determined by subtle immunodominance patterns influenced by many factors including other HLA molecules and previous pathogenic encounters [34]. To address these possibilities, we analyzed responses in HIV-1 naive individuals by priming PBMC of ten donors at high cell density with HIV-1 peptide-pool pulsed autologous Langerhans-like cells followed by restimulation with peptide pulsed fresh PBMCs. We tested the resulting HIV-1 peptide pool specific T cell lines by ELISPOT analysis. (Figure 1B) All donors generated IFNγ with a magnitude significantly greater ($p < 0.0001$) than ELISPOT responses generated by HIV-1-infected subjects.

Characterization of A0201 restricted responses in HIV-1 naive individuals

To further evaluate specific T cell responses, we selected the most common HLA subtype, A0201 for detailed analysis. Eight HIV-1 peptides with A0201 restriction (Table 2) were pulsed individually onto the TAP deficient A0201+ T2 hybridoma cell line to analyze functional peptide binding. A positive control peptide derived from the HTLV-TAX protein [(amino acids 11–19. LLFGYPVYV)] was used to normalize positive peptide binding (value = 1). A predicted A0301 restricted HIV-1 peptide was used as a negative control (value = 0). Each HIV-1 peptide was found to bind A0201 as shown by increased A0201 surface expression on peptide-pulsed T2 cells (Figure 2A). Subsequently, A0201 restricted HIV-1 peptides were combined in a pool and were used to prime an A0201 donor, resulting in selective proliferation to the HIV-1 peptide pool compared to control (Figure 2B). These cells produced IFNγ upon stimulation with HIV-1 peptide-pulsed antigen-presenting cells (PBMCs) in ELISPOT assays (Figure 2C). After three rounds of stimulation, 25% of the cells in culture were $CD8^+$ and 62% were $CD4^+$ (Figure 2D). Given that ~20–30% of MHCI binding peptides are predicted to bind to MHCII as well, this result is not unexpected (data not shown). We also generated two control cell lines: the first, a tetanus toxoid specific T cell line with 78% $CD4^+$ and no $CD8^+$ T cells elicited by whole protein stimulation; and the second, an EBV specific T cell line with 62% $CD8^+$ and 29% $CD4^+$ cells elicited by stimulation with an immunodominant EBV epitope BMLF1 (GLCTLVAML). $CD8^+$ T cells were sorted from the HIV-1

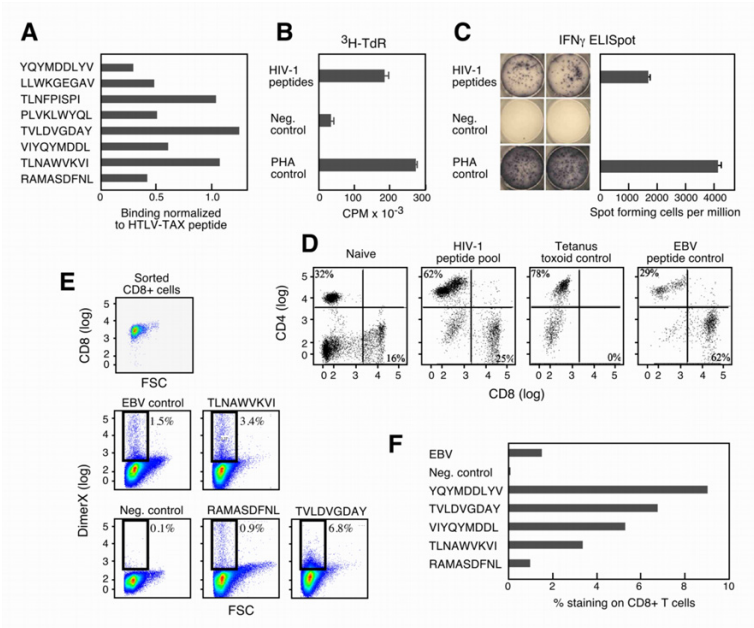

Figure 2

Generation and characterization of HLA-A0201 restricted HIV-1 peptide-specific T cell lines from uninfected individuals. Binding of HIV-1 peptides to A0201 was tested using the T2 cell line (panel A). These HIV-1 peptide-stimulated T cells generated antigen-specific proliferative responses (panel B). ELISPOT responses to HIV-1 peptide-loaded donor PBMCs or PHA stimulation (positive control) as well as to non-peptide loaded donor PBMCs (negative control) (Panel C). Flow cytometric analysis of CD4 and CD8 T cells in HIV-1 peptide-specific T cell lines and indicated control T cell lines (Panel D). $CD8^+$ T cells were sorted from the HIV-1 specific T cell line after multiple stimulations *in vitro* and their peptide specificities determined by individual peptide-loaded DimerX staining (Panel E). In Panel E, the negative control straining was obtained using the HIV-1 specific T cell line and a non-relevant peptide loaded DimerX. The EBV 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. One to nine percent of CD8 T cells were specific for each one of the selected HIV-1B peptides (Panel F).