**Supplementary Figure Legends**

**Supplemental Figure 1.** BTLA expression on NY-ESO-1-specific, MART-1-specific and virus-specific CD8+ T cells. Representative dot plots from melanoma patients (MP) showing *ex vivo* BTLA expression on NY-ESO-1-specific (A), virus-specific and MART-1-specific (B) CD8+ T cells. Values indicate the percentage of CD8+ T cells that express BTLA among tet+ CD8+ T cells. Data shown are representative of two independent experiments. (C) As shown for one melanoma patient (MP1), CD8+ T cells stained with A2/HIVpol 476-484 tetramers or ECD-labeled IgG control Abs were used to establish the threshold for identifying tet+ cells and BTLA+ cells, respectively.

**Supplemental Figure 2.** BTLA expression on NY-ESO-1-specific CD8+ T cells is upregulated as compared to naïve and memory CD8+ T cell subsets in melanoma patients. Pooled data showing the percentage (%) (A) and MFI (B) of BTLA expression on NY-ESO-1-specific CD8+ T cells, naïve (CCR7+CD45RA+), central memory (CCR7+CD45RA−), effector memory (CCR7−CD45RA−) and effector (CCR7−CD45RA+) CD8+ T cells detected *ex vivo* in PBMCs from eleven melanoma patients. The P values were calculated using the Wilcoxon signed rank test. Data shown are representative of two independent experiments performed in duplicate. (C) Correlation between the expression of BTLA and CCR7, CD45RA, CD27 by total CD8+ T cells assessed *ex vivo* in PBMCs from eight melanoma patients. For statistical analysis, a Pearson correlation test was performed. *, p < 0.05 was considered significant.
Supplemental Figure 3. BTLA, PD-1 and Tim-3 expression on virus-specific and total CD8+ T cells in healthy donors. (A) Pooled data showing the percentage and MFI of BTLA expression on Flu-, CMV-, EBV-specific and total CD8+ T cells from ten healthy donors. (B) Pooled data showing the distribution of Flu-, CMV-, EBV-specific and total CD8+ T cells from ten healthy donors according to BTLA, PD-1 and Tim-3 expression. Horizontal bars depict the mean percentage or MFI of BTLA and/or PD-1 and/or Tim-3 expression on tet+ CD8+ T cells. The P values were calculated using the Wilcoxon signed rank test. Data shown are representative of two independent experiments.

Supplemental Figure 4. BTLA+PD-1+Tim-3− and BTLA+PD-1+Tim-3+ NY-ESO-1-specific CD8+ T cells represent two dysfunctional T cell populations. Additional data from 8 melanoma patients showing the percentages of cytokine-producing A2/NY-ESO-1 157-165 tet+ CD8+ T cells according to BTLA, PD-1 and Tim-3 expression. The P values were calculated using the Wilcoxon signed rank test. These data correspond to the second set of independent experiments presented in Figure 2B. Experiments were performed in duplicates.

Supplemental Figure 5. HVEM expression and distribution on cell subsets isolated from PBMCs of melanoma patients. Histograms showing ex vivo HVEM expression on monocytes (CD14+), mDCs (CD11c+), B cells (CD19+), CD4+ T cells and CD8+ T cells isolated from PBMCs of two representative melanoma patients. Cells were surface stained with the following mAbs before flow cytometry analysis: CD19-FITC, CD14-ECD, CD8-PE-Cy7 (Beckman Coulter), CD4-PerCP-Cy5.5 (Biolegend), CD11c-PE
(eBioscience) and an HVEM-APC mAb (grey) or an APC-labeled isotype control antibody (black line) (eBioscience). A violet amine reactive dye was used to assess the viability of the cells.

**Supplemental Figure 6.** BTLA blockade increases the frequencies of cytokine-producing NY-ESO-1-specific CD8+ T cells and adds to PD-1 and Tim-3 blockades. Pooled data from melanoma patients ($n = 10$) showing the variation in the frequencies of IFN-$\gamma$-producing (A), TNF-producing (B) and IL-2-producing (C) NY-ESO-1 tet$^+$ cells for $10^6$ CD8+ T cells. PBMCs were incubated for 6 days with NY-ESO-1 157-165 peptide or with HIVpol 476-484 peptide and blocking mAbs against BTLA ($aBTLA$) and/or PD-1 ($aPD-1$) and/or Tim-3 ($aTim-3$) or an isotype control antibody ($IgG$), prior to evaluating intracellular cytokine production of A2/NY-ESO-1 157-165 tet$^+$ CD8+ T cells in response to cognate peptide. The $P$ values were calculated using the Wilcoxon signed rank test. Data shown are representative of two independent experiments performed in duplicate.

**Supplemental Figure 7.** BTLA blockade in combination with PD-1 blockade enhances NY-ESO-1-specific CD8+ T cell functionality. Pooled data from melanoma patients ($n = 10$) showing the variation in the percentages of cells that produce IFN-$\gamma$ (A), TNF (B) and IL-2 (C) among total A2/NY-ESO-1 157-165 tet$^+$ CD8+ T cells after a 6-day incubation with cognate peptide in the presence of blocking anti-BTLA ($aBTLA$) and/or anti-Tim-3 ($aTim-3$) and/or anti-PD-1 ($aPD-1$) mAbs and restimulation at day 6 with peptide, as compared to incubation with IgG control antibody and peptide. (D) Fold
changes in the percentages of cells that produce IFN-γ, TNF and IL-2 among total A2/NY-ESO-1 157-165 tet+ CD8+ T cells after a 6-day incubation with cognate peptide in the presence of blocking anti-BTLA and/or anti-PD1 and/or anti-Tim-3 mAbs and restimulation at day 6 with peptide, as compared to incubation with IgG control antibody and peptide. The P values were calculated using the Wilcoxon signed rank. Data shown are representative of two independent experiments performed in duplicate.

**Supplemental Figure 8.** BTLA blockade increases the frequencies of proliferating and total NY-ESO-1-specific CD8+ T cells and adds to PD-1 and Tim-3 blockades. Pooled data from melanoma patients (n = 10) showing the variation in the frequencies of CFSElo (A) and total (B) A2/NY-ESO-1 157-165 tet+ cells. CFSE-labeled PBMCs were incubated for 6 days with NY-ESO-1 157-165 peptide or HIVpol 476-484 peptide and blocking mAbs against BTLA (aBTLA) and/or PD-1 (aPD-1) and/or Tim-3 (aTim-3) or an isotype control antibody (IgG). The P values were calculated using the Wilcoxon signed rank test. Data shown are representative of two independent experiments performed in duplicate.