or positive control EBV peptide at 640 molar excess in PBS pH 7.2 overnight. HIV-1B specific $CD8^+$ cells were stained with peptide loaded A2:Ig fusion protein DimerX reagent and detected by PE-conjugated A85-1 mAb (anti-mouse IgG1, BD biosciences). DimerX staining was determined by flow cytometry (FACSAria, BD Biosciences). The negative control straining was obtained using the HIV-1 specific T cell line and a non-relevant HIV-1 peptide loaded DimerX. The positive control was obtained using donor PBMC's stimulated with 1uM BMLF-1 EBV peptide for one week and then stained with EBV peptide loaded Dimer X.

Generation of HLA allele specific transfectants for peptide binding and restriction analysis

Cell lines and RNA isolation

HLA-homozygous human B-lymphoblastoid cell lines (B-LCL) from the International Histocompatibility Working Group (IHWG) were thawed and grown in RPMI complete medium (RPMI supplemented with 10% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1% pen-strep). The HLA class I deficient 721.221 LCL line and the Amphopack 293, retroviral producer line, were maintained in DMEM complete medium (DMEM supplemented with 10% FCS, 2 mM L-glutamine, 1% pen-strep, 1 mM sodium pyruvate). Total RNA was isolated from 5×10^6 B-LCL using the RNeasy kit (Qiagen) according to the manufacturer's instructions. The purified RNA was eluted in 50 ul RNase-free water and 10 μl of this was used to synthesize cDNA using an oligo dT primer, dNTP mix, DTT, and M-MLV reverse transcriptase (200 u) (Gibco BRL).

Primer, PCR amplification and cloning of PCR products

HLA locus-specific primers were synthesized according to the information from [22]. Sense and antisense primers were made for each of HLA-A, HLA-B, and HLA-C with a HindIII site at the 5' end of sense and a NotI site at the 5' end of the antisense. PCR amplification was done using 2 μl of cDNA, 1× PCR buffer with Mg, 200 uM each dNTP, 0.3 uM of each primer and 0.5 μl Taq polymerase. The conditions for PCR were one cycle of 95°C for 5 min prior to addition of polymerase, followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, 68°C for 1.5 min and finally one cycle of 68°C for 4 min. PCR products were analyzed on a 1.2 % agarose gel and gel purified using a gel extraction kit (Qiagen # 28704). Subsequently, 6 μl of purified PCR product was ligated into the pCR2.1 vector using the TA cloning kit (Invitogen, K2000-01) following the manufacturer's directions. Transformation into INVaF' competent cells was carried out and plated onto LB/ampicillin (100 μg/ml) plates. After incubation at 37°C overnight, colonies were selected, grown and miniprep DNA prepared (QIAprep Spin Miniprep Kit (Qiagen 27104). Clones were checked for inserts by restriction digestion with EcoR1. Several clones for each were sent for DNA sequencing using the vector primers M13 Reverse and T7 promoter. Two additional primers designed to anneal in the middle of all HLA class I genes in the sense and anitsense directions were also used [22]. All sequences were verified by DNA sequencing.

Subcloning of HLA cDNAs into retroviral vector pLNCX2 and generation of viral supernatants

Sequence-verified HLA cDNA clones were digested with HindIII and Not I and the insert was gel purified and subcloned into the retroviral vector, pLNCX2 (BD Biosciences, cat# 6102-1) which had been linearized with HindIII and NotI. Plasmid DNA preps were done and sequence was confirmed once again using the forward and reverse sequencing primers (BD Bioscience #K1060-F) for the vector, pLNCX2. Retroviral producer cell lines were established by transfection of the Amphopack 293 cell line using the HLA/pLNCX2 DNA. Amphopack 293 cells (4×10^6) were seeded onto a 100 mm tissue culture dish in 15 ml of DMEM complete medium and incubated overnight at 37°C. Five minutes prior to transfection, the medium was replaced with complete medium containing chloroquine (25 μM). The semi-confluent cells were transfected using 20 μg of plasmid DNA in combination with 61 μ l2 M $CaCl_2$ (0.5 ml total volume) and 0.5 ml 2 × HBS, bubbled in. The solution was added dropwise to the cells and gently swirled. Plates were incubated at 37°C for 6–8 h, at which time the medium was removed and replaced with fresh DMEM complete medium. Plates were incubated further at 37°C for 48 h. At 48 h post-transfection the viral supernatant was removed from the plates and spun at 2000 rpm for 10 min, then filtered through a 0.45 um filter. The supernatant was stored at 4°C for immediate infection or frozen at -80°C for later infection.

Retroviral infection of 721.221 cells, flow cytometric analysis and cell sorting

721.221 cells (EBV transfected B cell line which is MHC class 1 A, B, C negative) were infected with the retroviral supernatant using Lipofectamine reagent (Invitrogen #18324-111). For each infection, $1–3 \times 10^6$ cells in log phase were pelleted, resupended in 100 μl of DMEM complete medium, and transferred to one well in a 24 well plate. 4 μl of lipofectamine reagent, 2 ml of viral stock, and 0.2 ml of l M Hepes were added. The plate was spun for 1–2 h at 2000 rpm and subsequently the medium was discarded, the cells were washed one time with fresh medium, and finally were resuspended in 2 ml of fresh medium. The following day, infected cells were put into neomycin (G418) selection at 800 μg/ml. Selection was continued for 5–7 days at which point the concentration was dropped down to 400 μg/ml. Approximately one week post-infection, the cells were analyzed for cell-surface expression of HLA. An aliquot of cells was stained using the monoclonal antibody W6/32 at 1:500, incu-