



Figure S1:TCR-Immunoscope analysis: T-cell lines were expanded following the procedures as described in this protocol. On day 10 of culture, cells were positively selected using Melan-A₂₆₋₃₅A27L-MHC-Pentamer-APC followed by incubation with anti-APC-beads as described in Methods. The positive and the negative fraction were isolated. Purity was >95% Multimer⁺ and <5% Multimer⁺ cells respectively. TCR-Immunoscope using 24 different BV-family specific primers was performed on both fractions: Multimer⁺: blue, Multimer⁻: red. Results from two different donors are shown. The number of detectable bands in each BV gene family is depicted. A similar repertoire was observed when analyzing non-purified Melan-A-specific T-cell-lines on day 10 of culture (not shown).

Supplementary Figure 2



Figure S2: Assessment of crossreactivity of Melan-A₂₆₋₃₅A27L-specific T-cells against the HM1.24₂₂₋₃₀ epitope. T-cells were stimulated with Melan-A₂₆₋₃₅A27L and expanded as described. On day 11 T-cells were restimulated using autologous monocytes, which had been cultured overnight with GM-CSF/IL-4 containing medium as described in methods. Monocytes were pulsed with the respective peptides as indicated for each plot.

Supplementary Figure 3



Figure S3: Phenotype of 10 day cultured MELOE-1-specific T-cells. Cells were gated according to live scatter plots, and then gated for CD8⁺ MELOE-1 MHC-multimer⁺ cells. Plots depicting the phenotype show CD8⁺ MELOE-1-MHC-multimer⁺ T-cells.



Figure S4: Interferon- γ responses after stimulation with peptide-pulsed autologous monocytes to different cancer-associated antigens after one and two rounds of stimulation. Data illustrate data from Table I. As negative control T-cells were stimulated with unpulsed monocytes.