Supplementary Figures and Materials and Methods

Bobisse et al., Figure S1

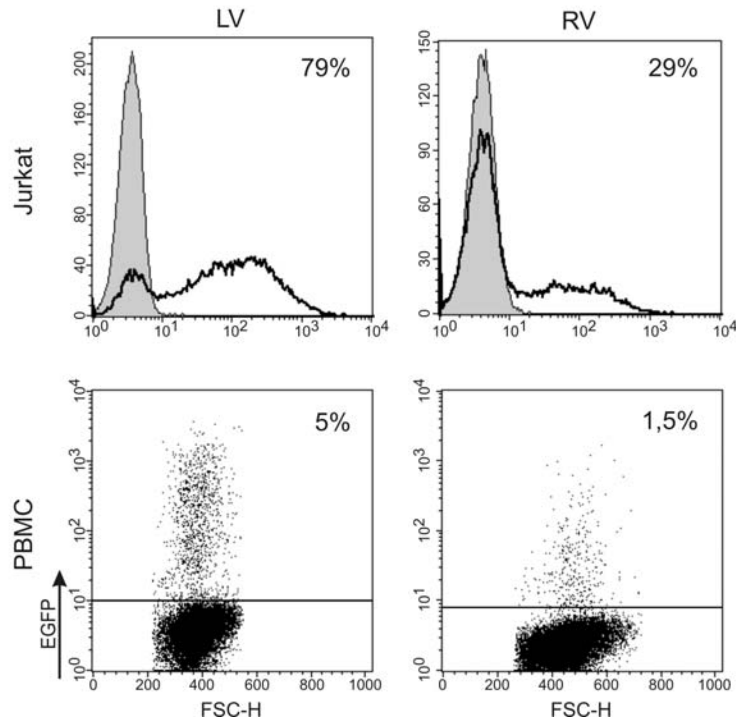

Figure S1. Gene transfer performances of EGFP-coding LV or RV vectors. Jurkat cells were transduced with 1 mL of non-concentrated supernatant containing LV or RV vectors harbouring EGFP (upper panels; dark lines, EGFP samples; shaded curves, untransduced samples); PHA-activated PBMC were infected once with either virus supernatant at a MOI of 2 (lower panels). For RV, PBMC transduction was conducted by spinoculation on Retronectin-coated plates. EGFP expression was measured 72 hours after transduction by cytofluorimetric analysis.

Bobisse et al., Figure S2

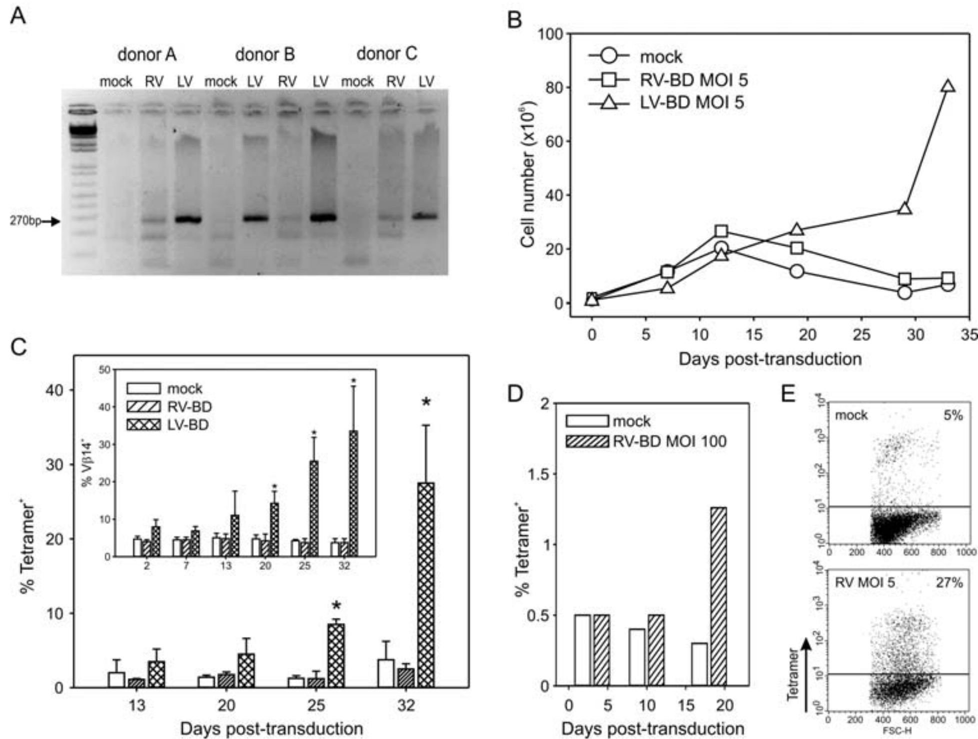

Figure S2. Comparative analysis of TCR expression induced in PBMC by LV and RV. *A,* Activated PBMC from 3 donors were transduced with either LV or RV carrying the identical BD cassette (MOI of 5). PCR was performed on genomic DNA isolated from antigen-expanded cultures 15 days after transduction. PCR primers were specific for the CDR3 hypervariable region of the cloned TCR-α2.2 chain. A non-transduced, antigen-expanded culture (mock), is reported as control. *B*, Growth kinetics of representative T cell cultures generated by transduction with LV-BD, RV-BD (MOI of 5) or left untransduced. *C,* Transduced cells described in *A* were stimulated weekly with antigen-loaded T2 cells and periodically analysed for transgenic TCR expression by staining with the Melan-A tetramer and anti-Vβ14 mAb (inset panel). Plotted results are mean ± SD of three donors. * refers to statistical significant differences ($P < 0.05$) between LV- and RV-transduced cells. *D,* Transgenic TCR expression in RV-BD-transduced PBMC infected at a MOI of 100. *E,* Anti-Melan-A TCR expression in long-term stimulated cultures generated by infection at a MOI of 5. The reported analysis refers to day 60.

Bobisse et al., Figure S3

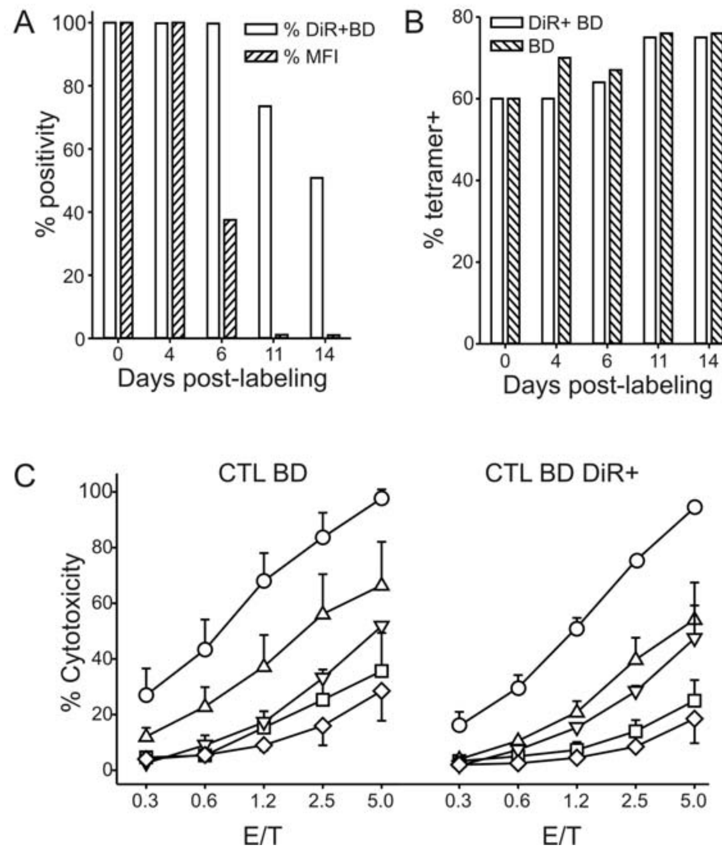

Figure S3. T cell labelling for optical imaging *A,* Transduced T lymphocytes were labelled with DiR, restimulated weekly and analysed over a period of 14 days. Histograms show the percentage of DiR^+ cells and the corresponding MFI at different timepoints. *B,* Expression of anti-Melan-A transgenic TCR on unlabelled or DiR-labelled dividing T lymphocytes. *C,* Cytotoxic activity of control and dye-labelled transduced T cells against Melan-A-pulsed T2 cells (circles), T2 cells (squares), Mel3/3 (triangles), A375 (diamonds) and SK-23 MEL (inverted triangles) melanoma cell lines.

Supplementary Materials and Methods

Plasmids. The LESN vector was a derivative of the LXSN RV containing the EGFP gene (1). A LESN fragment included between restriction sites ApaI and XbaI in the 3'LTR was deleted and replaced by standard cloning procedures with a 2.6 kbp SalI/SalI insert from the BD I-TCR construct. The resulting vector (RV-BD) is a SIN RV carrying the complete BD cassette in the 5'α-PGKminCMV-β 3' orientation substituting the EGFP and neomycin resistance genes.

Viral vector production. Monocistronic and bidirectional RV were used to produce vescicular stomatitis virus (VSV)-pseudotyped stocks of viral particles. Retroviral particles were generated by transient co-transfection of 293gp packaging cells stably expressing MLV gag and pol proteins, with 15 μg of transfer vector (LESN or RV-BD) and 5 μg of hCMV-G plasmid (2). 293gp adherent cells were grown to about 50% of confluency and transfected by calcium phosphate precipitation. Twelve-14 hours after RV transfection, the medium was replaced with 6 ml fresh complete medium. Viral supernatant was collected 30 hours later, spun to remove all producer cells, filtered though 0.45 μm pore size filters and stored at -80°C or directly used for transduction experiments. For LV and RV comparative experiments, virus stocks were produced in parallel. Where indicated, virus-containing supernatants were concentrated by ultracentrifugation at 104,000 x g for 2 hours at 4 °C. pCMV-EGFP and LESN vector titres were determined by limiting dilution on 293T cells in 6-well plates. Briefly, serial dilutions of virus supernatant were added to wells the day after cell seeding. Three days later, the percentage of EGFP-transduced cells was determined by flow cytometry, and transducing units (TU) per ml were calculated. Titres of 2-5x10^6/ml for LV and 2-4x10^5/ml for RV were routinely obtained.

RV transduction of PBMC. PHA pre-activated PBMC were transduced once with RV by spinoculation in retronectin-coated (RetroNectin™, Takara) 24-well tissue culture plates. Briefly, 1 ml of viral supernatant containing a known amount of vector particles was added to PBMC

($1x10^6$/well) and spun for 1 hour at 1200 x g, then incubated at 37°C overnight in the presence of IL-2 at 100 U/ml and protamine sulphate (8 µg/ml). Three days after transduction, PBMC were labelled for cytofluorimetric analysis or immediately stimulated with antigen-loaded T2 cells, as described for LV-transduced PBMC.

PCR. Proviral DNA integration levels were qualitatively analysed by PCR amplification of genomic DNA from transduced T cells. Genomic DNA was purified from $2x10^6$ cells using the Gentra Puregene Cell Kit (Qiagen), according to the manufacture's instructions. A 270-bp TRAV12-2/J35/C integrated fragment was amplified with the forward primer 5'-GTTGCTCCAGGCCACAGCACTGTT-3' and the reverse primer 5'-TGTGCCGTTTCGATA GGCTTTGGGA-3', complementary to the CDR3 region of the transgenic α-chain, under the following conditions: 94°C for 5 min, followed by 35 cycles of amplification (94°C for 1 min, 69°C for 1 min, 72°C for 2 min) and an extension phase of 15 min at 72°C.

Supplementary References

1. Indraccolo S, Habeler W, Tisato V, *et al.* Gene transfer in ovarian cancer cells: a comparison between retroviral and lentiviral vectors. Cancer Res 2002;62:6099-107.

2. Yee JK, Friedmann T, Burns JC. Generation of high-titer pseudotyped retroviral vectors with very broad host range. Methods Cell Biol 1994;43 Pt A:99-112.