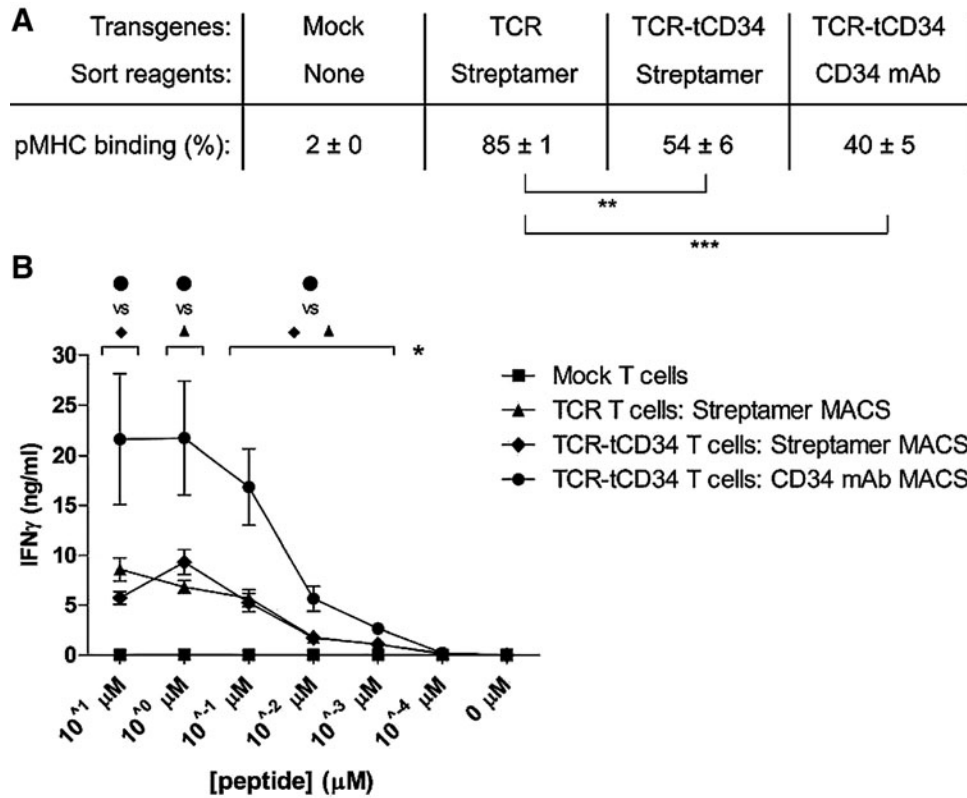
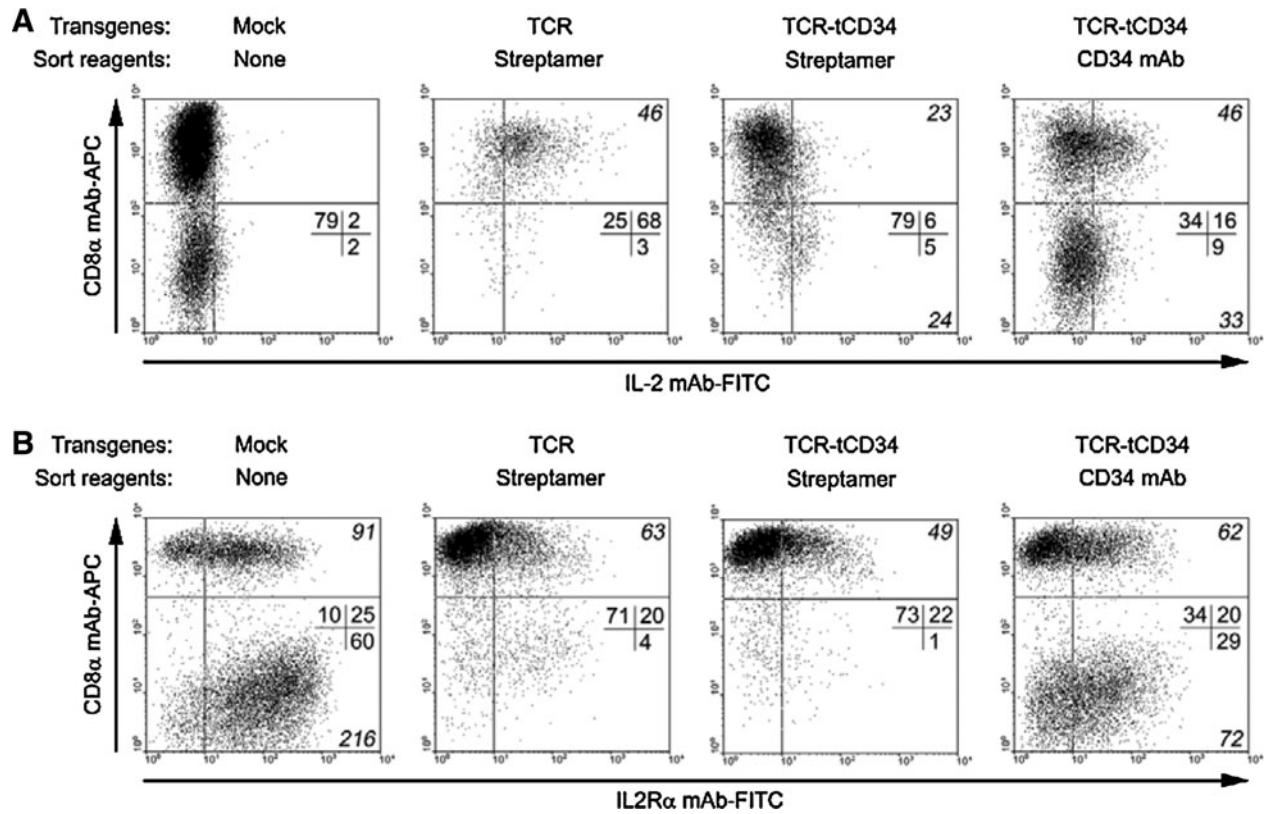


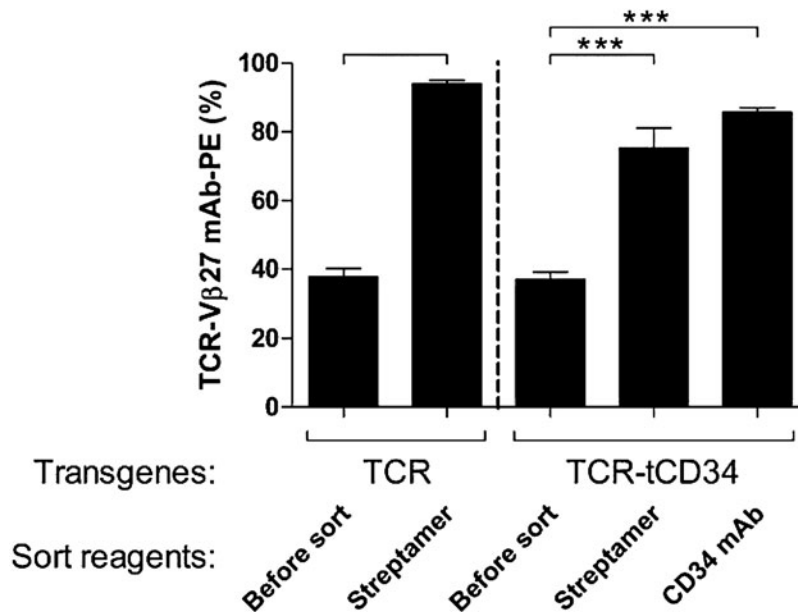
Supplementary Data



SUPPLEMENTARY FIG. S1. CD34 mAb-sorted T cells demonstrated enhanced antigen-specific IFN- γ production. Primary human T cells were transduced and MACSorted as described in the legend to Fig. 4. **(A)** T cells were analyzed for pMHC binding 3–6 weeks after MACS by flow cytometry (using gp100/A2 tetramer). Numbers represent the mean percentage \pm SEM of four repeat experiments with T cells from two healthy donors. **(B)** Sorted T cells from **(A)** were analyzed for IFN- γ production after antigen-specific stimulation. T cells were stimulated overnight with BLM cells loaded with titrated amounts of gp100 peptide and supernatants were tested for IFN- γ by ELISA. Graphs represent mean IFN- γ values (ng/ml) \pm SEM of two independent measurements from two healthy donors. IFN- γ data were corrected for differences in pMHC binding, with pMHC binding of CD34 mAb-sorted T cells [as in **(A)**] set to 100%. EC₅₀ values of Streptamer- and CD34 mAb-sorted T cells (0.05 and 0.03 μ M peptide, respectively) were calculated with MasterPlex ReaderFit software (MiraiBio, South San Francisco, CA). Statistically significant differences were calculated with a Student *t* test: ***p* < 0.005; ****p* < 0.0005.



SUPPLEMENTARY FIG. S2. CD34 mAb-sorted CD4⁺ T cells express IL-2 and IL-2R α . Primary human T cells were transduced and MACSorted as described in the legend to Fig. 4. T cells were stained with APC-conjugated anti-CD8 α mAb in combination with one of the following antibodies: **(A)** FITC-conjugated anti-IL-2 mAb ($n=2$); or **(B)** FITC-conjugated anti-CD25 mAb ($n=10$). In the case of IL-2 stainings, T cells were stimulated for 6 hr with BLM (gp100⁻/A2⁺) cells loaded with 10 μ M gp100 peptide, permeabilized, and stained with anti-IL-2 mAb, as described in more detail in Materials and Methods. Stainings were analyzed by flow cytometry and figures show representative dot plots, indicating percent (all quadrants) and MFI of stained T cells (upper and lower right quadrants when % ≥ 5 , in italic).



SUPPLEMENTARY FIG. S3. MACSort with Streptamer or CD34 mAb yields T cell populations with significantly enhanced percentages of TCR β expression. Primary human T cells were transduced and MACSorted as described in the legend to Fig. 4. T cells were stained with PE-conjugated anti-TCR-V β 27 mAb and analyzed by flow cytometry. Columns and error bars represent the mean percentage and SEM of two to four repeat experiments with T cells from two healthy donors. Statistically significant differences were calculated by Student t test: *** $p < 0.0005$.