Supplementary Material

A simple and rapid method for quality control of major histocompatibility complex-peptide monomers by flow cytometry

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1 Supplementary Figures

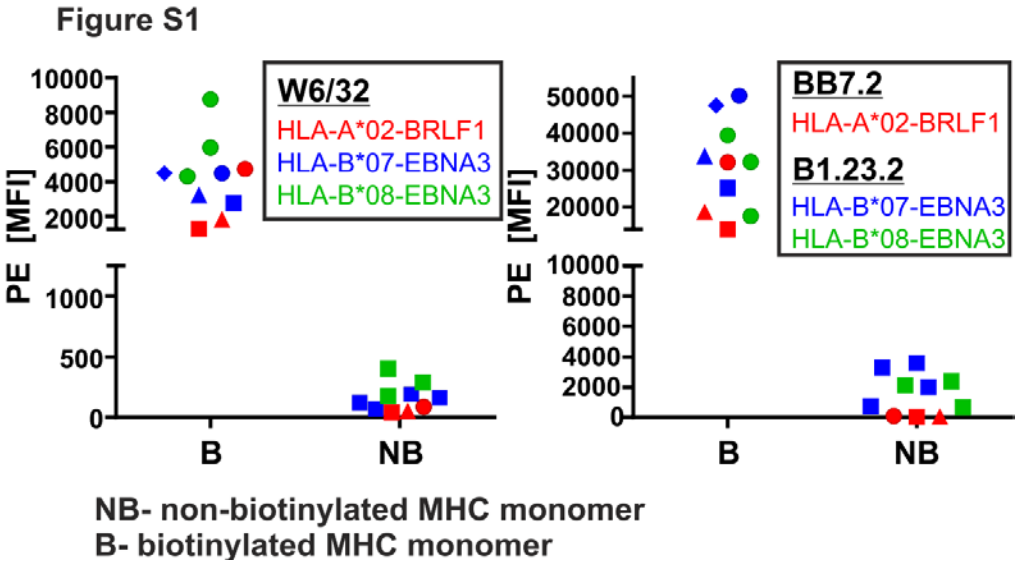

Figure S1. Detection of biotinylated MHC monomers. Recognition of the biotinylated/non-biotinylated monomers at a concentration of 0.05µg/ml, by the indicated anti-MHC antibodies is shown. Each dot represents one repeat of the assay (color-coded for the MHC allelic product).

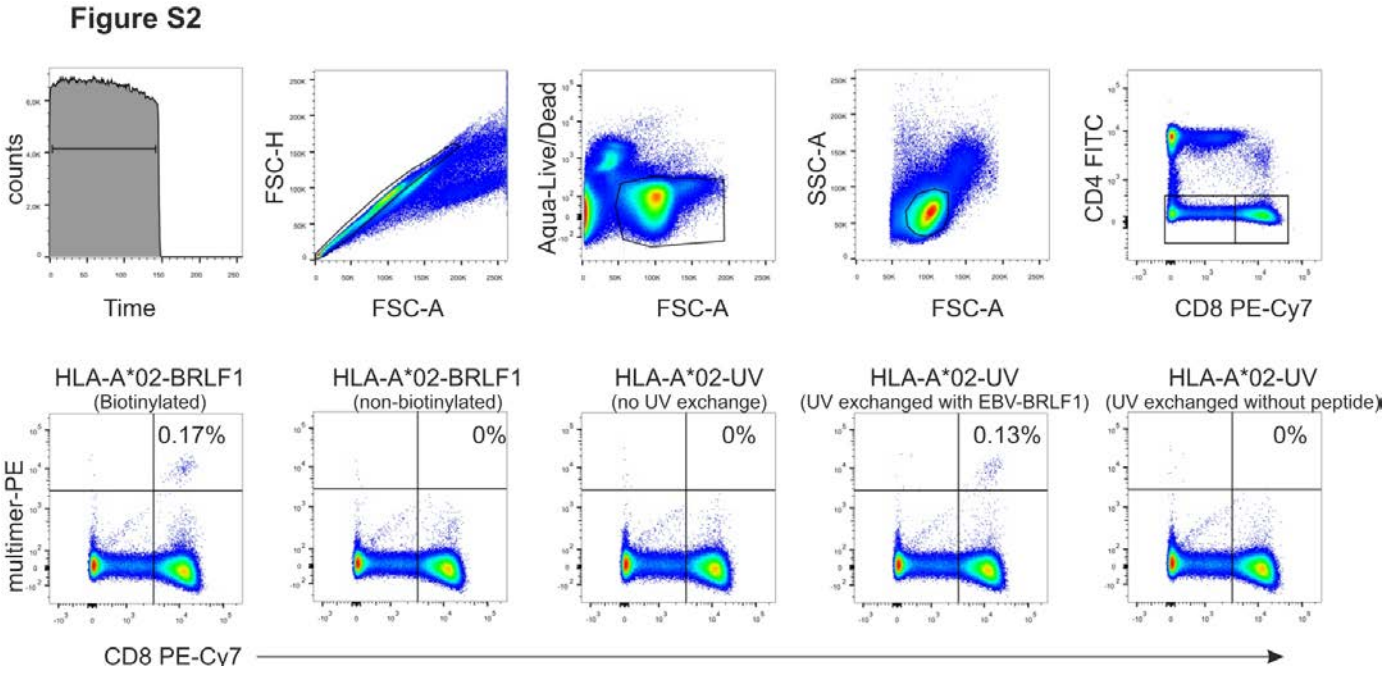

Figure S2. Multimer stainings. The top panel indicates the gating strategy as mentioned in the Section "Materials and Methods". Here, 1 x 10^6 PBMCs were analysed (donor 2). The bottom panel shows exemplary stainings performed on PBMCs from donor 3 (one of two donors tested) with the biotinylated/non biotinylated MHC multimers.

Figure S3

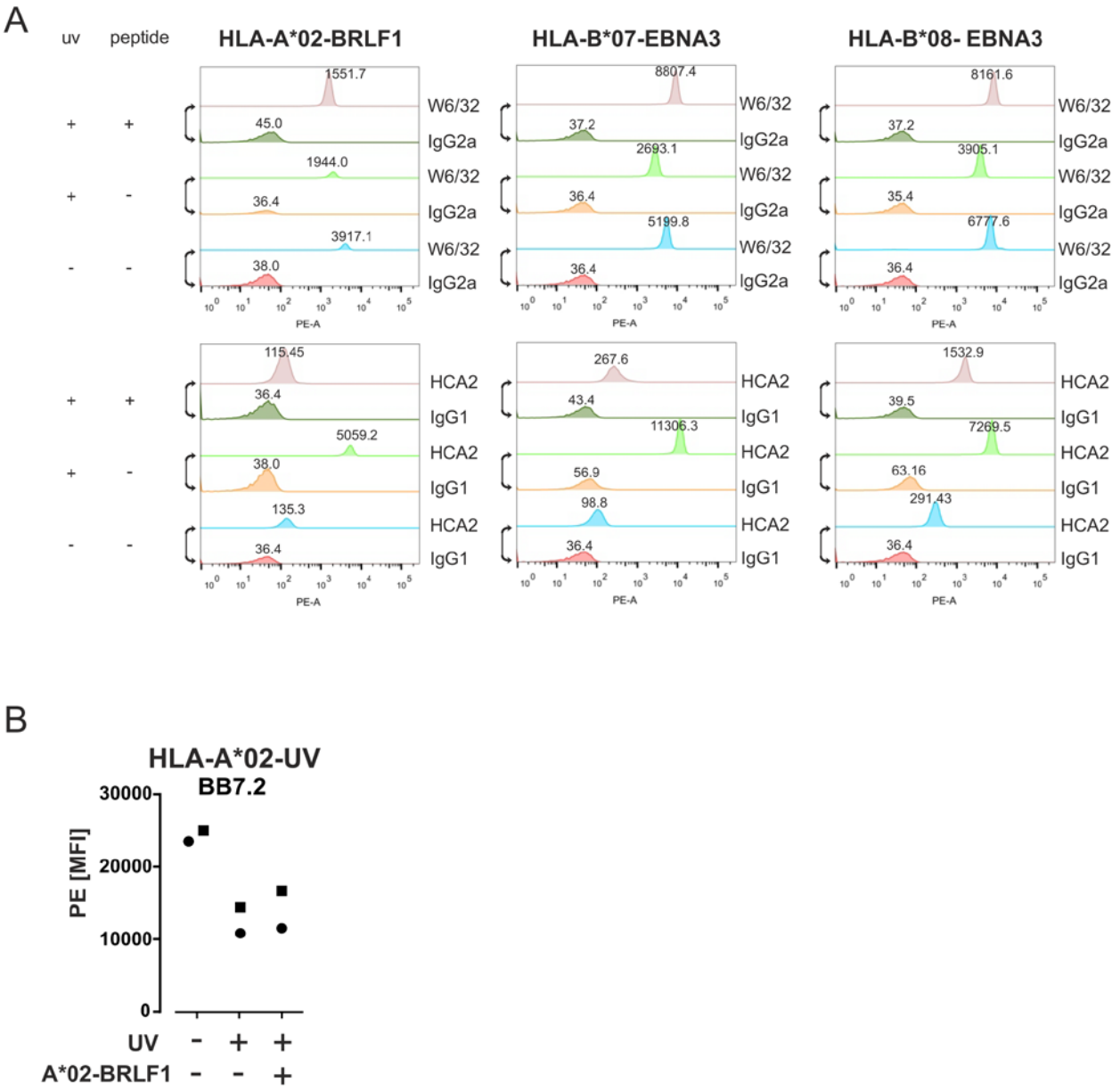

Figure S3: Quality control of UV peptide-exchanged monomers. A. The figure shows the fluorescence histograms of one of the two experiments summarized in **Figure 4**. UV-exchange was performed with the indicated peptides and the monomers were subsequently analysed using the beads. The PE MFI values are indicated above the respective peaks. **B**. Binding of the BB7.2 mAb to HLA-A*02 monomer was detected using a PE-labeled secondary antibody. Median fluorescence of the PE signal from two representative experiments out of three is plotted.

Figure S4

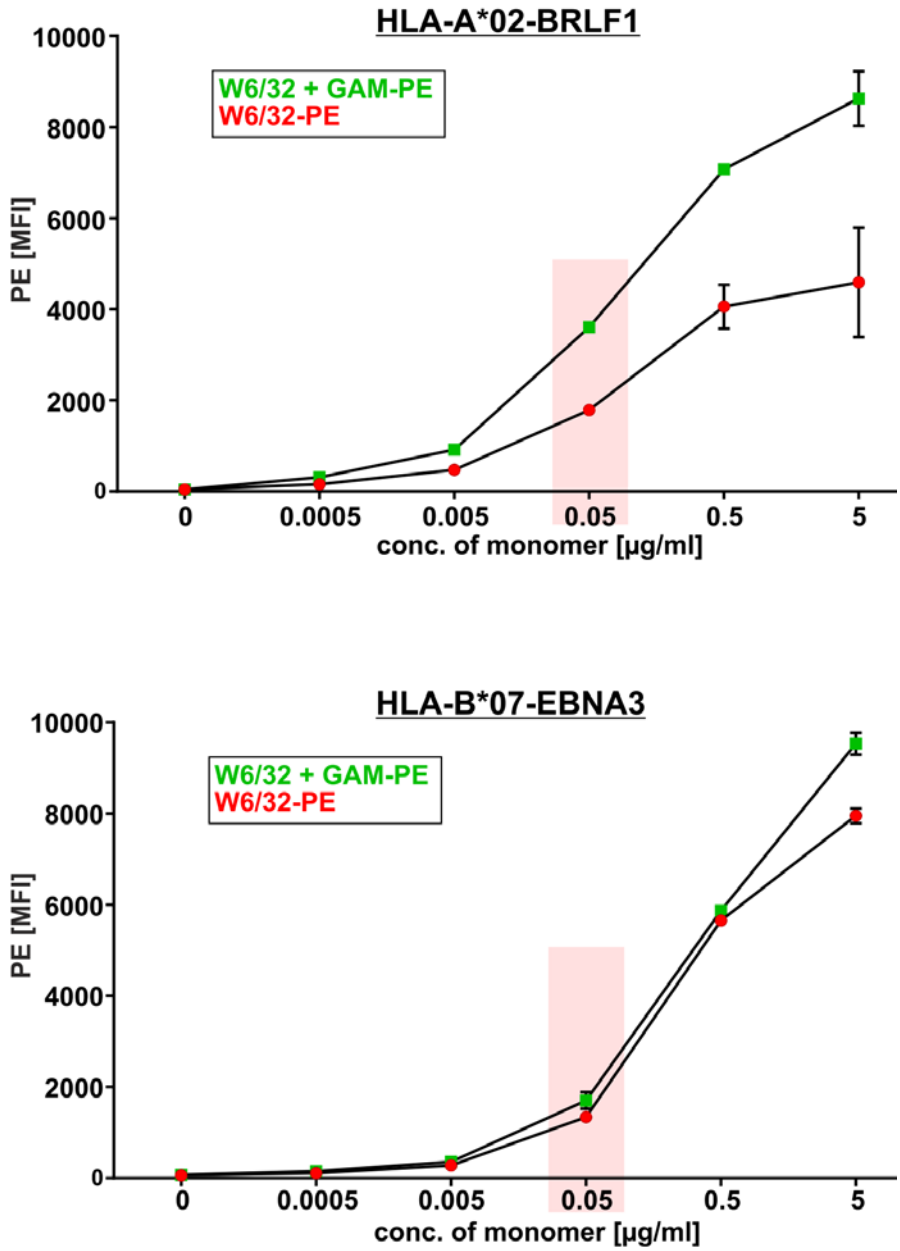

Figure S4: Comparison of fluorescence-coupled antibody to purified mAb in combination with a secondary Ab. Monomers were coupled to beads at the indicated concentrations and stained in duplicates either using a W6/32-PE antibody (Cat. #311406, Biolegend) or a combination of purified W6/32 antibody and a secondary antibody (GAM-PE) as described in the manuscript. The resulting PE fluorescence (MFI) is plotted. The concentration of monomer used in the study is indicated within the red box.