YMTHE, Volume *27*

Supplemental Information

A Potent Tumor-Reactive p53-Specific Single-Chain TCR without On- or Off-Target Autoimmunity *In Vivo*

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Supplemental Information

Figure Titles

Figure Legends

Supplementary Figure 1. Specificity and Functional Avidity of scTCR-Modified Murine T Cells. TCR-transduced T cells were incubated overnight with T2 cells loaded with the cognate peptide ($p53_{264-272}$) or irrelevant peptide ($gp100_{280-288}$) and stained for intracellular granzyme B (GzmB) (**A**) and TNFα (**B**). Representative histograms of p53TCR dc-(black bars) and p53TCR sc-(white bars) modified $CD8^+$ and $CD4^+$ T cells are shown. (**C**) The same TCR-transduced T cells were tested for their cytolytic activities in a 5h ^{51}Cr-release assay against T2 cells pulsed with the indicated concentrations of $p53_{264-272}$ peptide and effector to target ratio (E:T). The avidity of TCR-transduced $CD8^+$ (left panel) and $CD4^+$ (right panel) T cells was determined as the concentration needed to induce a half-maximum specific lysis (EC50). (**D**) Cytolytic activity of TCR-transduced T cells in response to T2 cells pulsed with 10μM of $p53_{264-272}$ or irrelevant $FluM1_{58-66}$ peptide at the indicated $CD8^+V\beta3^+$ or $CD4^+V\beta3^+$ to target ratios.

Supplementary Figure 2. Histological Analysis of GvHD in Mice Infused with TCR-Modified T Cells. Spleens were harvested from mice showing early onset of GvHD symptoms after ACT and processed for histological examination. Tissue sections from mice injected with mock, dc, βc, αc and scTCR-modified T cells (a, b, c, d and e, respectively). Micrographs show frozen sections immunohistochemically stained with anti-CD4 mAb. Very few infiltrating $CD4^+$ T cells (brown spots) were detected in controls and scTCR-treated animals as opposed to high number of T cell infiltrates in tissues of mice with GvHD, in particular recipients adoptively transferred with dc and βcTCR-T cells. Sections are shown at an original magnification of ×20. Scale bar = 50 μm.

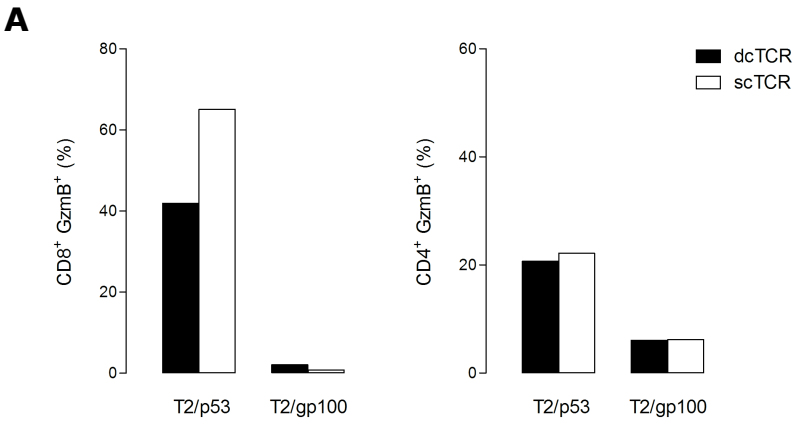
Supplementary Figure 3. Evaluation of On-Target Reactivity of scTCR-Modified Murine T Cells. TCR-transduced T cells were tested for their cytolytic activity in a standard 5h ^{51}Cr-release assay against (**A**) $p53^+A2K^{b+}$ mouse (MEF/R172H and MEF/R270H) and $p53^+A2^+$ human (Saos2/143) tumor cell lines, $p53^-A2^+$ control targets (MEF and Saos2), and (**B**) MEFs and T cells from HupkiA2 or p53-null $A2K^b$ mice, pulsed or not with 1μM of $p53_{264-272}$ at the indicated $CD8^+V\beta3^+$ to target ratios.

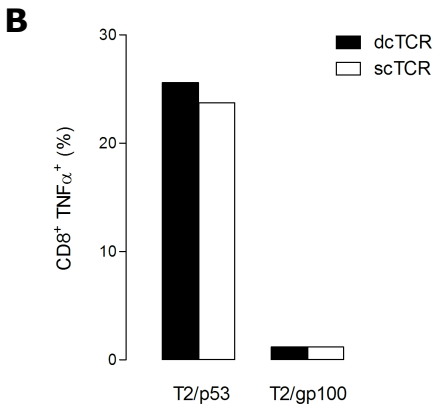
Supplementary Figure 4. Analysis of the Memory State of T Cells in Mice which have Mounted a Complete Tumor Response. Spleens from mock control or mice which have

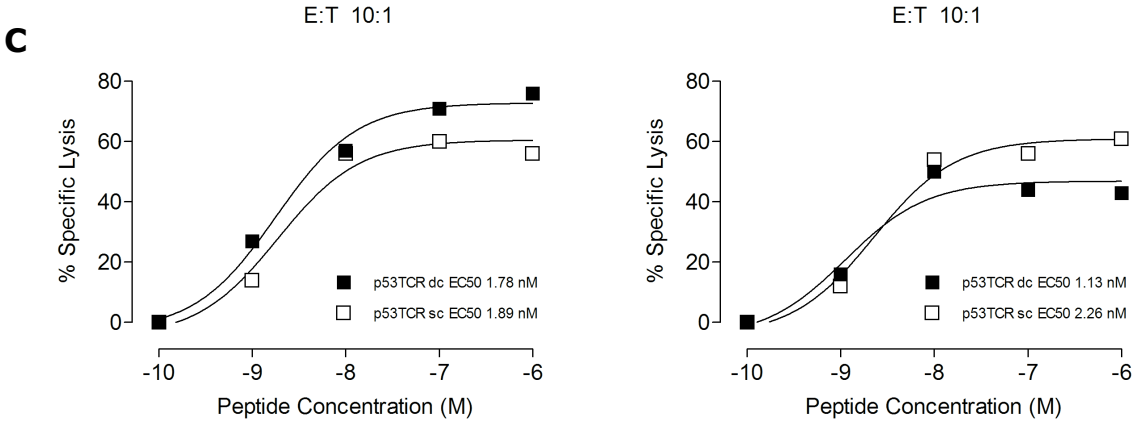
shown complete tumor eradication after transfer of p53 scTCR-modified T cells were harvested, pooled, homogenized, filtered and separated by MACS for $CD8^+$ T cell population. The antigen memory state of ex-vivo $CD8^+$ T cells was analyzed by an IFNγ ELISPOT assay. Briefly, titrated numbers of MACS-purified $CD8^+$ T cells (from mock control or p53 scTCR-treated mice) were incubated with T2 cells loaded with $p53_{264-272}$ peptide ($2.5x10^{-7}$M). A murine T cell line stably expressing the p53 scTCR was used as positive control effector cells. (**A**) Antigen specific IFNγ-producing T cells were detected in p53 scTCR-treated mice as opposed to background spots in mock control animals. (**B**) Spot quantification using C.T.L. ImmunoSpot S5 Versa Analyzers (C.T.L. Europe GmbH, Bonn, Germany).

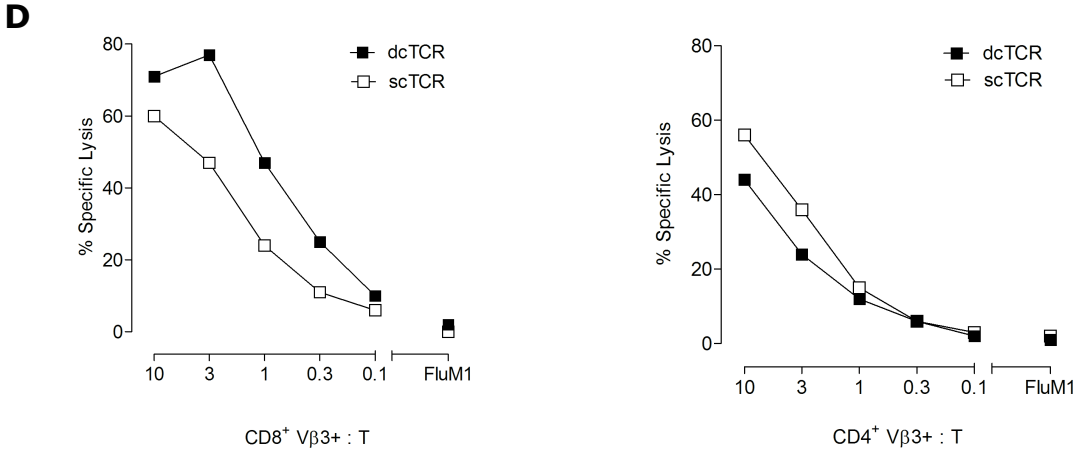
Supplementary Figure 5. Specificity and Functional Avidity of scTCR-Modified Human T Cells. (**A**) Avidity (K_D) of peptide-MHC pentamer binding to T cells determined by Scatchard analysis. ScTCR-transduced human $CD8^+$ (open squares, left plot) and $CD4^+$ (closed squares, right plot) T cells were tested for binding to pentameric p53(264-272)A2.1 complexes by flow cytometry. (**B**) scTCR-transduced human $CD8^+$ T cells were tested for their cytolytic activities in a 5h ^{51}Cr-release assay against $p53^+A2.1^+$ (Saos2/143) and $p53^-A2.1^+$ (Saos2) human tumor cell lines at the indicated $CD8^+V\beta3^+$ to target ratio.

Supplemental Figure 1

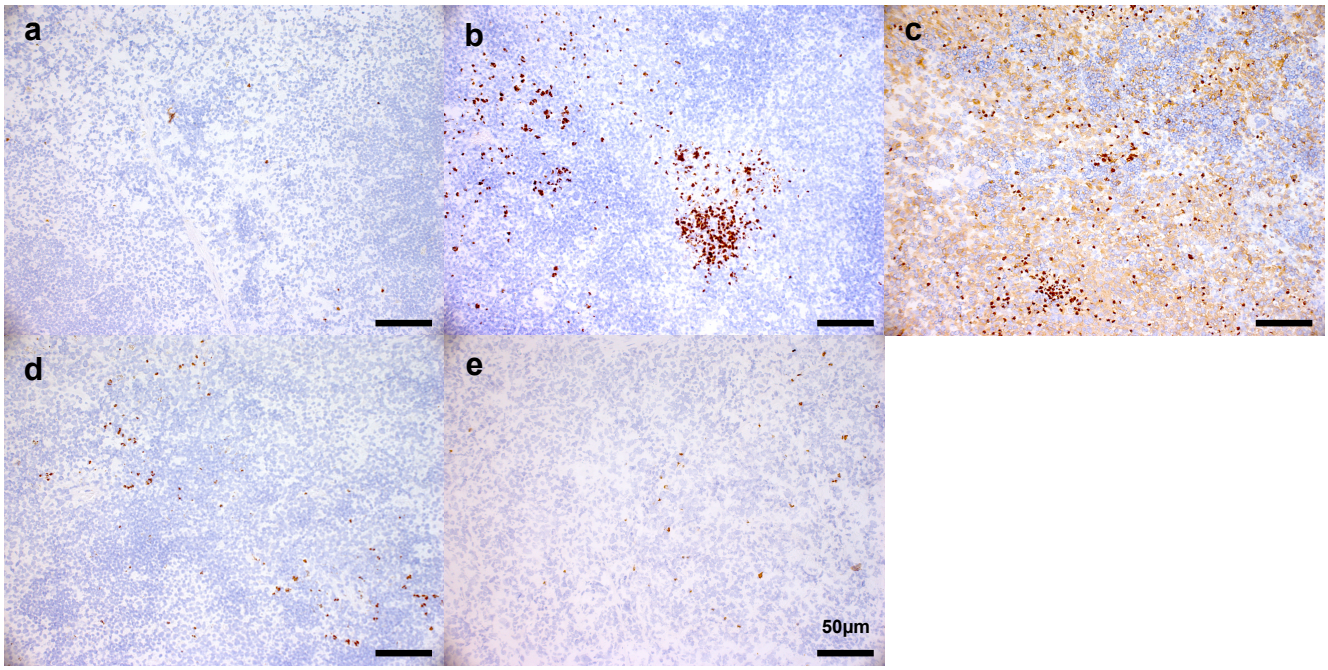






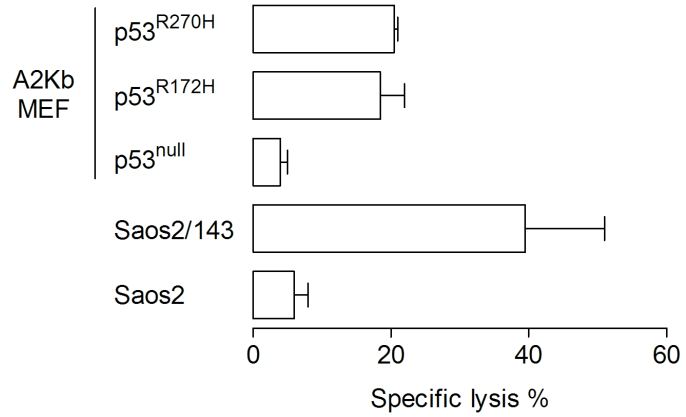


Supplemental Figure 2



Supplemental Figure 3

A



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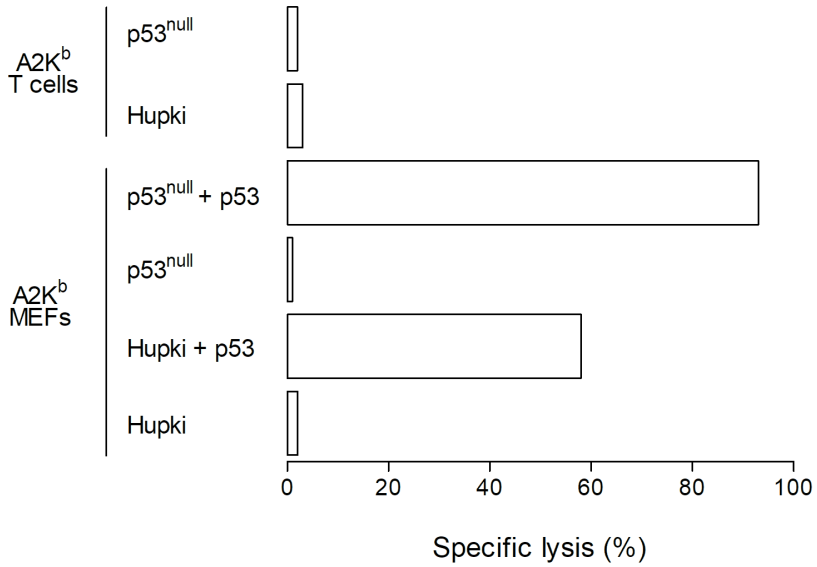
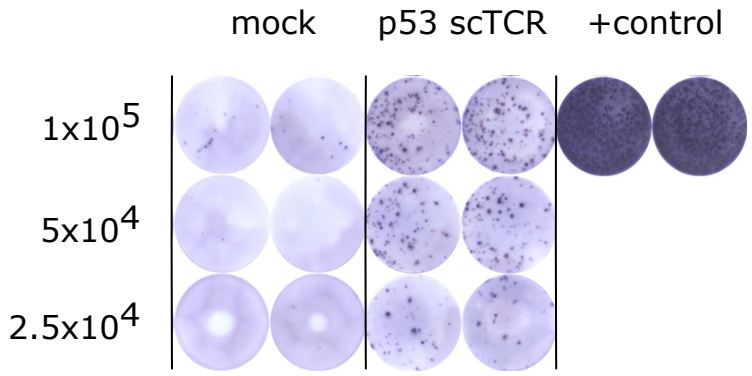


Supplemental Figure 4

A



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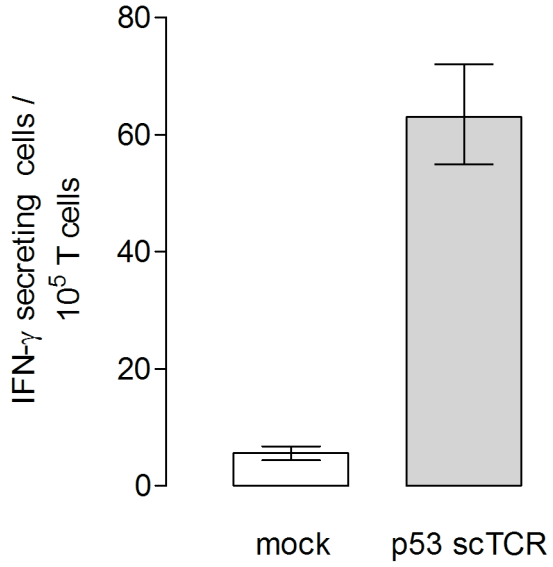

Supplemental Figure 5