

Figure S1. OT-I-transduced and OT-I transgenic T cells display comparable antigen-specific functionality *in vitro*

(A) OT-I-transduced B10.A T cells were stimulated with either C1498, C1498-OVA or without stimulators in a 10:1 ratio and incubated for 14 hours. For the last 4 hours Brefeldine A was added. Dot plots display IFN γ (upper row) and granzyme B (lower row) expression of gated CD8 $^+$ GFP $^+$ T cells. (B) The left graph depicts IFN γ expression of OT-I-transduced T cells (gated on GFP $^+$ CD8 $^+$ (Δ)) or OT-I transgenic T cells (gated on CD8 $^+$ (\square)). Either OT-I-transduced

or OT-I transgenic B6 T cells were incubated with SIINFEKL-pulsed B6 splenocytes for 7 hours at the indicated peptide concentrations. For the last 4 hours Brefeldine A was added. On the right in vitro cytotoxicity of OT-I-transduced (Δ) and OT-I transgenic (\square) T cells against C1498-OVA is comparatively graphed at the stated E:T ratios. Values are shown \pm 1 SE.

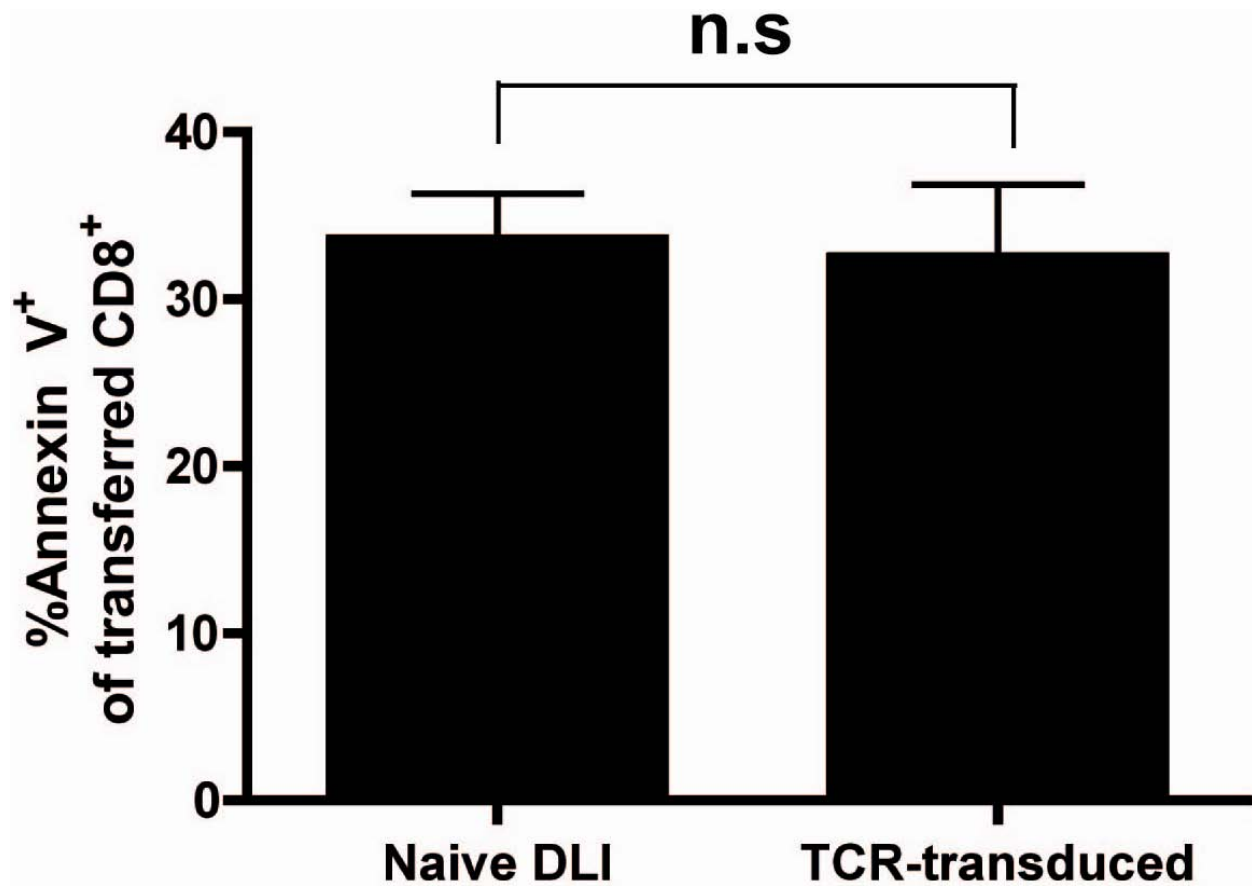


Figure S2. TCR-transduced T cells show comparable apoptosis rates to naïve DLI after three days *in vivo*

(A) 2.5×10^6 TCR gene-transduced allogeneic (B10.A) T cells were adoptively transferred late into established allogeneic (B10.A \rightarrow B6) transplant recipients. Freshly isolated splenic CD8⁺ T cells from B10.A donors (purified by MACS column separation) were CFSE labelled and used as controls. Three days later mice recipient spleens were analyzed. Transferred T cells were either gated on GFP⁺CD8⁺ (for TCR-transduced T cells) or on CFSE⁺CD8⁺ (for naïve T cells) and analyzed for annexin V expression.

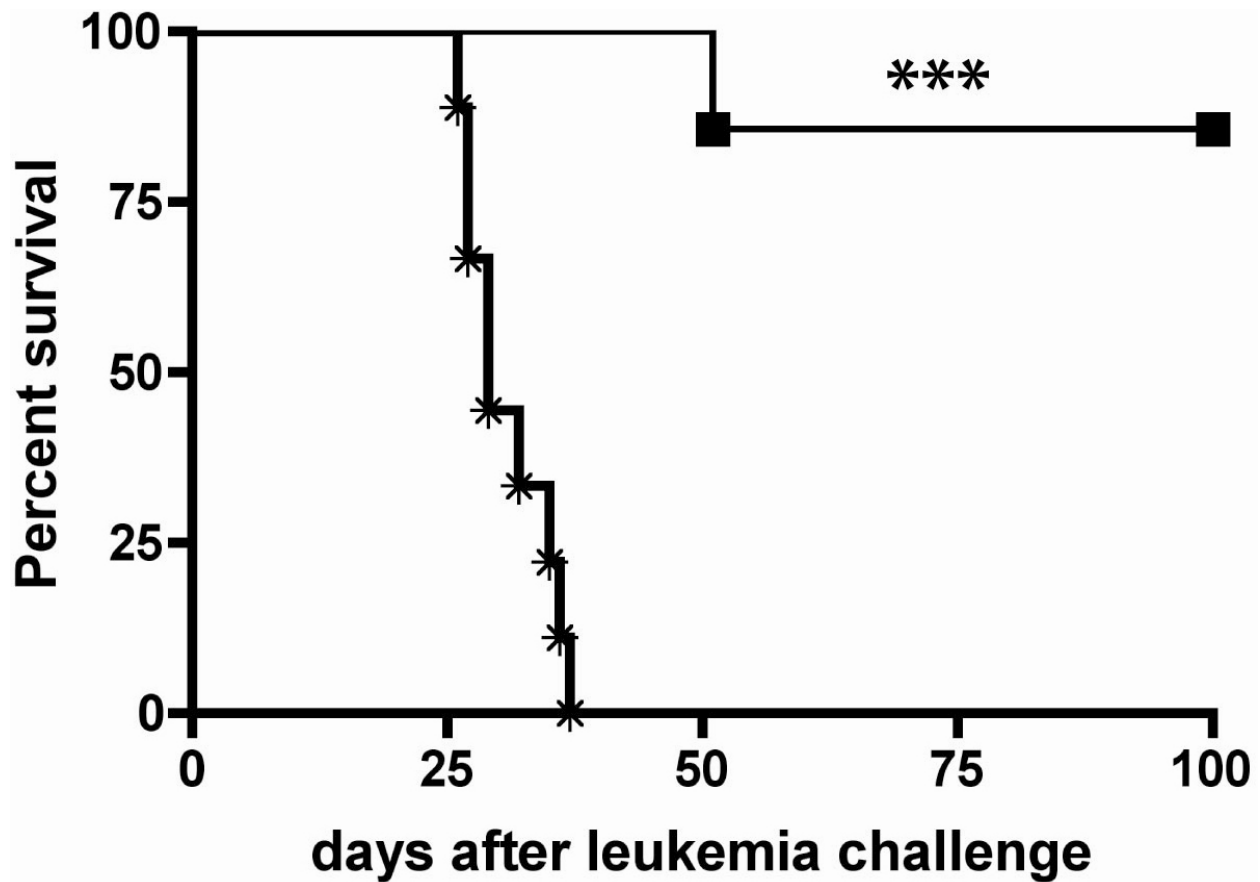


Figure S3. Allogeneic TCR-transduced T cells mediate strong GVL effects when the leukemia challenge is given very early after AT

40×10^6 TCR gene-transduced allogeneic (B10.A) T cells (-*-) were adoptively transferred late (56 days) after HCT into allogeneic (B10.A \rightarrow B6) transplant recipients. Mice receiving PBS were used as controls (-■-). One day after AT mice were challenged with 1.2×10^6 C1498-OVA cells intravenously (***, $p < 0.0001$ between treatment groups; $n = 7/\text{group}$).

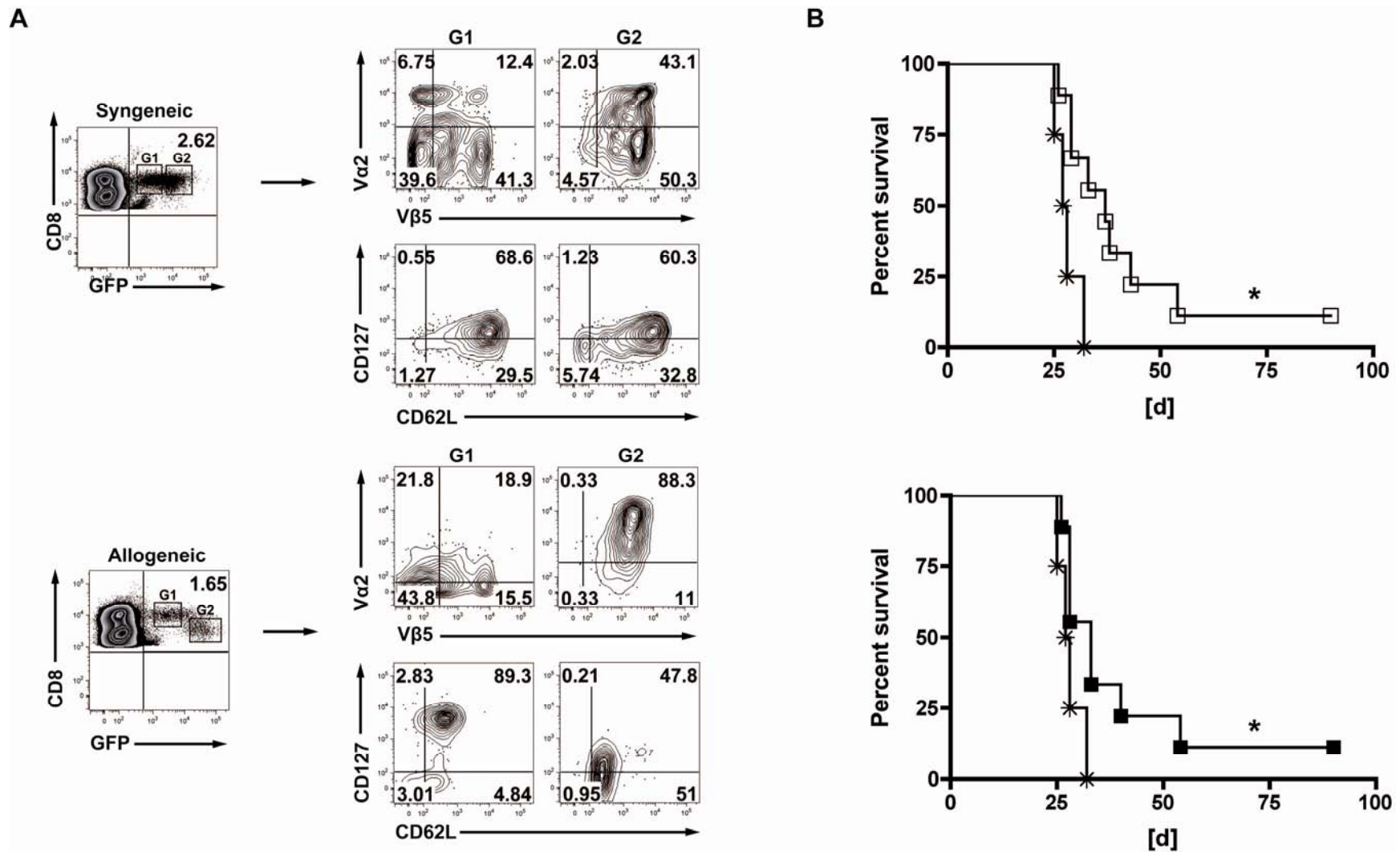


Figure S4. Long-term persisting TCR-transduced CD8⁺ T cells display central memory phenotype and mediate GVL effects *in vivo*

(A) 40×10^6 TCR gene-transduced allogeneic (B10.A) or syngeneic (B6) T cells were adoptively transferred late after HCT into either allogeneic (B10.A \rightarrow B6) or syngeneic (B6 \rightarrow B6) transplant recipients. Mice were sacrificed 180 days after AT and spleens were analyzed by flow cytometry. Results from one representative syngeneic and allogeneic mouse are depicted. Cells were gated on CD8⁺ and GFP (G1, G2) and either costained for V β 5/V α 2 or CD127/CD62L. (B) Mice received TCR-transduced allogeneic (-*-) or syngeneic (-□-) T cells as in (A). 50 days after AT mice were challenged with 1.2×10^6 C1498-OVA cells intravenously. A PBS-treated cohort (-■-) was used as control (n = 9 / group).