Material and Methods

Xenograft GVHD model

NSG-A2^{+} mice were irradiated (250 cGy) and were transplanted with HLA-A2^{-} human PBMCs (10-13 × 10^{6})(1). Recipient mice were injected with vehicle alone or with human RTrx1 at 5μg/mouse/day from day -1 to day 14. Recipients were monitored for clinical score and survival until 60 days post transplantation.

Histologic analysis. Representative samples of liver, small intestine, large intestine, lung, and skin were obtained from transplanted recipients 21 days post transplant, fixed in 10% neutral-buffered formalin, and washed with 70% ethanol. Samples were then embedded in paraffin, cut into 5-μm thick sections, and stained with H&E. A semi quantitative scoring system was used to account for histologic changes consistent with GVHD in the colon, liver, and lung as previously described. Data were presented as individual GVHD target organ. All slides for GVHD analysis were coded and read in a blinded fashion.

Flow cytometry. Mononuclear cells were isolated from mouse recipient spleen or liver. The livers and spleens were processed using 100μm and 40μm nylon mesh cell strainers and syringe plunger respectively from Fisher Scientific (cat# 22363549 and 22363547). Splenocytes were briefly lysed and quenched to remove red blood cells whereas liver mononuclear cells were isolated using Ficoll separation followed washes, and cells were either re-stimulated with PMA/Ionomycin for intracellular staining or directly surface stained for flow cytometric analysis.

The live cells were determined with flow cytometric analysis by using live/dead fixable yellow dead cell stain kit from Invitrogen (cat # L34968). This dye permeates into dead cells producing a fluorescent signal, but is excluded from entering into live cells. The cells were stained for surface markers and intracellular cytokines using standard flow cytometric protocols. Stained cells were analyzed using FACSDiva software, LSR II (BD Biosciences, San Jose, CA), and FlowJo (Tree Star, Ashland, OR).The following Abs were used for cell-surface staining: anti-CD4–V450 (RM4-5), -APC, and -PEcy7 (BD Biosciences), anti–CD8-PEcy5 (53-6.7), -APCcy7 and -AF700 (BD Biosciences,); anti–CD45.1-FITC (A20), - and -APC (BD Biosciences), anti–B220-FITC (RA3-682) and -PE (eBioscience,), anti-CD44–APC,PE(eBioscience,) (IM7) anti-CD62L-Pecy5,-FITC(eBioscience) (MEL-14),CD25–FITC (7D4) ,Pecy7(eBioscience) (PC 61.5) and anti- H2Kb (AF6-88.5.5.3). Intracellular staining was carried out using anti–IFN-γ–PE or Per-cp 5.5 (XMG1.2; BD Biosciences), anti–IL-4–PE (11B11; BD Pharmingen), anti–IL-5–PE (TRFK5; BD Pharmingen), anti-Foxp3–PE (FJK-16s; eBioscience), T-bet-PE (4B10; sc-21749) and PE rat anti-mouse IgG1 (BD 562027), anti-human Trx1-pE (ATRX-03-IMCO Corp Ltd), pNFκB-p65-APC (S536, Cell signaling) and appropriate isotypes. The human antibodies used in this study are as follows. Anti-CD4– eFluor V450 (OKT-4), Anti-CD4-PE-cy7 (OKT-4), anti–CD8 FITC (HIT8a), anti–CD8- APC-cy7 (SK1), anti–IFN-γ-PE-cy7 (4S.B3).

Figure S1

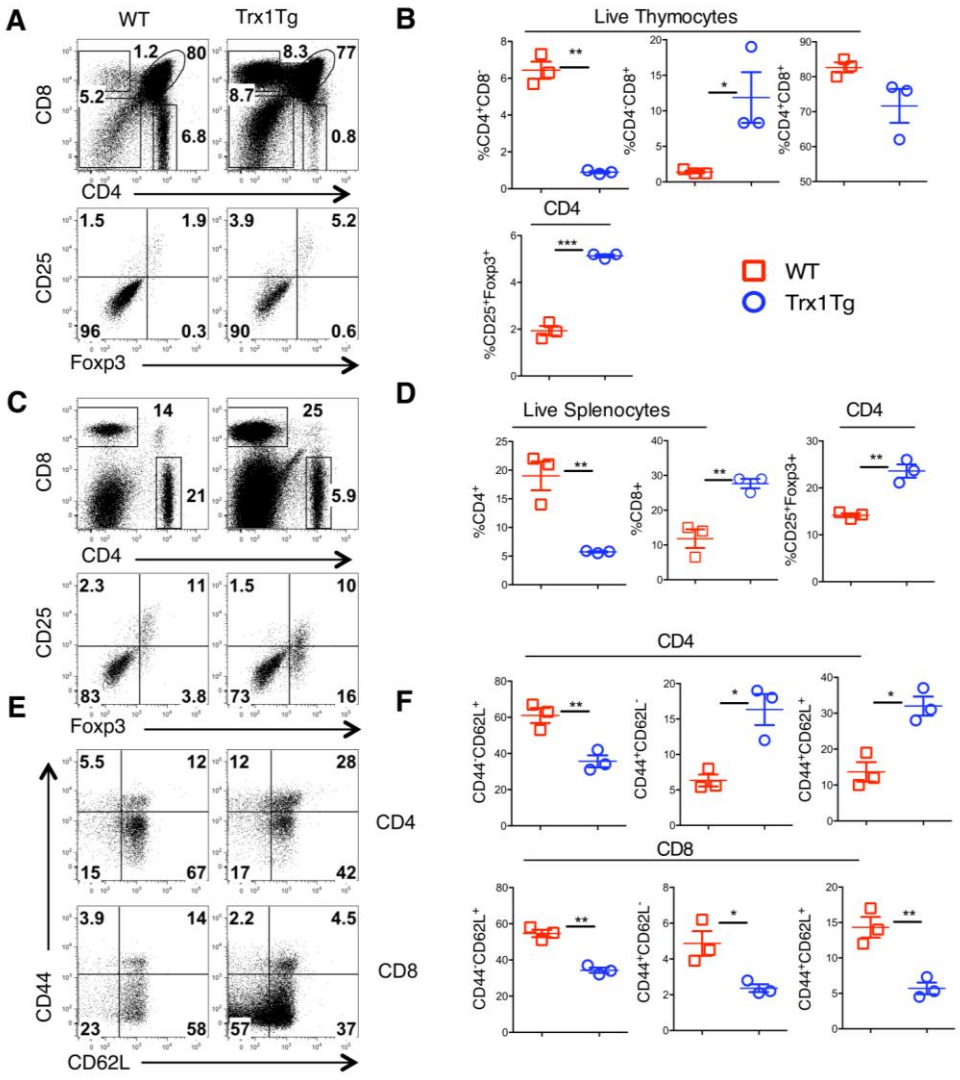


Figure S1: Effects of Trx1 on T-cell phenotype. Thymus and spleens were obtained from un-manipulated age and sex-matched WT and Trx1Tg mice on B6 background. These organs were individually processed, counted and stained for the expression of CD4, CD8 CD44, CD62L, and Foxp3. (A) CD4 and CD8 expression are shown on total live thymocytes and CD25 and Foxp3 expression on gated live $CD4^+$ cells were shown in thymus. (B) The mean ± SD of each cell subset is shown from 3 mice in each group. (C) CD4 and CD8 expression were shown on total live splenocytes, CD25 and Foxp3 expression on gated $CD4^+CD8^-$ cells, and CD44 and CD62L expression on gated $CD4^+$ or $CD8^+$ cells in spleen. One representative mouse (C and E) or flow chart means ± standard deviations (SD) with 3 mice per group (D and F) are displayed. The data is one representative of 2 independent experiments. Significance determined by Student's *t* test. Asterisks indicate statistical significance *p<.05; ** p<.01; *** p<.001.

Figure S2

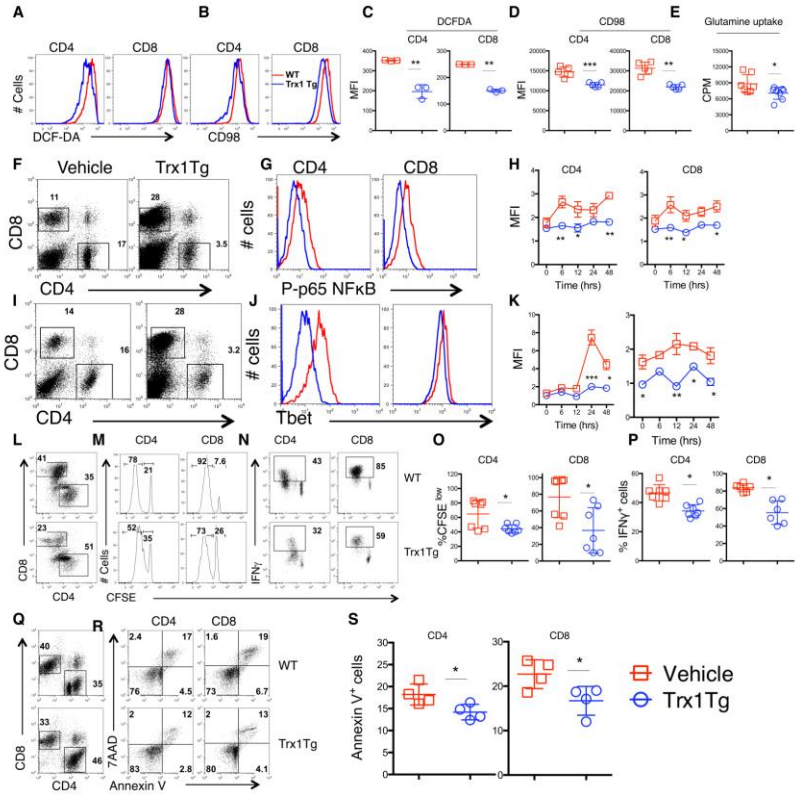


Figure S2: Effect of Trx1 on T-cell activation and function in vitro: Splenocytes from WT and Trx1-Tg mice on B6 background were stimulated with anti-CD3 plus anti-CD28 antibodies each at 1µg/ml for 48 hr. Cells were washed and stained with CD4, CD8, CD98 and DCF-DA (A and B), or mean fluorescence intensity ± SD of 3-6 samples per group (C and D). Data shown here is one representative of at least 2 independent experiments. T cells were stimulated in vitro with anti-CD3 plus anti-CD28 antibodies for 48 hr and measured for glutamine uptake reflected by radioactivity (CPM) (E). Data represents 2 independent experiments. In separate experiments with the same setting, splenocytes from WT and Trx1-Tg mice on B6 background were stimulated with anti-CD3 plus anti-CD28 antibodies for 48hrs. Cells were washed and stained for CD4 and CD8 expression on the surface followed by intracellular staining for phospho-p65 subunit of NFκB activity and T-bet expression at various time points. The levels of phospho-p65 NFκB (F-H) and T-bet (I-K) are shown on gated donor CD4 and CD8 cells, respectively. (H and K) shows mean fluorescence index. Purified T cells from WT and Trx1-Tg mice on B6 background were labeled with CFSE and were co-cultured with T-depleted allogenic APCs (1:3 ratio) for 5 days. T-cell proliferation, IFNγ production and apoptosis are shown in one representative sample in each group (L, M, N and Q), or mean ± SD of 4-7 samples per group (O, P and S). Data represents 2 independent experiments. Significance determined by Student's *t* test Asterisks indicate statistical significance *p<.05. ** p<.01; *** p<.001.

Figure S3

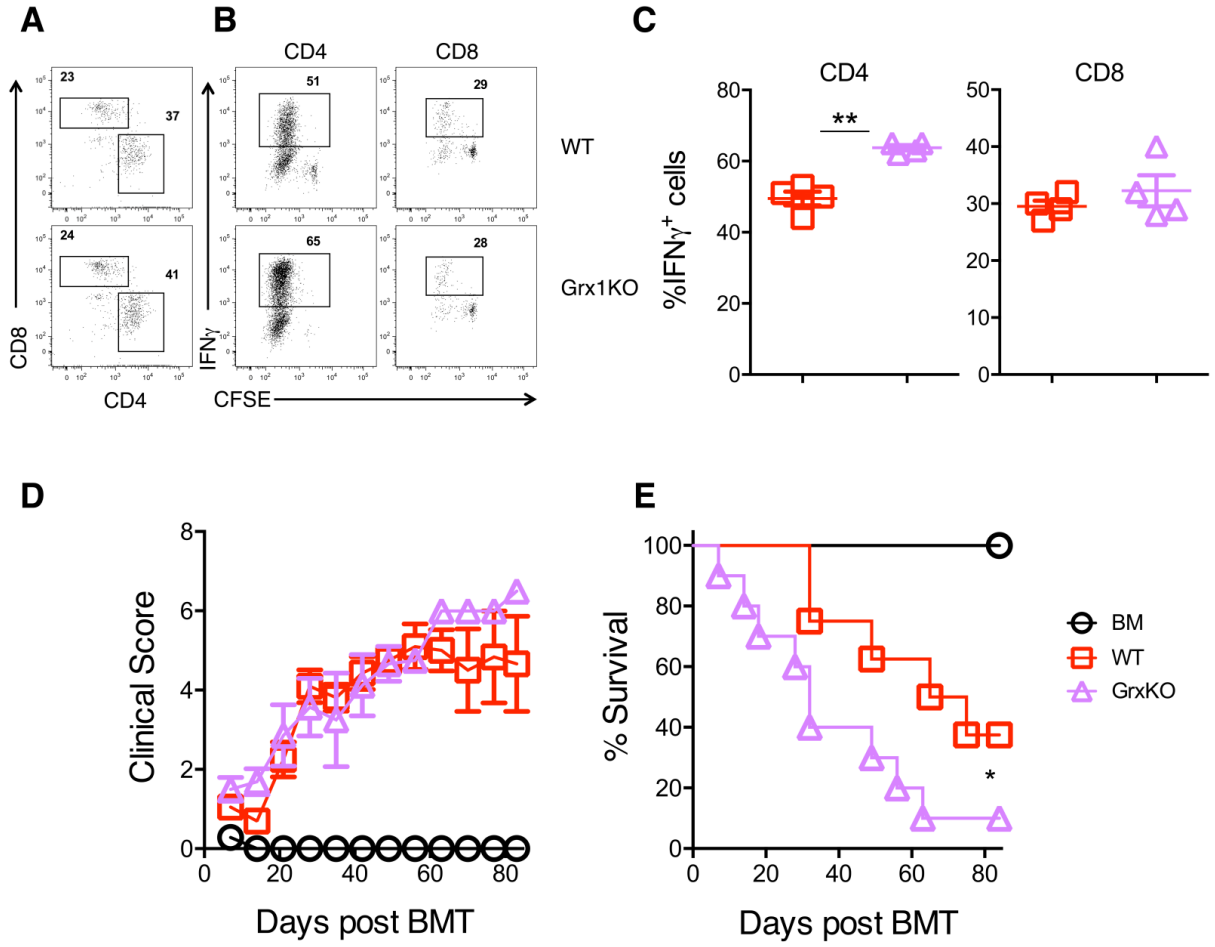
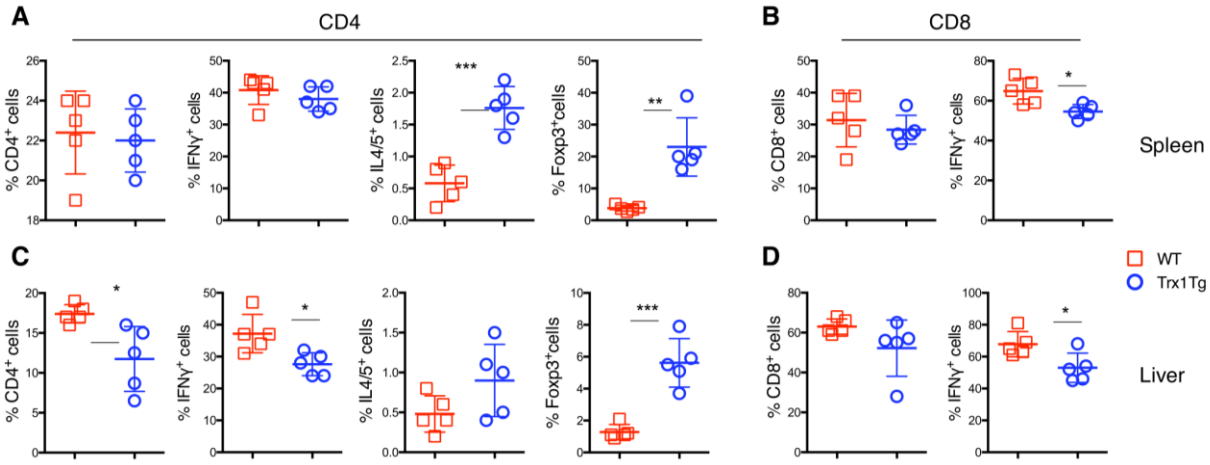


Figure S3: T cells deficient for Grx1 increase GVHD mortality after allo-BMT. CD25 depleted T cells were isolated from WT and Grx1KO mice on B6 background, labeled with CFSE and injected intravenously into lethally irradiated BALB/c mice at 2×10^6/mouse. Four days after cell transfer, spleens were collected from recipient mice and subjected to cell counting and FACS staining. (A-B) $CD4^+$, $CD8^+$ and IFNγ expression are shown among gated live donor cells ($H2K^{b+}$). (C) Summary data shown is from one of two representative experiments. The mean ± SD of $\%IFN\gamma^+$ cells is depicted for 4 mice per group. In the separate experiments, BALB/c mice were lethally irradiated and transplanted with 5×10^6/mouse TCD-BM ($Ly5.1^+$) or plus purified T cells ($Ly5.2^+$) (0.5×10^6/mouse) from WT or Grx1 KO mice. Recipients were monitored for clinical score and survival (D and E) until 80 days post BMT (n = 10 per group). Data shown is from 2 combined experiments. For comparison of recipient survival among groups, the log-rank test was used to determine statistical significance. Clinical scores were compared using a nonparametric Mann-Whitney *U* test. Asterisks indicate statistical significance *p< .05.

Figure S4



Figures S4: Effect of Trx1 overexpression on donor T-cell expansion and migration after allo-BMT. BMT was carried out as outlined in figure 3. Three weeks post-BMT, recipient spleens and livers were collected and mononuclear cells were isolated and subjected to cell counting and FACS staining (A&C). Summary of overall intracellular expression of IFNγ, IL-4/5 and Foxp3 expression are shown on gated donor CD4 or CD8 cells among recipient live spleen or liver cells (B&D). Data shown here are from one of two representative experiments. Significance determined by Student's *t* test. Asterisks indicate statistical significance *p < .05, **p < .01, *** p<.001.

Figure S5

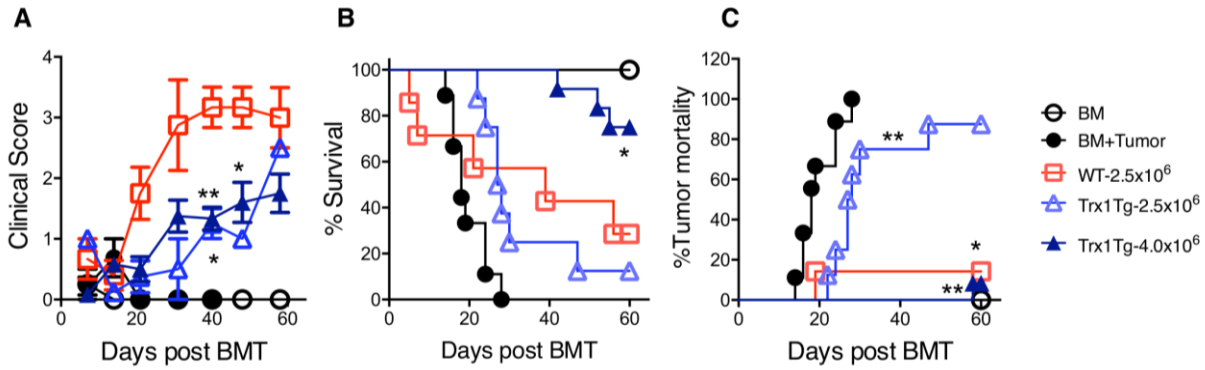


Figure S5: Overexpression of Trx1 in T cells affects GVHD and GVL effect. BDF1 mice were lethally irradiated and transplanted with 5×10^6/mouse TCD-BM ($Ly5.1^+$) or plus purified T cells ($Ly5.2^+$) from WT or Trx1-Tg mice at the doses indicated. Recipients were monitored for clinical score (A), survival (B), and tumor mortality (C) until 60 days post BMT (n = 7-10 per group). For comparison of recipient survival among groups, the log-rank test was used to determine statistical significance. Clinical scores and tumor mortality were compared using a nonparametric Mann-Whitney *U* test. . Asterisks indicate statistical significance *p< .05, **p < .01, ***p < .001.

Figure S6

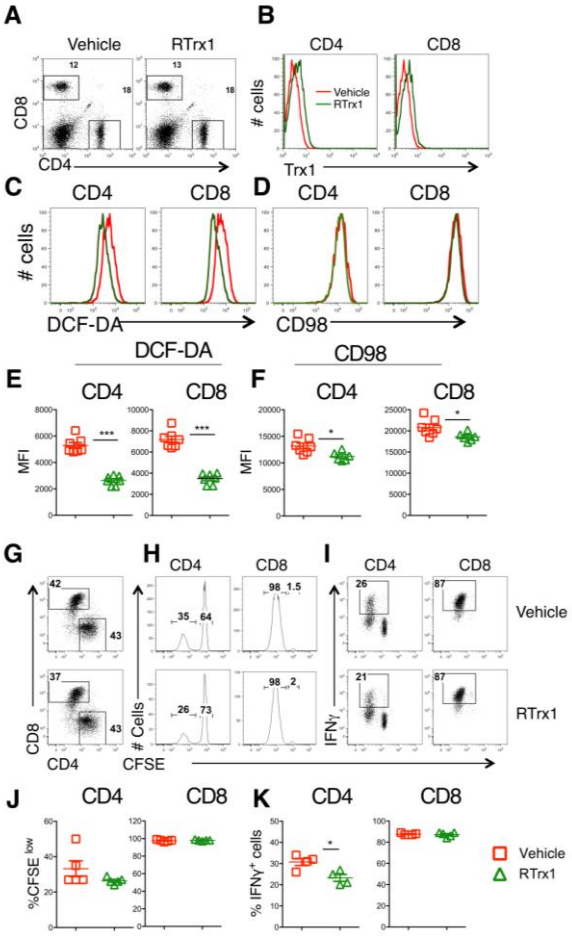


Figure S6: Effects of RTrx1 on T-cell allogeneic responses. Splenocytes from mice on B6 background were stimulated with anti-CD3 plus anti-CD28 antibodies each at 1µg/ml for 6 hrs in the presence or absence of 2 µg/ml RTrx1. Cultured cells were washed and stained for surface CD4, CD8, and intracellular RTrx1 expression (A and B). RTrx1 expression is shown on gated CD4 or CD8 cells (B). In a separate settings splenocytes from mice on B6 background were stimulated with anti-CD3 plus anti-CD28 antibodies each at 1µg/ml for 48 hr in the presence or absence of 2 µg/ml RTrx1. Cells were washed and stained with CD4, CD8, CD98 and DCF-DA (C and D), with mean fluorescence intensity ± SD of 3-6 samples per group (E and F). CFSE-labelled T cells from B6 mice were stimulated with allogeneic APCs in the presence or absence of 2 µg/ml RTrx1 for 5 days. Cells were subjected to FACS staining and analyzed for T-cell proliferation and cytokine expression. (G, H and I) CFSE dilution and % IFNγ$^+$ cells are shown on gated CD4$^+$ or CD8$^+$ cells. (J and K) The mean ± SD for CFSE diluted or IFNγ$^+$ cells are shown, respectively. Data shown here is one of at least 2 independent experiments. Significance was determined by student's *t* test. Asterisks indicate statistical significance *p < .05, **p < .01, ***p < .001.

Figure S7

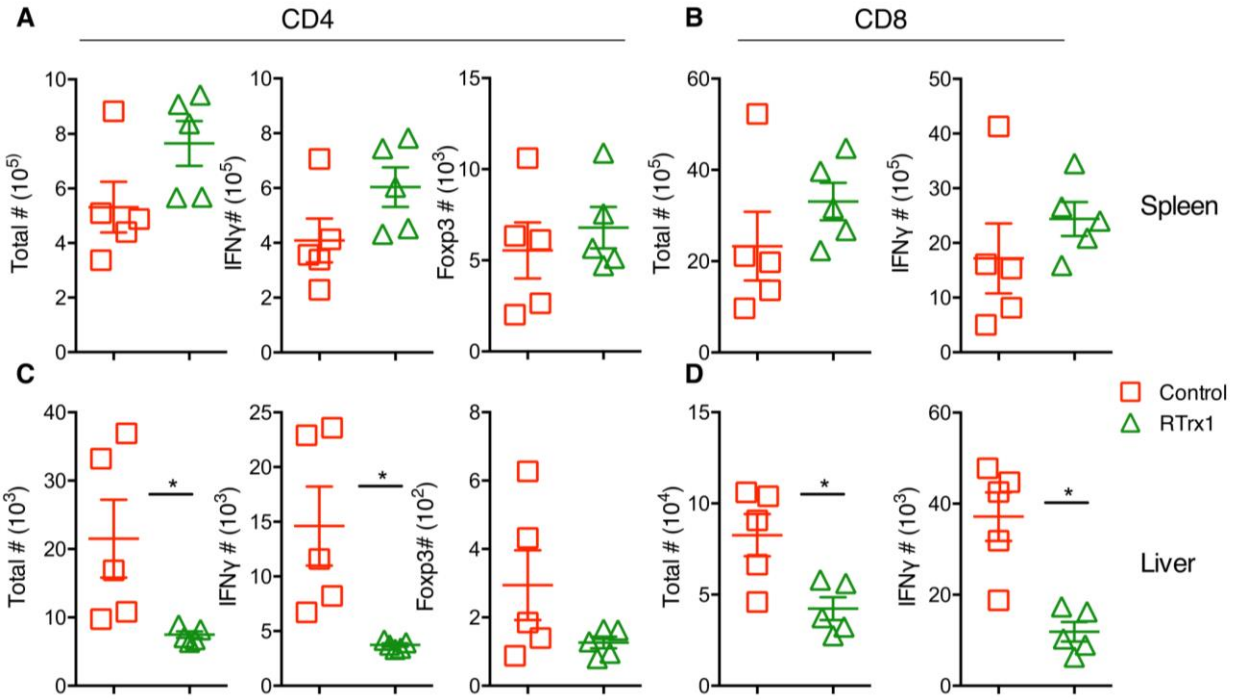


Figure S7: Effect of Trx1 overexpression on donor T-cell expansion and migration after allo-BMT. BMT was carried out as outlined in figure 3 using BALB/c mice as the recipients. The absolute numbers of IFNγ$^{+,}$ IL-4/5^{+}, or Foxp3^{+} donor (H2K^{b+}Ly5.1^{-}) CD4^{+} (A and C) and IFNγ^{+} CD8^{+} cells (B and D) in recipient spleen and liver, respectively. Data shown here are from one of two representative experiments Significance determined by Student's *t* test. Asterisks indicate statistical significance. *p < .05

Figure S8

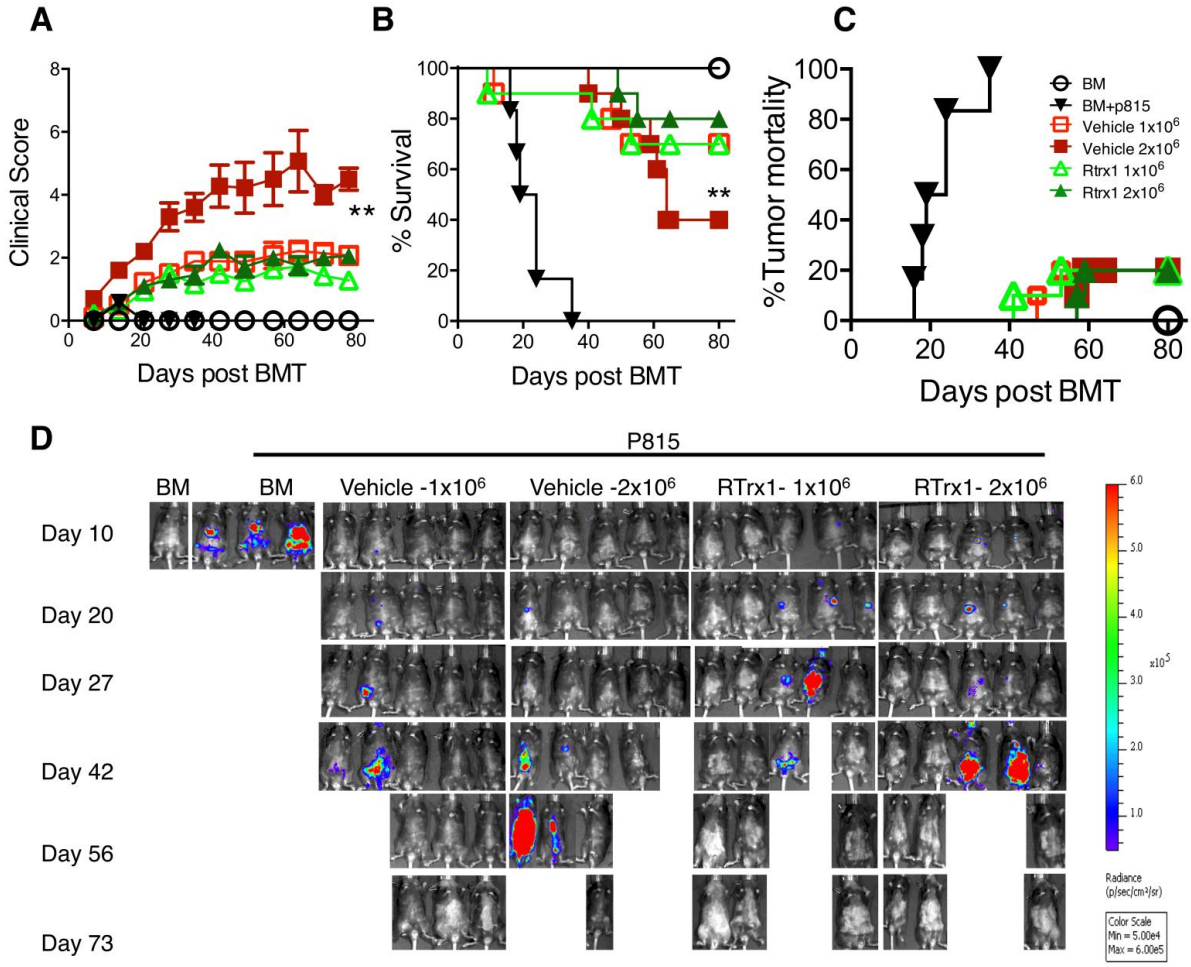


Figure S8: RTrx1 attenuates GVHD while preserving the GVL activity. BDF1 mice were lethally irradiated and transplanted with 5×10^6/mouse TCD-BM or plus purified T cells ($1\text{-}2\times10^6$/mouse) from B6 mice. Recipients with different T-cell dose were injected with vehicle alone or with RTrx1 from day -1 to day 14. Recipient mice were also infused with luciferase-transduced p815 (5000 cells/mouse) cells at the day of BMT. Recipients were monitored for clinical scores (A), survival (B) and tumor mortality (C) until 80 days post BMT. Tumor growth was monitored using BLI on the dates indicated (D) and is shown from one representative experiment. Data shown here (A-C) is from 2 combined experiments (N=10). For comparison of recipient survival among groups, the log-rank test was used to determine statistical significance. Clinical scores and tumor mortality were compared using a nonparametric Mann-Whitney *U* test. Asterisks indicate statistical significance *p < .05, **p < .01.

1. Nguyen H, Kuril S, Bastian D, Kim J, Zhang M, Vaena SG, Dany M, Dai M, Heinrichs JL, Daenthanasanmak A, et al. Complement C3a and C5a receptors promote GVHD by suppressing mitophagy in recipient dendritic cells. *JCI insight.* 2018;3(24).