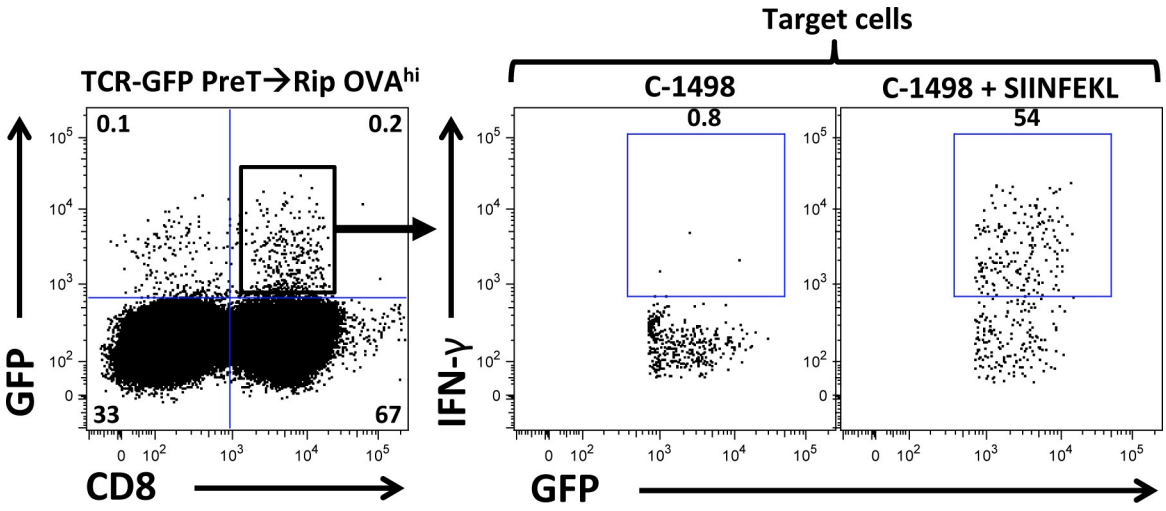
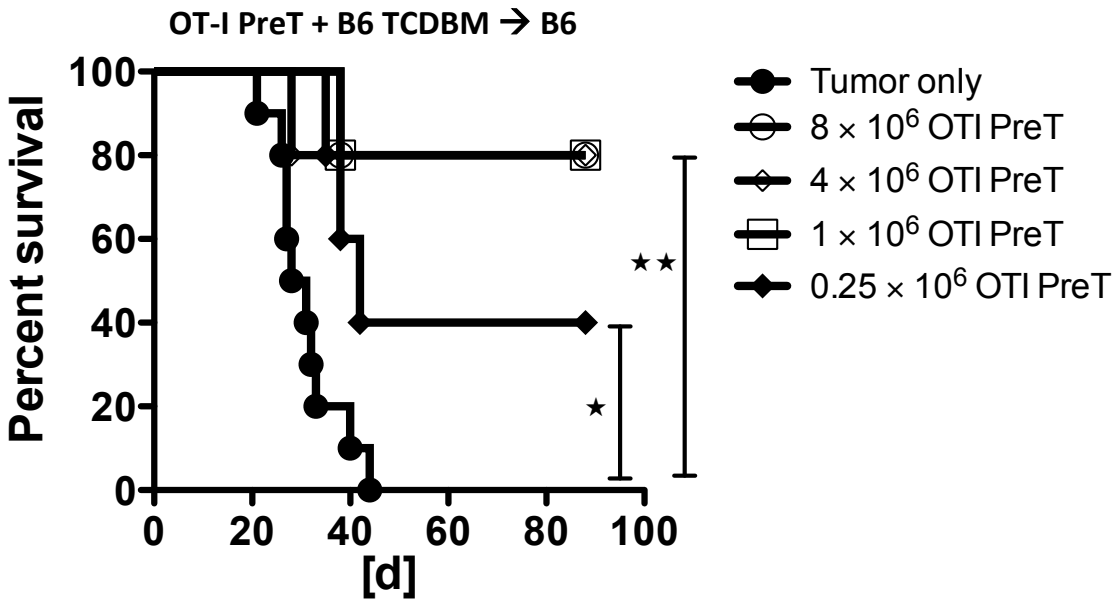
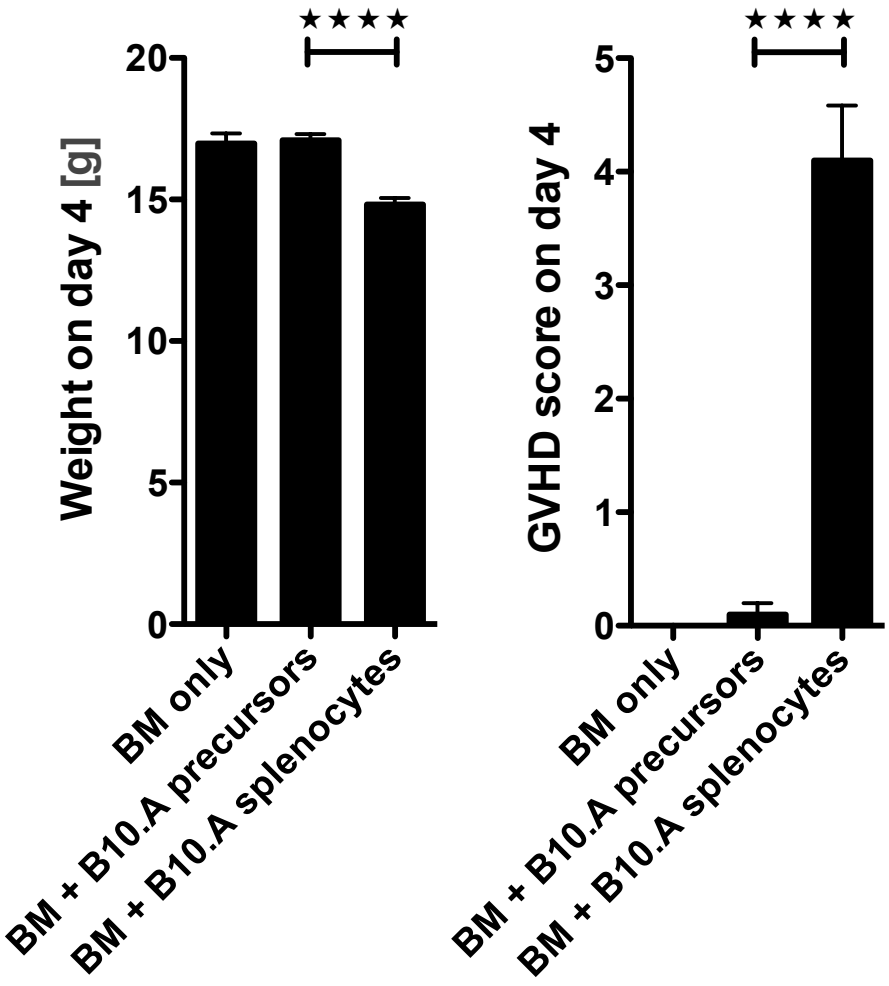


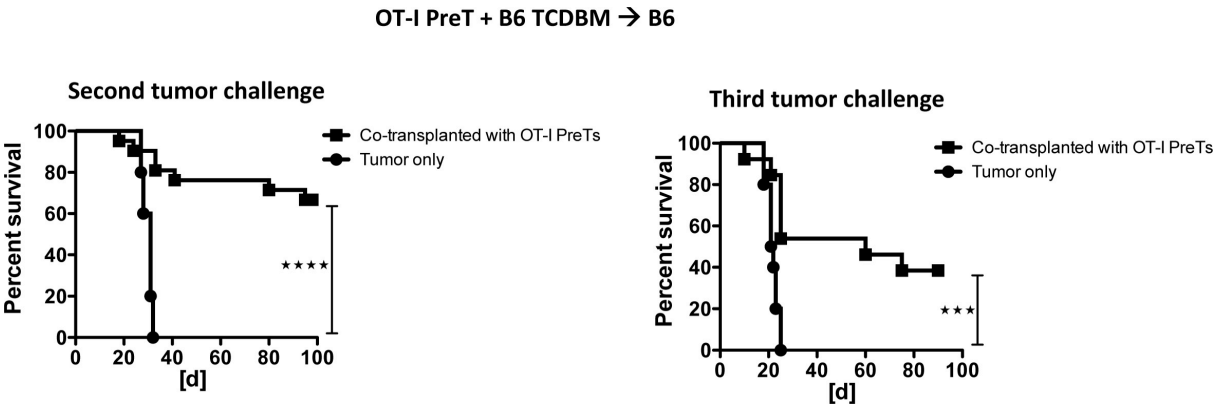
Supplementary figure S1. **T cell progenies of model TCR-transduced preTs escaping thymic negative selection are functional in vivo**. Lethally irradiated Rip-OVA^{hi} mice received 3 × 10^6 syngeneic TCDBM cells and 8 × 10^6 preTs that had been transduced to express a TCR against OVA which represents a self-antigen in this experimental context. Splenocytes were harvested on day 28 after transplantation and cultured in vitro for another four days in the presence of doxycycline. Antigen-specific functionality was assessed by flow cytometry-based intracellular IFNγ detection assays using either naïve or peptide-coated (SIINFEKL) leukemia cells (C1498) as stimulators (middle and right panel). For determining the IFN-γ response CD8$^+$/GFP$^+$ double positive cells were gated and analyzed (right upper quadrant of the left panel). Results of a representative plot are shown.



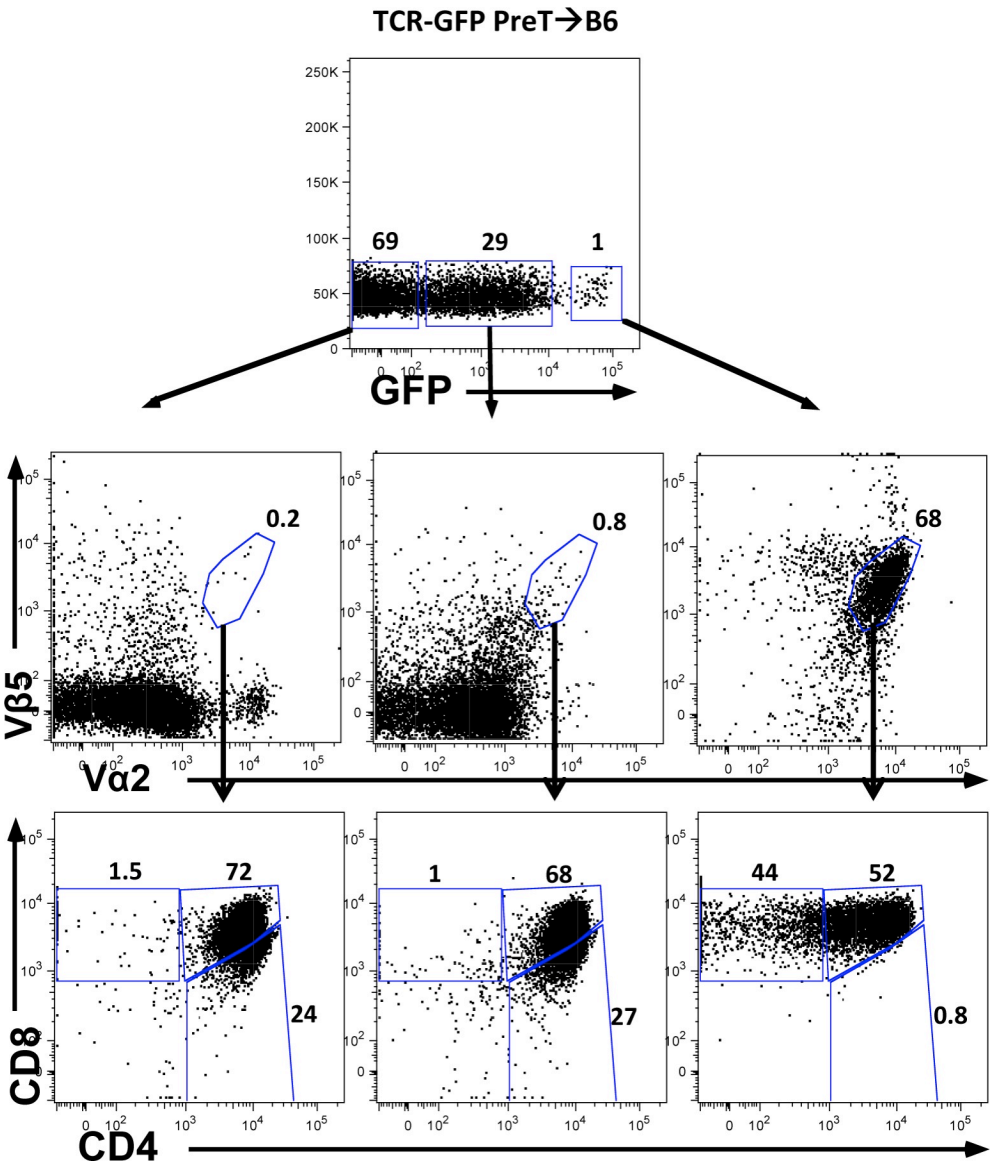
Supplementary figure S2. **Anti-leukemia effects of co-transplanted preTs are dose dependent.** Lethally irradiated B6 mice were reconstituted with 3×10^6 syngeneic TCDBM cells and co-transplanted with increasing doses of OT-I TCR transgenic preTs. Three weeks later, transplant recipients were intravenously injected with 1.2×10^6 C1498-OVA leukemia cells. PBS-treated mice served as controls. n = 5 per group.



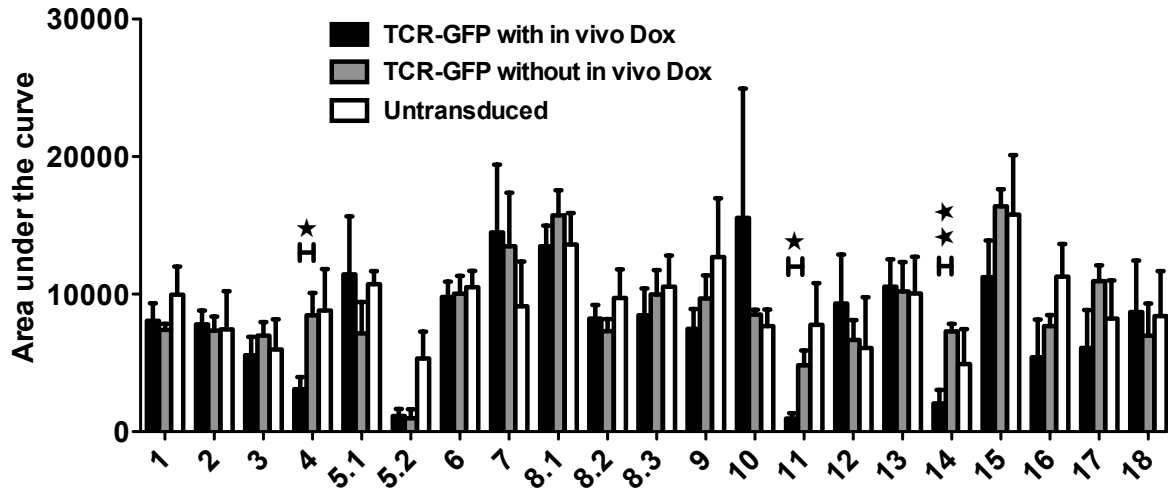
Supplementary figure S3. **Third party preTs can be transplanted across MHC-barriers without evoking GVHD**. Lethally irradiated B6 mice received 15 × 10^6 BALB/c-derived TCDBM cells together with either 8 × 10^6 third party (B10.A) preT cells or 8 × 10^6 third party (B10.A) splenocytes (n = 10 per group). Four days after transplantation, recipients were weighed (left panel) and GVHD was clinically scored in a blinded fashion (right panel).



Supplementary figure S4. **Adoptive transfer of TCR transgenic preTs confers long-term anti-leukemia protection *in vivo***. Surviving mice of the experiment shown in figure S2 were pooled and re-challenged with 1.2×10^6 C1498-OVA leukemia cells (left panel, n = 21). Survivors of this second exposure to leukemia were injected with 1.2×10^6 C1498-OVA leukemia cells for a third time (right panel, n = 13). Non-treated animals served as controls (n = 5 to 10).



Supplementary figure S5. **Forced expression of an introduced MHC-I restricted TCR on precursor T cells instructs CD8 lineage commitment.** Lethally irradiated B6 mice received 3×10^6 syngeneic TCDBM cells and 8×10^6 sorted TCR-transduced preTs expressing high levels, low levels or no GFP (upper plot). For transgene induction recipients received doxycycline in their drinking water starting the day of co-transplantation. Two weeks after transplantation, thymi were harvested and thymocytes assessed using flow cytometry by first gating on GFP^{hi}, GFP^{low} and GFP^{neg} cells followed by gating on the TCR (Vα2Vβ5) positive population (second row). Within this gate the population of $CD8^+/CD4^+$ double-positive, CD8 single-positive (SP), and CD4 SP lymphocytes were determined (lower row).



Supplementary figure S6. **TCR Vβ spectratype analysis of preT-derived T cells.** Lethally irradiated B6 mice received 3×10^6 syngeneic TCDBM cells together with either 8×10^6 non transduced or model TCR gene-transduced preTs (n = 4 for each group). For one group doxycycline was added at the day of transplantation while the second group did not receive doxycycline. One month after transplantation, splenocytes were harvested and cultured *ex vivo* in the presence of doxycycline. After four days of culture 10^5 GFP^+CD3^+ cells were sorted and analyzed by complete TCR Vβ spectratyping as described previously [1]. Data were analyzed by GeneMapper software (Life Technologies) comparing the area under the curve (AUC) representing various CDR3-size lengths. Peak Scanner software (Life Technologies) was used for calculations. Statistical analysis was done applying the Student's t-test.

References

1. Koestner W, Hapke M, Herbst J, Klein C, Welte K, Fruehauf J, *et al.* PD-L1 blockade effectively restores strong graft-versus-leukemia effects without graft-versus-host disease after delayed adoptive transfer of T-cell receptor gene-engineered allogeneic CD8+ T cells. *Blood* 2011 Jan 20; **117**(3)**:** 1030-1041.