SUPPLEMENTAL MATERIAL

Kuball et al., http://www.jem.org/cgi/content/full/jem.20082487/DC1

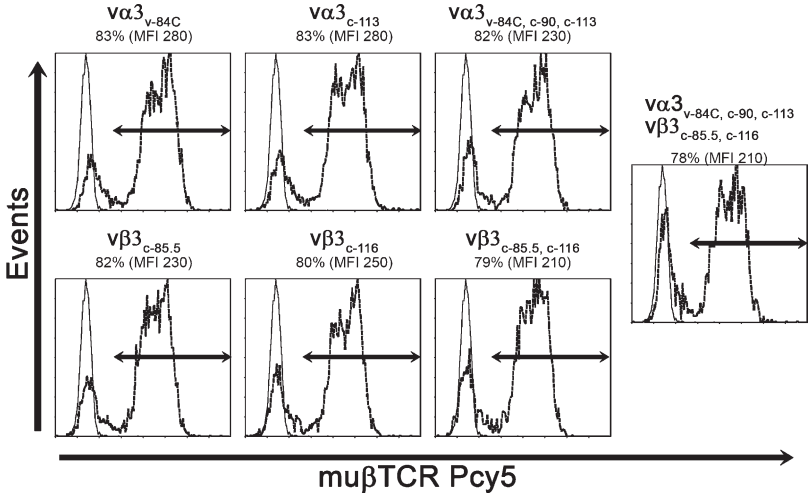

Figure S1. TCR β chain staining of ΔTCR-transduced 58α⁻β⁻ hybridoma cells. Mock-transduced 58α⁻β⁻ cells (continuous line) and 58α⁻β⁻ cells transduced with the indicated ΔTCR chain (dashed line) in combination with the corresponding WT TCR chain or, where indicated, the corresponding ΔTCR chain were stained with anti-muβTCR–PE at 4°C, and were analyzed by flow cytometry. The percentage and MFI of muβTCR-positive cells are indicated. All data are representative of at least two independent experiments.

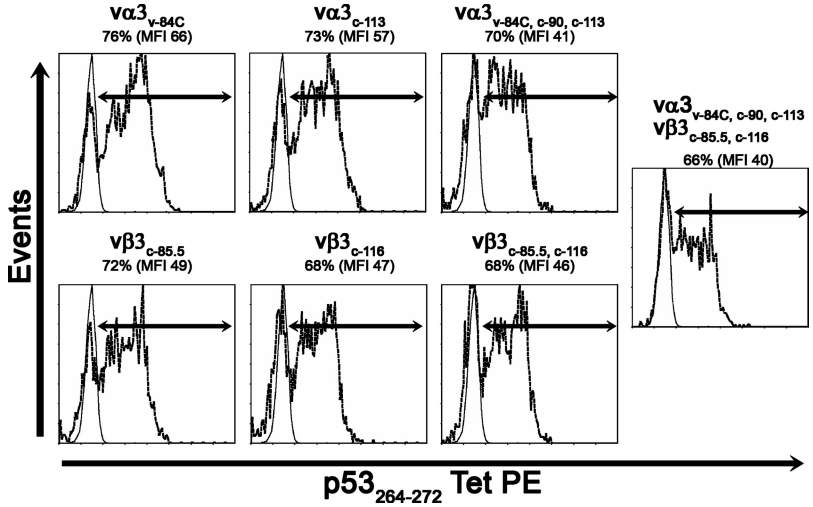

Figure S2. Tetramer staining of ΔTCR-transduced 58α⁻β⁻ hybridoma cells. Mock-transduced 58α⁻β⁻ cells (continuous line) and 58α⁻β⁻ cells transduced with the indicated ΔTCR chain (dashed line) in combination with the corresponding WT TCR chain or, where indicated, the corresponding ΔTCR chain were stained with $p53_{264-272}$Tet–PE at 4°C, and were analyzed by flow cytometry. The percentage and MFI of tetramer-positive cells are indicated. All data are representative of at least two independent experiments.

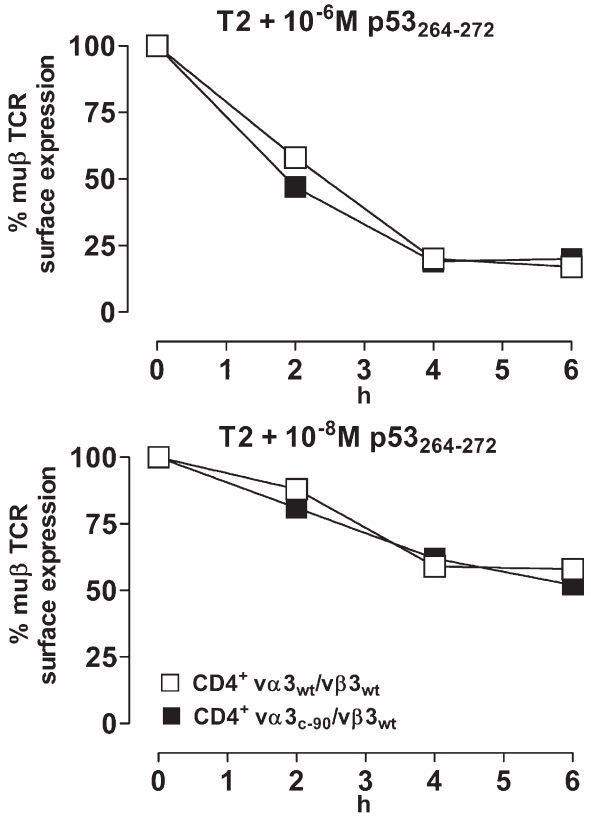

Figure S3. TCR internalization after antigen encounter. Surface expression of the introduced mouse WT and ΔTCR β chain in $CD4^+$ T cells transduced to acquire specificity for p53 before and after coincubation with T2 cells pulsed with (top) 10^{-6} or (bottom) 10^{-8} M $p53_{264\text{-}272}$ peptide was assessed by flow cytometry. Surface expression after antigen encounter was calculated as the percentage of total introduced muβTCR surface expression. All data are representative of two independent experiments.

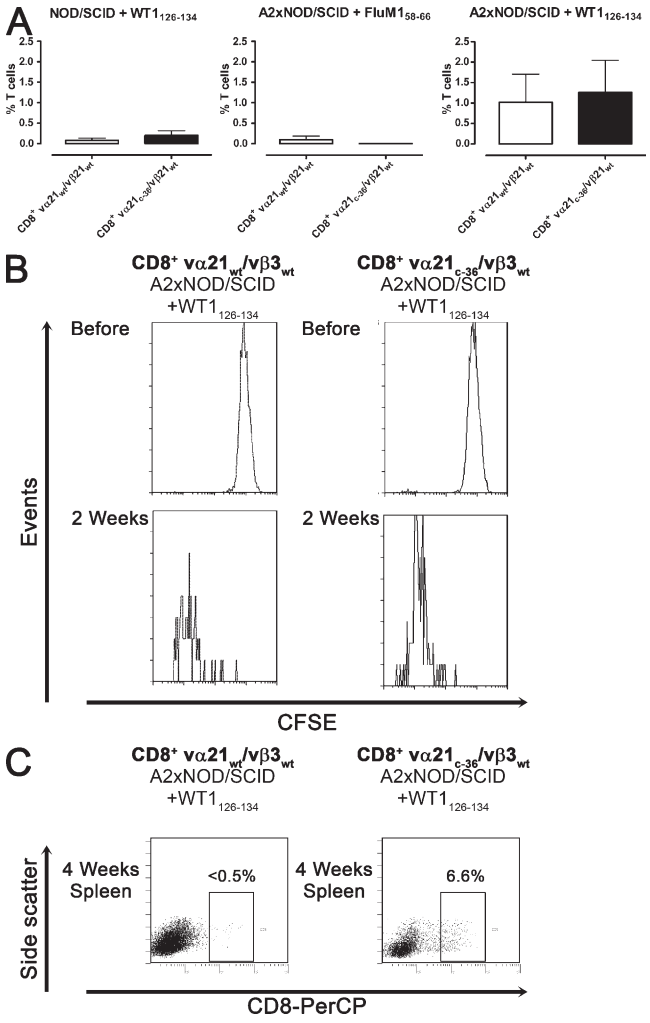

Figure S4. Antigen-specific in vivo proliferation of TCR-transduced T cells. 2×10^6 $vα21_{WT}/vβ21_{WT}$ and $vα21_{c\text{-}36}/vβ21_{WT}$ CFSE-labeled TCR-transduced T cells were injected intravenously into NOD/SCID and HLA-A2 × NOD/SCID mice ($n = 24$). Simultaneously, mice were immunized subcutaneously once with 10 µg of the indicated peptide and received 6×10^5 IU rhIL-2 resuspended in incomplete Freund's adjuvant subcutaneously once 6 h later. 2 wk later, PBMCs were obtained from peripheral blood by tail bleeding, (A) stained with anti-CD8 antibody (data are means ± SEM), and (B) tested for dilution of CFSE. (C) 4 wk later, spleen cells from mice with peripheral engraftment of transduced T cells at 2 wk (the HLA-A2 × NOD/SCID mice recipients that had been immunized with $WT1_{126\text{-}134}$ peptide after cell transfer) were stained with anti-CD8 antibody and were analyzed by flow cytometry. Data are representative of two independent experiments.

Table S1. Primers used for site-directed mutagenesis

Location (IMGT)		Primers (5′ to 3′)
	α chain species	
v-24	Mouse	Forward: CGCAGAGCTGCAGTGTCAATTTTCCT
		Reverse: GGAAAATTGACACTGCAGCTCTGCGTTCT
v-83	Human	Forward: GTCTTCTTACAAAAAAGTGCCAAGCACCTC
		Reverse: GGTGCTTGGCACTTTTTTGTAAGAAGACAG
v-84C	Mouse	Forward: GTTCAGCAAGAGTCAGTCTTCCTTCAACC
		Reverse: GGTGGAAGGAAGACTGACTCTTGCTGAAC
c-36	Human	Forward: TATTCACCGATTTTGATTCTCAAACACAAGTGTCACAAAG
		Reverse: TTGTGACACTTGTGTTTGAGAATCAAAATCGGTGAATAGG
c-90	Mouse	Forward: GCCTGGAGCCAGCAGACAAGCTT
		Reverse: AGCTTGTCTGCTGGCTCCAGGCA
c-90	Human	Forward: GTGCTGTGGCCTGGAGCCAAAAATCTGAC
		Reverse: AAAGTCAGATTTTTGGCTCCAGGCCACAGCAC
c-109	Human	Forward: GACTTTGCATGTGCAAACGCCTTCCAAAACAGC
		Reverse: GCTGTTTTGGAAGGCGTTTGCACATGCAAAGTC
c-113	Mouse	Forward: TCTTCAAAGAGACCCAGGCCACCTA
		Reverse: GTAGGTGGCCTGGGTCTCTTTGAAGATA
	β chain species	
c-1.3	Mouse	Forward: AGAGGATCTGAGACAAGTGACTCCA
		Reverse: TTGGGTGGAGTCACTTGTCTCAGAT
c-84.5	Mouse	Forward: GGCCTACAAGGAGAGCCAGTATAGC
		Reverse: AGGCAGTAGCTATACTGGCTCTCCTTGTAG
c-85.6	Human	Forward: AGCAGCCCGCCCTCCAAGACTCCAG
		Reverse: TGGAGTCTTGGAGGGCGGGCTGCTCCTTGA
c-116	Mouse	Forward: ACCCAAACCTGTCACACAGCAGATCAGT
		Reverse: AGGCCTCTGCACTGATCTGCTGTGTGAC

Primers used for site-directed mutagenesis of TCR chains to change asparagine to glutamine in the glycosylation sites indicated in Fig. 1.