

Supplemental Figure S1.

Determination of EC50 for NY-ESO-1-specific TCRs.

(A and B) ELISA measuring secretion of IFN- γ from TCR-transduced PBMCs following 48 hours coincubation with K562 engineered to express HLA-A*02:01 and pulsed with varied concentrations of (A) MART₂₆₋₃₅ or (B) NY₁₅₇₋₁₆₅ peptide. (C-E) ELISA measuring secretion of IFN- γ from TCR-transduced PBMCs following 48 hours coincubation with K562 engineered to express (C) HLA-B*07:02, (D) HLA-B*18:01, or (E) HLA-C*03:04 and pulsed with varied concentrations of indicated peptides. Means ± SD for two technical replicates are shown. EC₅₀ values and associated errors determined by non-linear curve fitting are indicated.



Supplemental Figure S2.

Establishment of xenograft tumor line and function of input T cells for in vivo experiment.

(A) ELISA measuring secretion of IFN- γ from TCR-transduced PBMCs following 48 hours coincubation with derivatives of the PC-3 prostate cancer cell line engineered to express (left) HLA-A*02:01 and NY-ESO-1 full protein, (middle) HLA-A*02:01 alone, or (right) NY-ESO-1 full protein alone. Means ± SD for two technical replicates are shown. (B) ELISA comparing secretion of IFN- γ from TCR-transduced PBMCs following 48 hours coincubation with indicated M257 or PC-3 target cells. On the 4th day post-transduction, TCR-transduced PBMCs were sorted for CD3⁺/LNGFR⁺ and then expanded for 13 additional days prior to the co-culture/ELISA assay and the *in vivo* experiment. Means ± SD for a representative experiment with two technical replicates is shown. (C) Flow cytometry contour plots comparing the transduction (LNGFR⁺) levels of TCR-transduced PBMCs used for the *in vivo* experiment.