Supplementary material

Flow cytometry and peptide-MHC multimer staining

Dextramers specific for EBV HLA-A*0201/GLCTLVAML, EBV HLA-B*07:02/RPQGGSRPEFVK or an irrelevant peptide HLA-A*0201/ SLYNTVATL (NEG), APC-labeled, (all from Immudex, Copenhagen, Denmark) were used for the detection of EBV-specific CD8+ T cells. PBMCs were stained according to the manufacturer's recommendation. The following antibodies were added for appropriate gating of the cells; anti-CD3-FITC (eBioscience, Thermo Fisher Scientific, Waltham, MA, USA), anti-CD4-PE-Cy7 (eBioscience), anti-CD8-BV605 (BioLegend, San Diego, CA, USA. Cells were acquired on a BD FACSCanto flow cytometer (BD Biosciences, San Jose, CA 95131, USA) and the data analyzed using FlowJo software (Treestar Inc., Ashland, OR, USA).

Gating strategy

Cells were gated on live cells based on forward scatter (FSC) and side scatter (SSC) (gate A). CD3+ T cells (gate B) were selected and analyzed for the expression of CD4 and CD8 (gate C). CD8+ T cells were further selected and the percentage of identified multimer$^+$ CD8$^+$ T cells reported of the total number of CD8+ T cells (gate D) (see Supplementary figure 1).

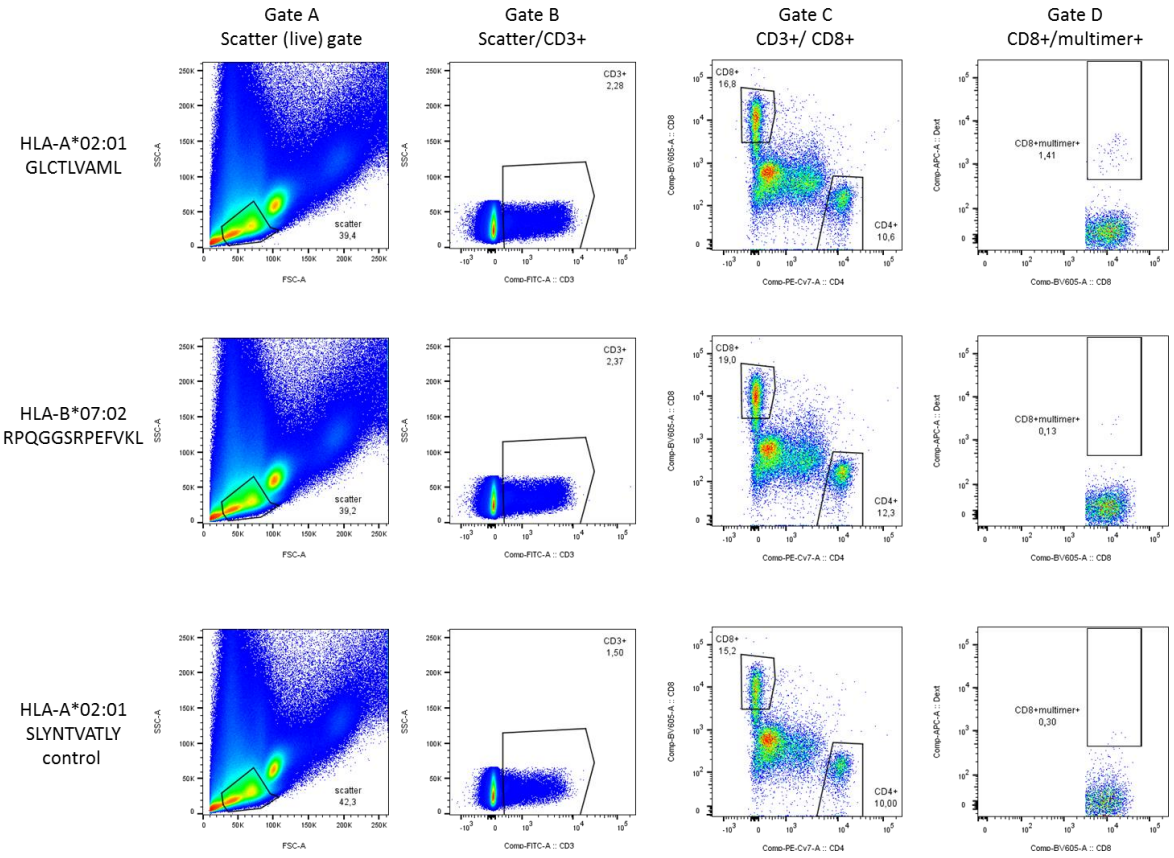
IFN-γ ELISPOT assay

IFN-γ ELISPOT assays were performed on PBMCs *ex vivo*. Anti-IFN-γ coating antibody was purchased from Mabtech (Nacka Strand, Sweden) and used to coat MultiScreenHTS HA filter plates (Merck Millipore Burlington, MA, USA). PBMCs were tested against overlapping 15-mer peptide library PepMix™ Collection EBV (JPT Peptide Technologies, Berlin, Germany) with 14 peptide pools spanning the corresponding antigenic epitopes. Negative controls with 1% DMSO and positive controls with SEC-3 superantigen (0.1µg/ml) (Toxin Technology Inc, USA) and CEF I PepMix™ Peptide Pool (JPT

Peptide Technologies) were included. PBMCs were seeded at $2x10^5$ cells per well and all conditions were tested in triplicates. Plates were incubated for 24 hours prior to the addition of detection reagents anti-IFN-γ-biotin and streptavidin-alkaline phosphatase enzyme conjugate (both from Mabtech) and BCIP/NBT substrate (Sigma-Aldrich, Saint-Louis, Missouri, USA). Spots were enumerated using an automated analyzer, CTL IMMUNOSPOT S5 VERSA-02-9030 (Cellular Technology Ltd, Shaker Heights, USA). Specific spots were calculated by subtracting the mean number of spots + (2×SD) of the medium-only control from the mean number of spots of experimental wells.

Statistical analysis

Statistics were made with 1-way ANOVA (multiple comparisons) with Dunnett correction using GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA). * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.



Supplementary figure 1. Gating strategy for detection of EBV-specific CD8+ T cells.

Peripheral blood mononuclear cells were gated on live cells based on forward scatter (FSC) and side scatter (SSC) (gate A). CD3+ T cells (gate B) were selected and analyzed for the expression of CD4 and CD8 (gate C). CD8+ T cells were further selected and the percentage of identified multimer^{+} CD8^{+} T cells reported of the total number of CD8+ T cells (gate D).