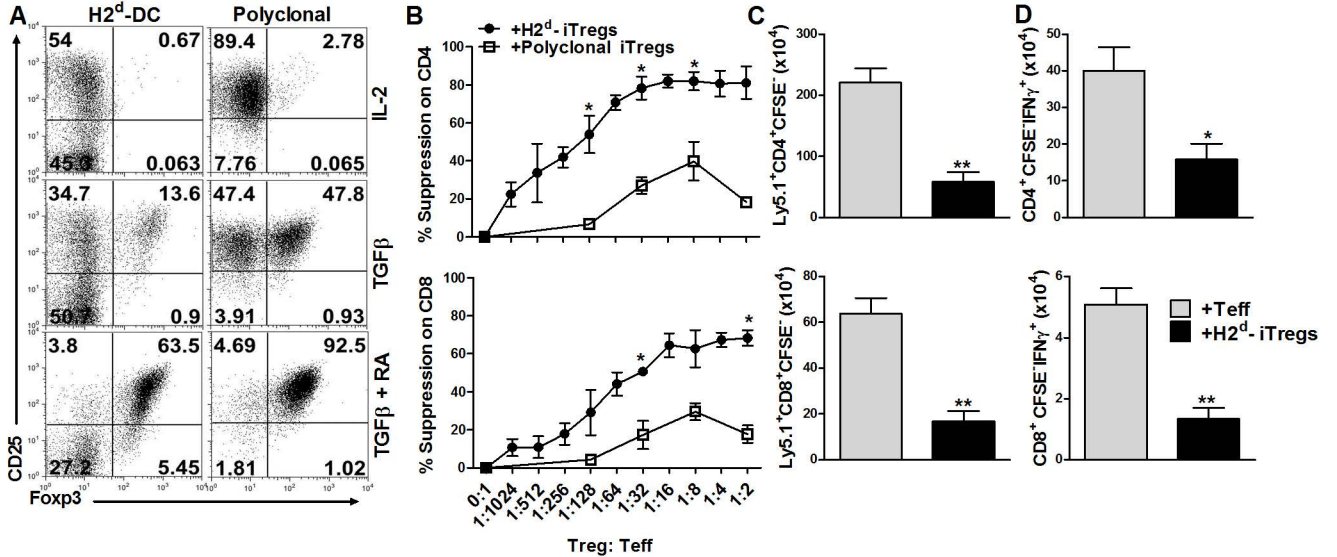

Fig. S1: Generation and Function of Alloreactive CD4 iTregs. Naïve $CD4^+CD25^-$ T cells were isolated from B6 mice and plated with either BALB/c DCs (alloreactive) or with α-CD3 + B6 APCs (polyclonal) with IL-2 (5ng/mL), TGF β (5ng/mL), and retinoic acid (40nM). Foxp3 expression was assessed through Flow cytometry (A). Activity of iTregs was assessed by suppressing CFSE dilution of B6 Teffs in vitro (B) and in vivo (C and D).* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Error bars indicate the mean of standard error.

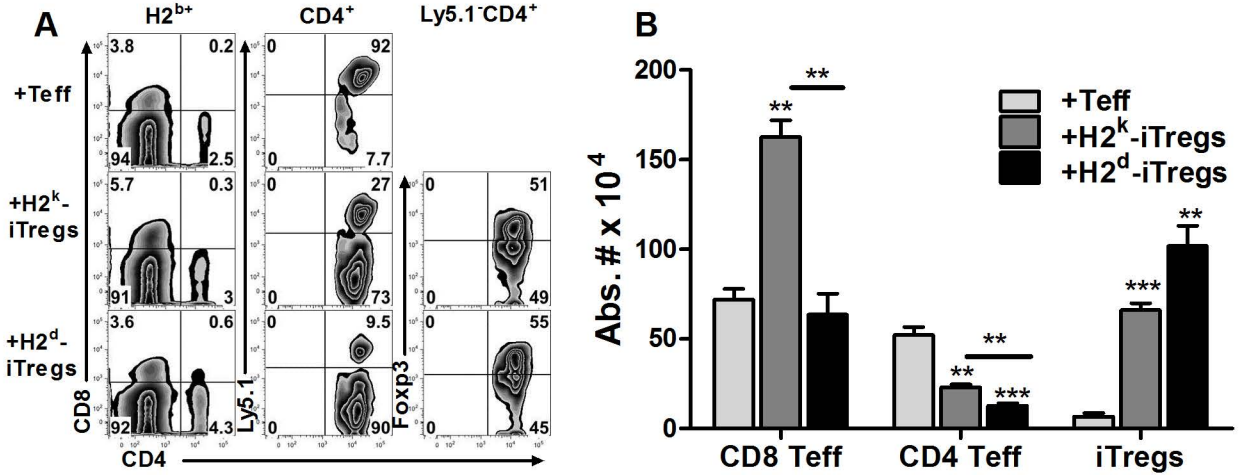

Fig. S2: Alloantigen specificity is essential for iTreg function and stability. BALB/c mice were lethally irradiated and transplanted with TCD-BM and either H2 ^{d} or H2^{k} iTregs as described in Fig. S1. Three days later Ly5.1 $^{+}$ CD25-depleted Teffs were injected. Fourteen days later recipient's spleens were excised, mononuclear cells isolated, and stained for indicated surface molecules, representative flow gating strategy is shown from spleen (A). Based on flow percentages absolute numbers of Teffs and iTregs were calculated for the spleen (B) * P<.05; ** P<.01; *** P<.001. Error bars indicate the mean of standard error.

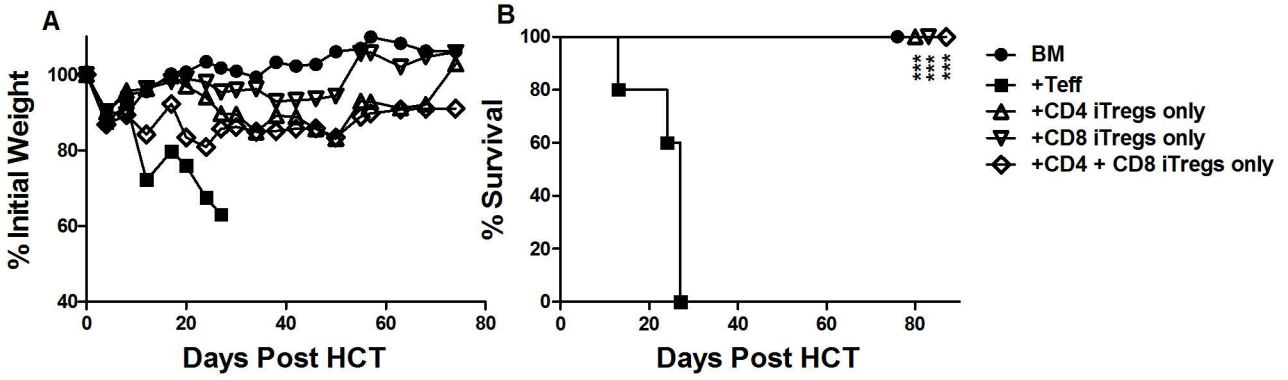

Fig. S3: Non-enriched iTregs are not pathogenic .: BALB/c mice were lethally irradiated and transplanted with 5 x 10^6 TCD-BM, and non-enriched 2 x 10^6 CD4 iTregs, 2 x 10^6 CD8 iTregs, or 1 x 10^6 CD4 + 1 x 10^6 CD8 iTregs. Three days later, 0.5 x 10^6 CD25-depleted Teffs were injected. Recipients were monitored for survival and body weight loss for 80 days (A), n= 8/group.

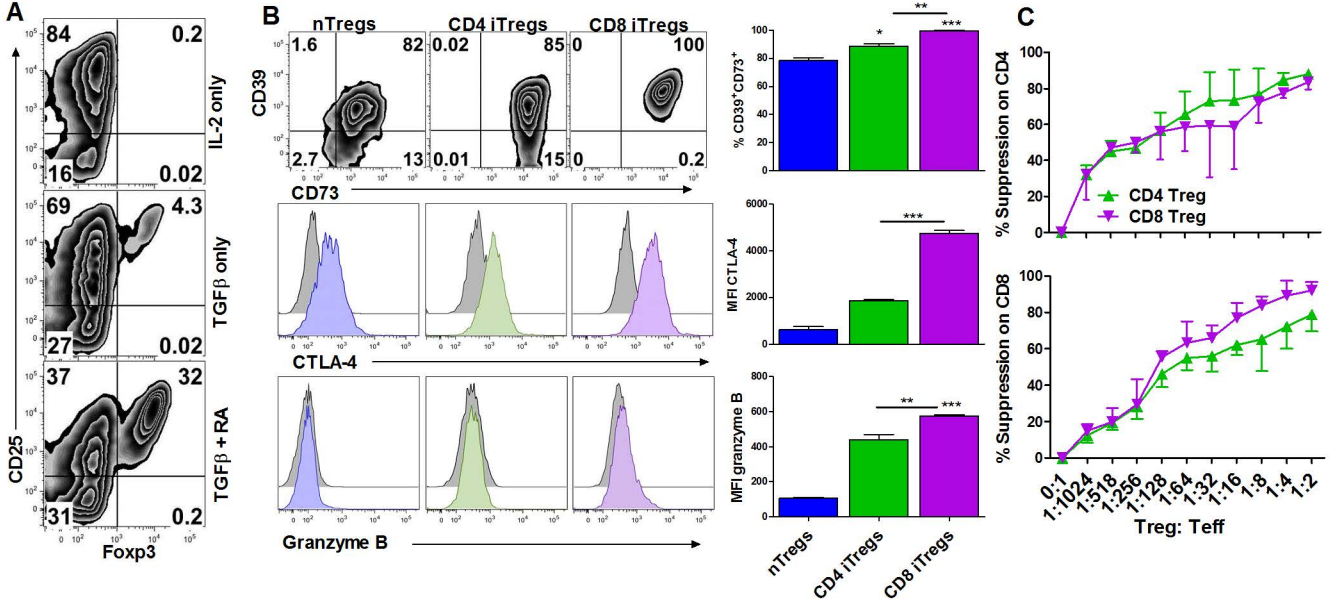

Fig. S4. Generation and Function of Alloreactive CD8 iTregs . Naïve $CD8^{+}CD25^{+}$ B6 T cells were stimulated with BALB/c DCs with IL-2 (5ng/mL), TGFβ (5ng/mL) and retinoic acid (40nM) for 4 days (A). Expression of CD39, CD73, CTLA4, and granzyme B were assessed between nTregs, alloreactive CD4 iTregs, and alloreactive CD8 iTregs post generation (B) and mean fluorescence intensity quantified ,n=3 (B). In vitro suppressive function between alloreactive CD4 and alloreactive CD8 iTregs was assessed through suppressive of CFSE labeled alloreactive B6 T cells n=3 (C). Error bars indicate the mean of standard error.

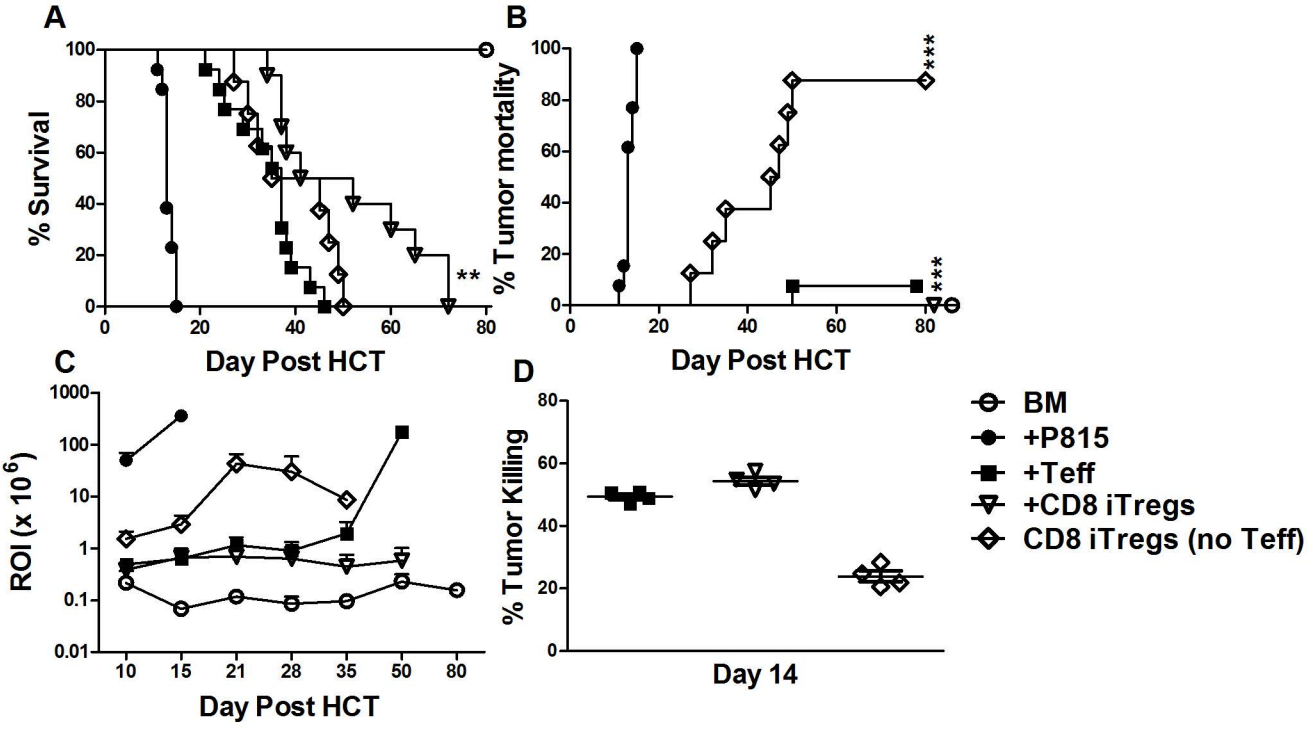

Fig. S5. CD8 iTregs moderately attenuate GVHD and maintain GVL responses . BDF1 recipient mice were lethally irradiated (1200cGY,split doses) and transplanted with 5 x 10^6 B6 TCD-BM, 4 x 10^6 CD8 iTregs, and 5 x 10^3 luc-P815 cells. Three days later 2 x 10^6 CD25-depleted Teff cells were injected. Recipients were monitored for survival (A) and tumor mortality (B). Every week to every other week mice were subjected to whole body imaging, region of signal intensity was quantified using IVIS software for each group (C). BDF1 mice were transplanted as described in (A), fourteen days post HCT splenocytes were plated with either $CFSE^{hi}$ P815 or $CFSE^{low}$ EL4 tumor cells for 16 hours. Percentage tumor killing was calculated as ratio EL4:P815 (no splenocytes control) / EL4: P815 (each group) and normalized to total CD8 donor T cells within splenocyte population n=4 (D). n=15. * P<.05; ** P<.01; *** P<.001. Error bars indicate the mean of standard error .

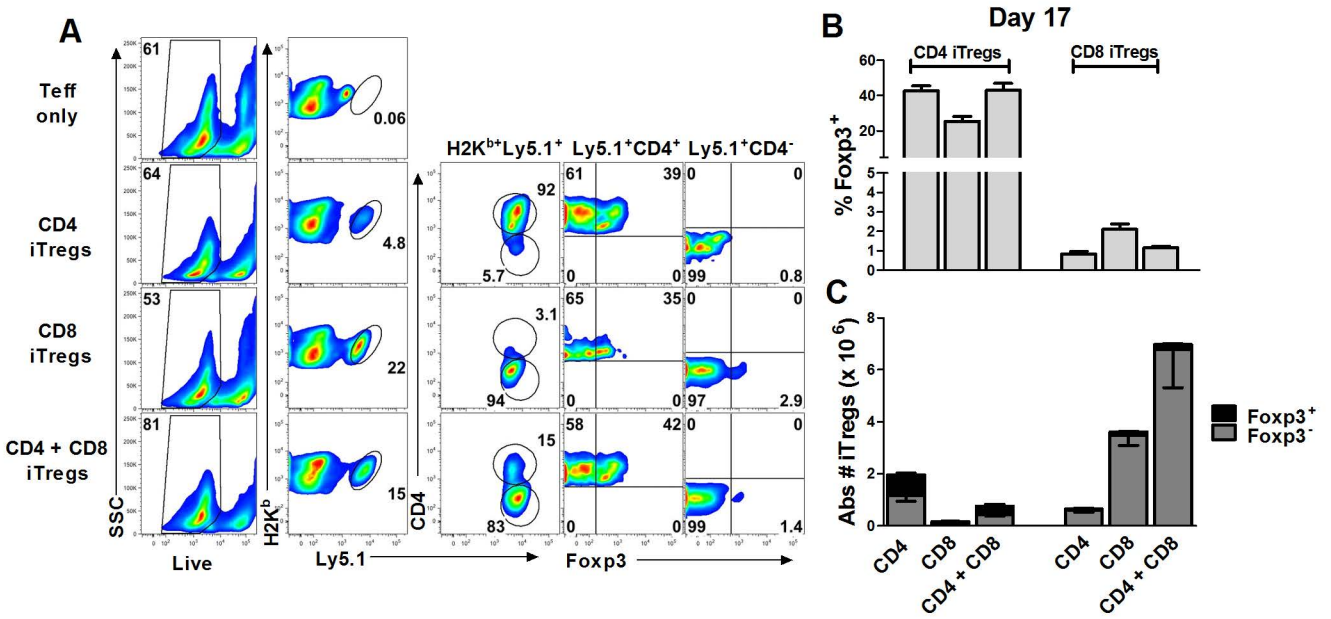

Fig. S6. CD4 iTregs display greater stability than CD8 iTregs. BALB/c mice were lethally irradiated and transplanted with 5 x 10 ^{6} B6 TCD-BM, 1 x 10 ^{6} CD4, 1 x 10 ^{6} CD8, or 0.5 CD4 + 0.5 x 10 ^{6} CD8 iTregs generated from Ly5.1 $^{+}$ mice. Three days later, CD25-depleted 0.5 x 10 ^{6} Teff cells were injected. Seventeen days post -transplant, spleens were collected and iTregs analyzed as described in the representative flow diagram (A). Quantification of percentage of Foxp3 (B) and absolut e number (C) per mouse n=4. Error bars indicate the mean of standard error.