) from Tc immune and naïve BALB/c mice were transferred i.v. into naïve SCID mice. One day later, these mice were orally challenged with *T. cruzi*. **(B)** Recipient SCID mice were sacrificed 12 days following *T. cruzi* challenge and serum was collected from individual mice. TS-specific serum IgG was measured from recipient mice via ELISA. Serum samples previously collected from SCID mice receiving *T. cruzi*-specific total SC were used as positive controls. N=4 mice/group. Data are representative of two independent experiments with similar results.



Supplemental Figure 4: B cells are important for the prevention of $CD8^+$ T cell exhaustion in our TS immune model. B cell depleted (α-CD20) and isotype control treated BALB/c mice were vaccinated first with TS-DNA i.m. twice, two weeks apart, rested for one month, and then vaccinated with Adeno-TS i.n. and s.c. twice, two weeks apart. Mice were treated with α-CD20 or isotype control antibody every 2-3 weeks for the duration of the study and assessed for B cell depletion via flow cytometry and *T. cruzi*-specific serum IgG via ELISA (data not shown). Approximately 4 months after the last vaccination, naïve and TS immune mice were systemically challenged with *T. cruzi* BFT s.c. **(A)** Prior to (pre) and fifteen days after systemic challenge (post), mice were bled and the percentage of $CD8^+TSKd1^+Lag\text{-}3^+$ T cells measured via flow cytometry. N=3-7 mice/group. * $p < 0.05$, ** $p < 0.01$ Mann-Whitney U test. Data are

representative of two independent experiments with similar results. Error bars represent SEM.