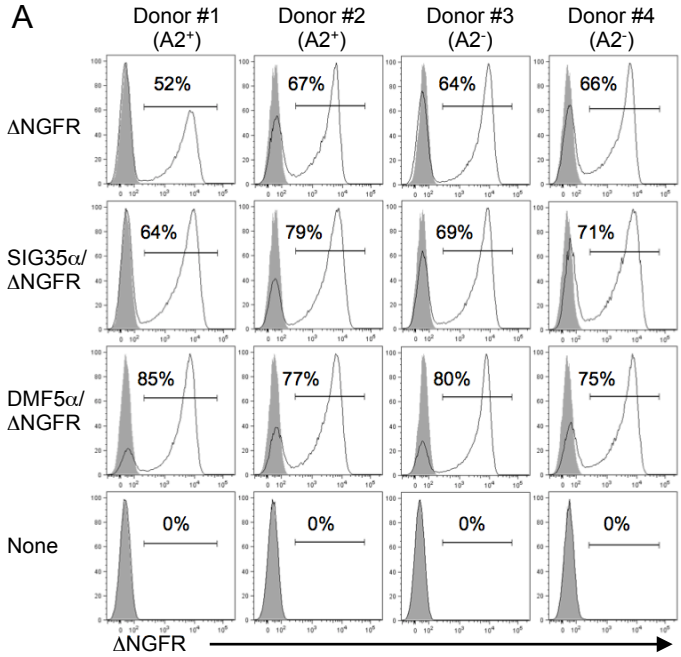
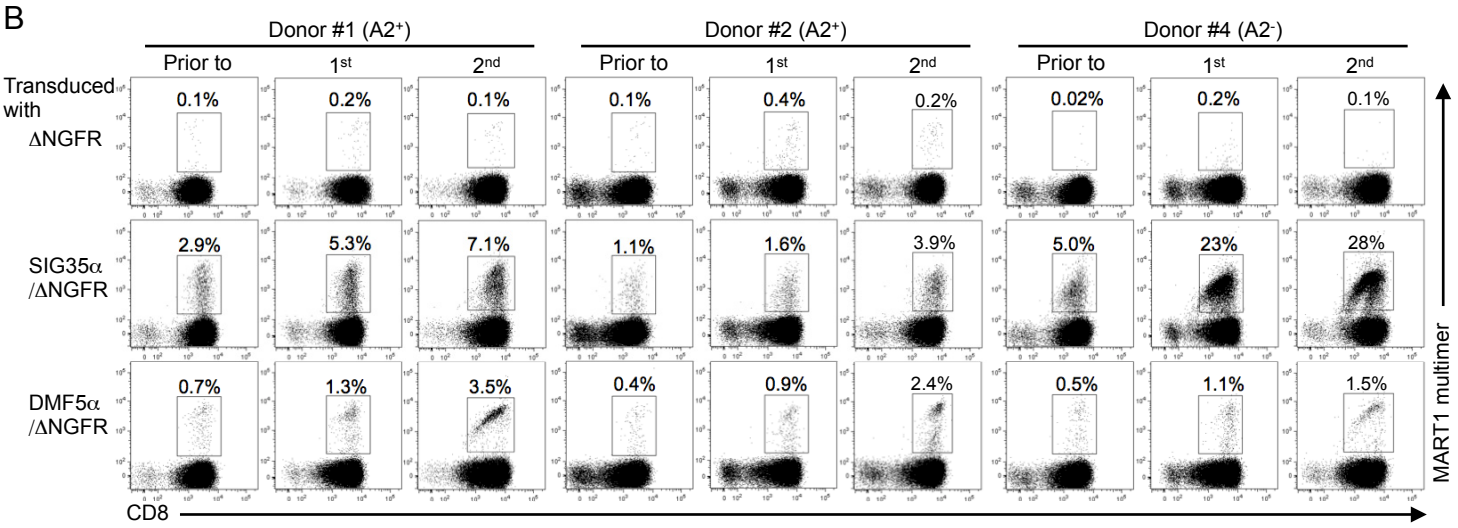
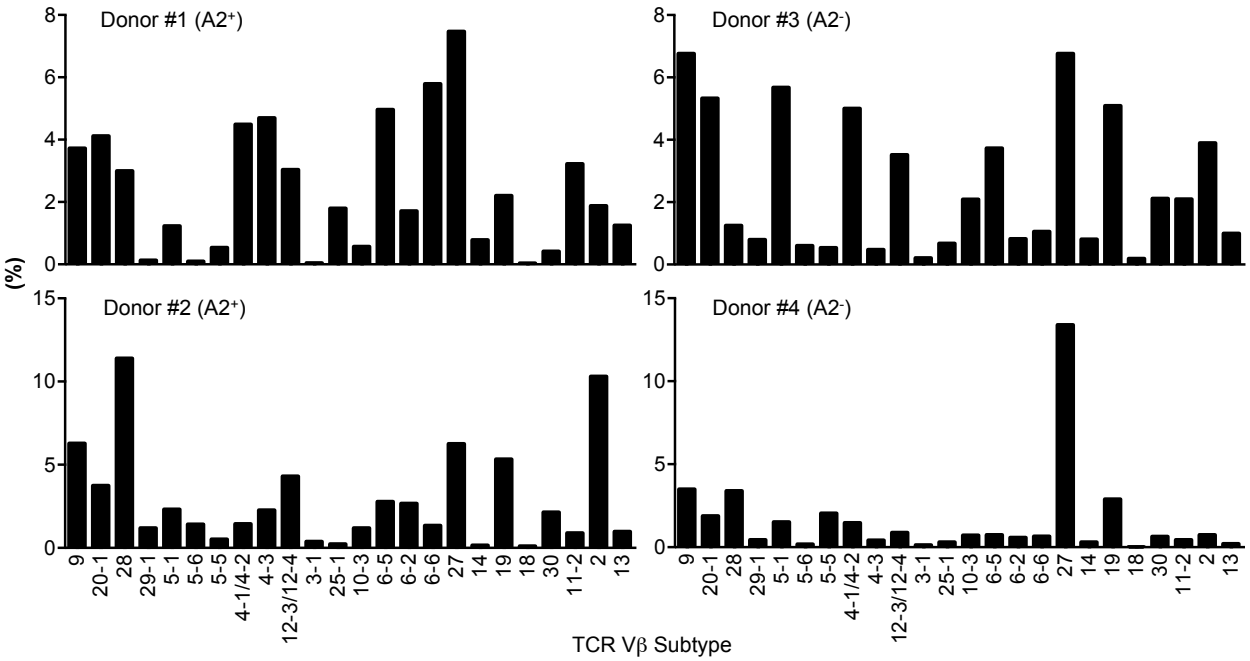
Supplementary Fig. 1





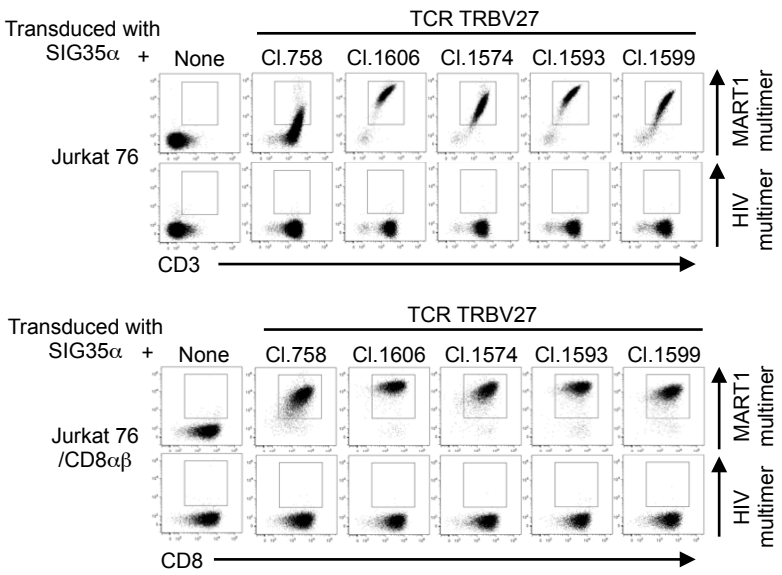
Supplementary Fig. 1. Compared to DMF5α, SIG35α requires a lower contribution from TCRβ chains to recognize A2/MART1. (A), Retroviral transduction efficiency of peripheral T cells with TCRα hemichain genes. Peripheral T cells isolated from two HLA-A2$^+$ donors (#1 and #2) and two A2$^-$ donors (#3 and #4) were retrovirally transduced with ΔNGFR, SIG35α/ΔNGFR or DMF5α/ΔNGFR and stained with anti-NGFR mAb. TCRα genes were fused with the ΔNGFR gene by the F2A sequence. (B), ΔNGFR-, SIG35α/ΔNGFR- or DMF5α/ΔNGFR-transduced CD8$^+$ T cells were stimulated with wt-aAPC pulsed with wild type A2/MART1 peptide once a week. Between stimulations, the T cells were supplemented with IL-2 (10 IU/ml) and IL-15 (10 ng/ml) every 3 days. ΔNGFR gene alone was utilized as a control. Data for A2/MART1 multimer staining performed prior to and following first and second stimulations are shown. Data for A2/MART1 multimer staining of an A2$^-$ donor #3 are shown in Fig. 2D. Data shown are gated on ΔNGFR$^+$ cells.

Supplementary Fig. 2



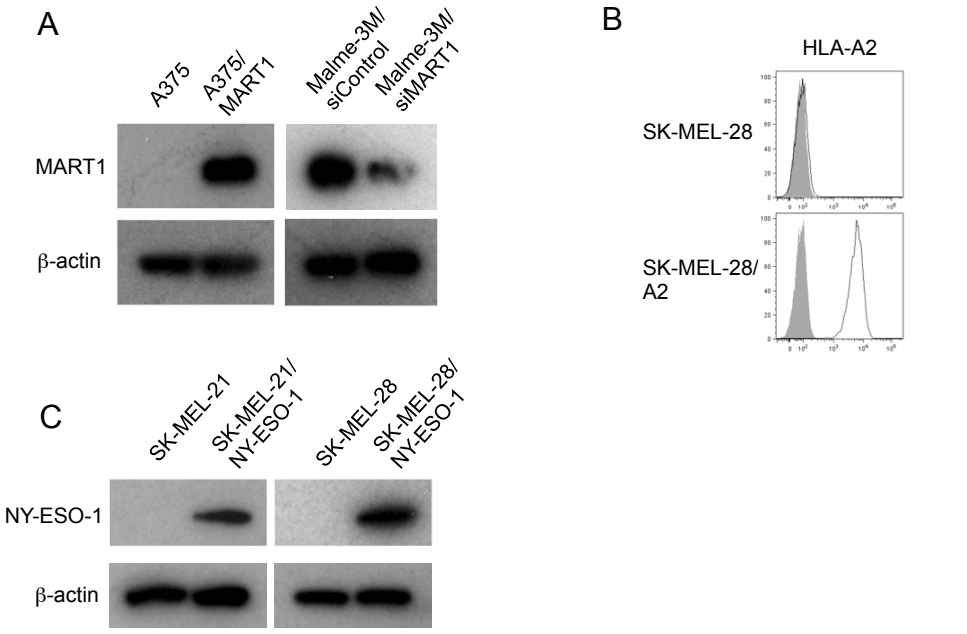
Supplementary Fig. 2. TCR Vβ subtype analysis of SIG35α/ΔNGFR-transduced CD8⁺ T cells in the HLA-A2⁺ and A2⁻ donors. SIG35α/ΔNGFR-transduced CD8⁺ T cells from two HLA-A2⁺ and two A2⁻ donors were stained with A2/MART1 multimer, mAbs for TCR Vβ subtypes, and anti-CD8 mAb. The percentage of A2/MART1 multimer⁺ CD8⁺ T cells expressing each subtype is shown in Fig. 3A. The percentage of the overall transduced CD8⁺ T cells expressing each subtype is shown. Data shown are gated on ΔNGFR⁺ cells.

Supplementary Fig. 3



Supplementary Fig. 3. The structural avidity range of A2/MART1 TCRs consisting of SIG35α is very broad and enhanced in the presence of CD8. Jurkat 76 or Jurkat 76/CD8αβ cells were individually transduced with eleven distinct TRBV27 TCRβ chains along with SIG35α or with DMF5 TCR. All Jurkat 76- or Jurkat 76/CD8αβ-derived A2/MART1 TCR transfectants were stained by 2 μg/ml A2/MART1 multimer. Data for multimer staining of seven representative Jurkat 76 or Jurkat 76/CD8αβ transfectants are shown in Fig. 5A. Data for multimer staining of the remaining 5 Jurkat 76 or Jurkat 76/CD8αβ transfectants are shown.

Supplementary Fig. 4



Supplementary Fig. 4. Expression of HLA-A2, MART1 and NY-ESO-1 in gene-engineered tumor cells. (A), Western blot analysis was performed with anti-MART1 mAb to detect MART1 expression in A375 transduced with full-length MART1 and Malme-3M transduced with siMART1 or siControl. β-actin expression was used as a positive control. (B), Surface expression of HLA-A2 on SK-MEL-28 transduced with HLA-A2 was analyzed by flow cytometry following staining with anti-HLA-A2 mAb. (C), Western blot analysis was performed with anti-NY-ESO-1 mAb to measure NY-ESO-1 expression in SK-MEL-21 and SK-MEL-28 transduced with full-length NY-ESO-1 cDNA. β-actin expression was used as a positive control.