Supplemental Figure legends

Supplemental Figure 1.: Levels of donor Treg expansion. Donor mice were injected i.p. with TL1A-Ig (50 µg) on days 1-4; rmIL-2 (1.5 µg) bound to the a-IL-2 mAb (JES6-5H4; 8ug) on days 4 and 6. Treg expansion levels are shown for B6-FoxP3^{rfp} mice (major model; **A**) (n = 6) and B10.D2 mice (minor model; **B**) (n = 3). Data are expressed as means ± SEM and were analyzed by a two-tailed unpaired *t* test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Supplemental Figure 2.: Comparable outcomes with lower amounts of PTCy. (**A-C**) A HSCT utilizing a B6 → BALB/c donor/recipient mouse model involving a complete MHC mismatch was performed on day 0. Lethally irradiated (8.5 Gy) BALB/c mice received 5x10^6 TCD B6-CD45.1 BM cells and spleen cells from expanded (TL1A-Ig/IL-2; TrED group) or untreated B6-FoxP3^{rfp} (GVHD and PTCy group) donor mice adjusted to contain 1.1x10^6 total T cells. Cyclophosphamide was given on day 3 and 4 post-HSCT at 50 mg/kg ip. Weights (**A**), clinical scores (**B**) and survival (**C**) are shown (GVHD: n = 5; TrED and PTCy: n = 10). Survival was analyzed by log-rank test (ns = not significant). TrED cells show advantages over PTCy treatment early post-transplant even when using lower/clinically more relevant amounts of Cy (50 mg/kg) and are comparable to 80 mg/kg in a major MHC mismatch model of pre-clinical HSCT.

Supplemental Figure 3.: Treg assessment in blood three and four weeks post-HSCT. (**A**) Percent Foxp3$^+$ Tregs out of total CD4$^+$ T cells (upper graphs) as well as Treg / CD4 ratios (lower graphs) are shown for day 21 (left) and 30 (right). Data are shown as mean ± SEM; ANOVA with Bonferroni correction was applied for multiple comparisons on day 21. Data are expressed as means ± SEM and were analyzed by a two-tailed unpaired *t* test on day 30. *p<0.05; **p<0.01; ***p<0.001. Day 21: n = 6; day 30: GVHD: n = 1; PTC: n = 3; TrED: n = 4. Data shown are from two experiments for day 21 and from one experiment for day 30. (**B**) Treg engraftment over time shows a faster engraftment in TrED compared to PTCy recipients on day 30. On day 21: GVHD: n = 2; PTCy and TrED n = 3. On day 30: GVHD: n = 1; PTCy: n = 3: TrED: n = 4. Data are expressed as means ± SEM and were analyzed by a two-tailed unpaired *t* test. *p<0.05.

Supplemental Figure 4.: Massons's trichrome staining from recipient lungs in a Minor HSCT mouse model on day 200 post-HSCT. Representative staining (chosen from 2 independent experiments) exhibited multifocal areas of moderate chronic, active inflammation and fibrosis in the PTCy compared to the TrED group which was within normal limits. Magnification = 200x. The pathology score is shown on the right (n = 6-8). Data are shown as mean ± SEM; ANOVA with Bonferroni correction was applied for multiple comparisons. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. Data shown are from one experiment. Scale bars: 100 μm.

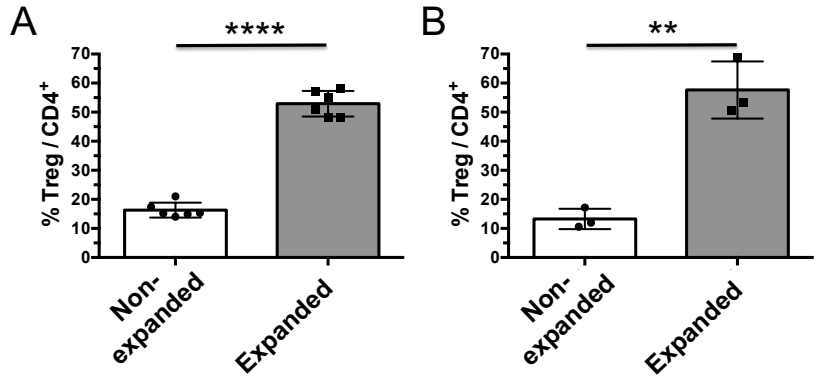
Supplemental Figure 5.: Naïve/memory compartment over time (1-3 months post-HSCT). A HSCT utilizing a B6 → BALB/c donor/recipient mouse model involving a complete MHC mismatch was performed as described in Fig. 1. The naïve/memory compartment was analyzed by flow cytometry (CD44/CD62L) in PB at 30, 60 and 90 days post-HSCT. The naïve compartments of CD4$^+$ and CD8$^+$ cells are significantly larger in TrED recipients compared to PTCy treated animals at 1 and 2 months post HSCT. At 3 months post HSCT a significant difference is only seen in CD8$^+$ cells. GVHD: n = 3; PTCy and TrED: n = 8-10. Data are expressed as means ± SEM and were analyzed by a two-tailed unpaired *t* test. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Supplemental Figure 6.: Assessment of the DN CD44/CD25 subsets at 3 weeks and 1 month post allogeneic HSCT by flow cytometry. A HSCT utilizing a B6 → BALB/c donor/recipient mouse model involving a complete MHC mismatch was performed as described in Fig. 1. (**A**) At 3 weeks post-HSCT no significant difference could be detected in DN3 (CD44$^-$CD25$^+$) and DN4 (CD44$^-$CD25$^-$) subtypes between the TrED and the PTCy group (n = 3-4). (**B**) However, at 1 month post HSCT there

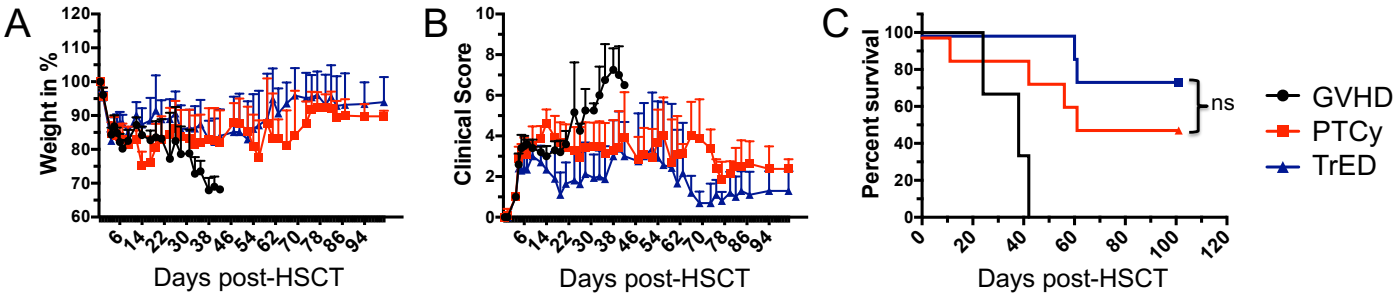
are significant differences detectable in DN3 ($CD44^{-}CD25^{+}$) and DN4 ($CD44^{-}CD25^{-}$) subtypes between the TrED and the PTCy group (n = 6). Data are expressed as means ± SEM and were analyzed by a two-tailed unpaired *t* test. *p<0.05; **p<0.01.

Supplemental Figure 7.: Assessment of recent thymic/marrow emigrants (RTEs/RMEs) at one and two months post-HSCT. A HSCT utilizing a B6 → BALB/c donor/recipient mouse model involving a complete MHC mismatch was performed on day 0. Lethally irradiated (8.5 Gy) BALB/c mice received $5x10^6$ TCD B6-RAG2p-GFP BM cells and spleen cells from expanded (TL1A-Ig/IL-2; TrED group) or untreated B6-$FoxP3^{rfp}$ (GVHD and PTCy group) donor mice adjusted to contain $1.1x10^6$ total T cells. Cyclophosphamide was given on day 3 and 4 post-HSCT at 80 mg/kg ip. RTEs/RMEs were analyzed in PB by flow cytometry one (**A**) and two (**B**) months post-HSCT (n = 2-3). (**A**) One month post-HSCT no significant differences were detectable between the TrED and the PTCy group in $CD4^{+}$, $CD8^{+}$ and $CD19^{+}$ cells. (**B**) Two months post-HSCT the only significant difference was evident in the $CD8^{+}$ compartment between the 2 groups. Data are expressed as means ± SEM and were analyzed by a two-tailed unpaired *t* test. *p<0.05; **p<0.01.

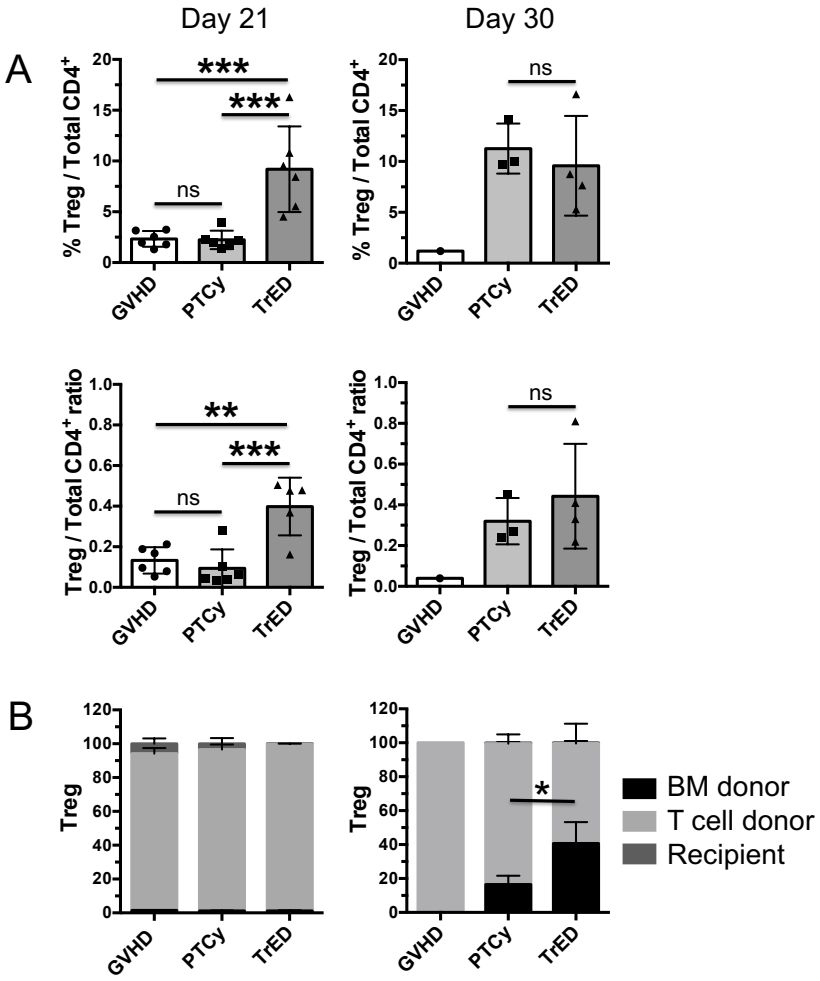
Supplemental Figure 1: Wolf et al.



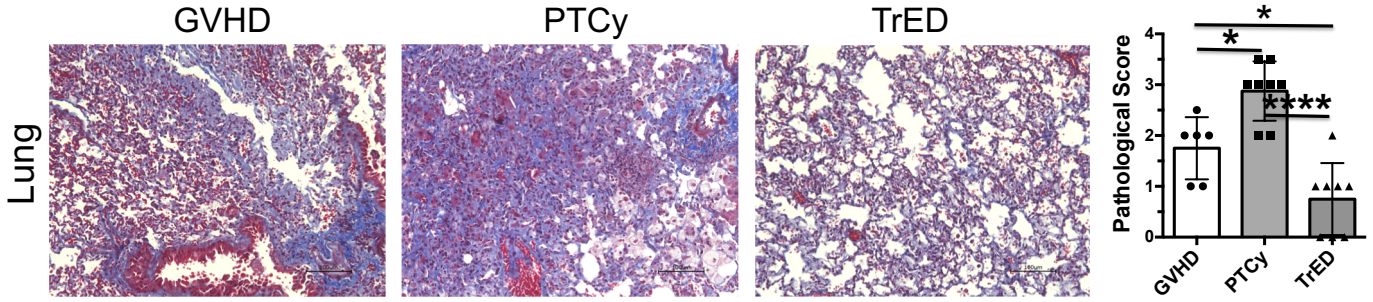
Supplemental Figure 2: Wolf et al.



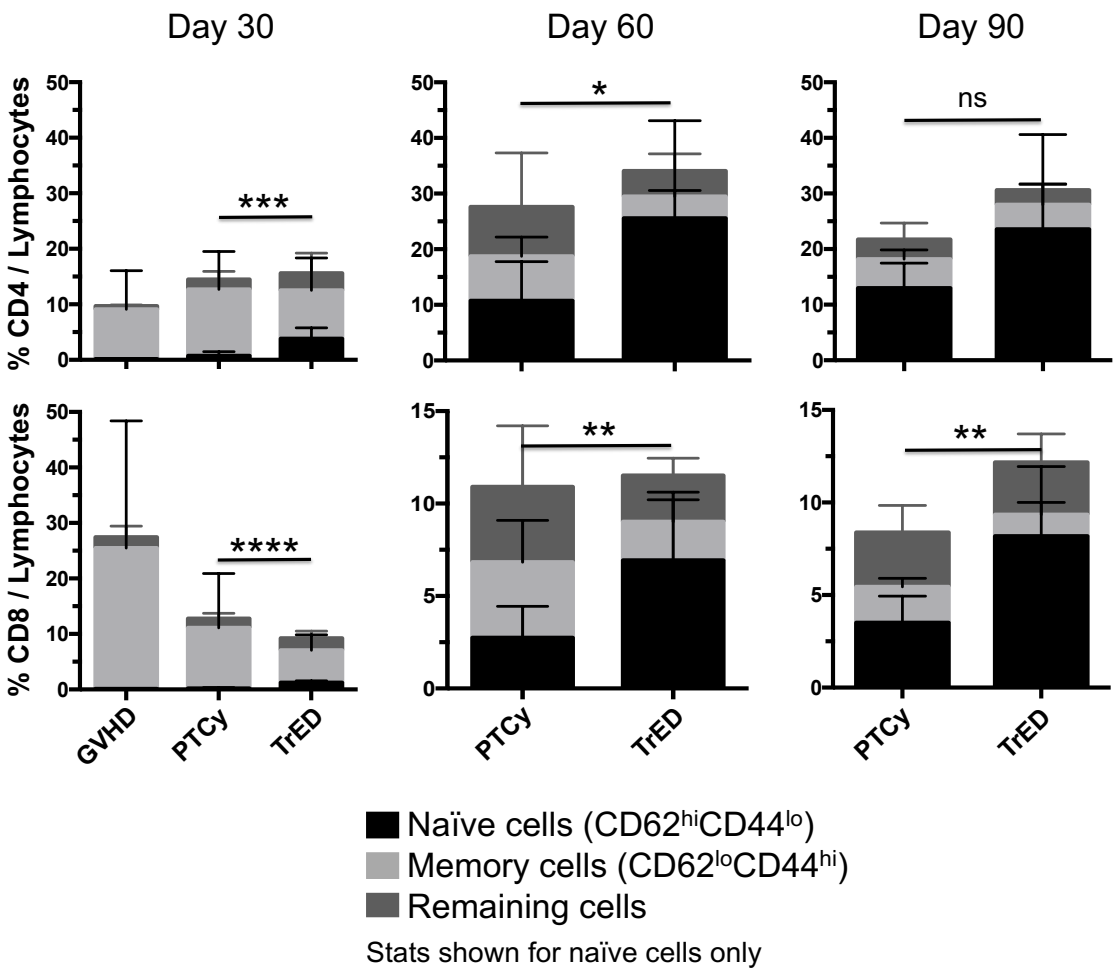
Supplemental Figure 3: Wolf et al.



Supplemental Figure 4: Wolf et al.

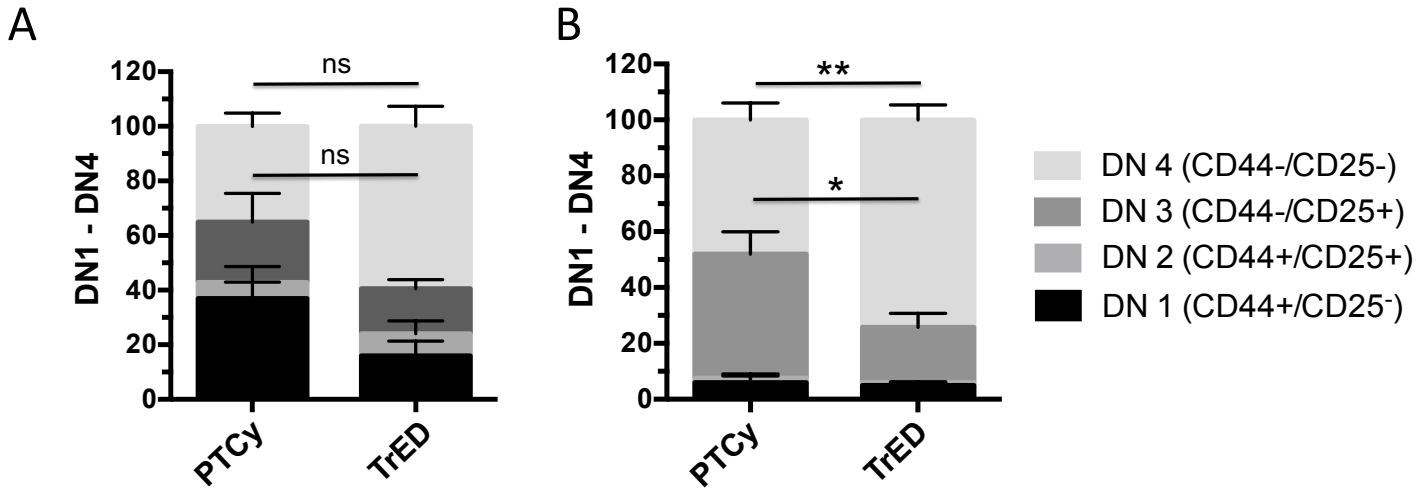


Supplemental Figure 5: Wolf et al.



Stats shown for naïve cells only

Supplemental Figure 6: Wolf et al.



Supplemental Figure 7: Wolf et al.