

Supplementary Material & Methods:

TNF- α and IL-10 secretion assays

Briefly, PBMCs from CMV and HHV-6 co-infected individuals were stimulated with either CMV or HHV-6 lysates and their respective Mock controls for 45 hours and productions of TNF- α and IL-10 were measured with an ultra-sensitive single molecule enzyme-linked immunoarray (Simoa,TMQuanterix Corporation, Lexington, MA) according to manufacturer's recommendations. This method utilizes both analogue and digital array equipment to detect much smaller amounts of solute compared to traditional ELISA methods. We used the commercially-available TNF- α and IL-10 kits. All samples were run in duplicate. In order to measure concentration of culture supernatant, the samples were diluted 1/30. The lower limit of detection for TNF- α and IL-10 were 0.015 pg/mL and 0.003 pg/mL, respectively. Only intra-assay coefficients of variance (CVs) < 10% were included in the analysis.

Supplementary Table 1

| ID donors | Unstimulated | Mock HHV-6A | Mock HHV-6B | CMV lysate | HHV-6A lysate | HHV-6B lysate | pp65 | IE1 | U54 | U90 |
|-----------|--------------|-------------|-------------|------------|---------------|---------------|-------|-------|-------|-------|
| #1 | 0.006 | 0.018 | 0.009 | 0.143 | 0.019 | 0.010 | 0.124 | 0.007 | 0.007 | 0.007 |
| #2 | 0.001 | 0.021 | 0.010 | 0.041 | 0.024 | 0.014 | 0.169 | 0.199 | 0.040 | 0.039 |
| #3 | 0.001 | 0.031 | 0.040 | 0.283 | 0.032 | 0.041 | 0.724 | 0.528 | 0.007 | 0.002 |
| #4 | 0.004 | 0.018 | 0.013 | 0.468 | 0.019 | 0.014 | 2.393 | 0.853 | 0.005 | 0.005 |
| #5 | 0.001 | 0.010 | 0.049 | 0.147 | 0.011 | 0.050 | 0.293 | 0.059 | 0.319 | 0.002 |
| #6 | 0.005 | 0.036 | 0.009 | 0.086 | 0.037 | 0.010 | 0.056 | 0.037 | 0.017 | 0.006 |
| #7 | 0.001 | 0.013 | 0.021 | 0.181 | 0.038 | 0.020 | 1.055 | 0.161 | 0.011 | 0.126 |
| #8 | 0.006 | 0.030 | 0.040 | 0.094 | 0.031 | 0.041 | 0.270 | 0.073 | 0.007 | 0.102 |
| #9 | 0.002 | 0.020 | 0.021 | 0.111 | 0.057 | 0.022 | 0.942 | 0.129 | 0.021 | 0.015 |
| #10 | 0.004 | 0.051 | 0.067 | 0.068 | 0.052 | 0.068 | 0.144 | 0.005 | 0.005 | 0.005 |
| #11 | 0.002 | 0.005 | 0.008 | 0.004 | 0.007 | 0.009 | 0.031 | 0.003 | 0.003 | 0.003 |

Supplementary Table 1A: Frequency of IFN-g secreting CD8+ T cells obtained for 11 donors post PBMCs stimulation with either Mock for HHV-6A or HHV-6B or in unstimulated conditions. Virus-specific stimulations were performed with lysates (CMV, HHV-6A, HHV-6B) or peptides (pp65, IE1, U54, U90). Data are expressed in percentage.

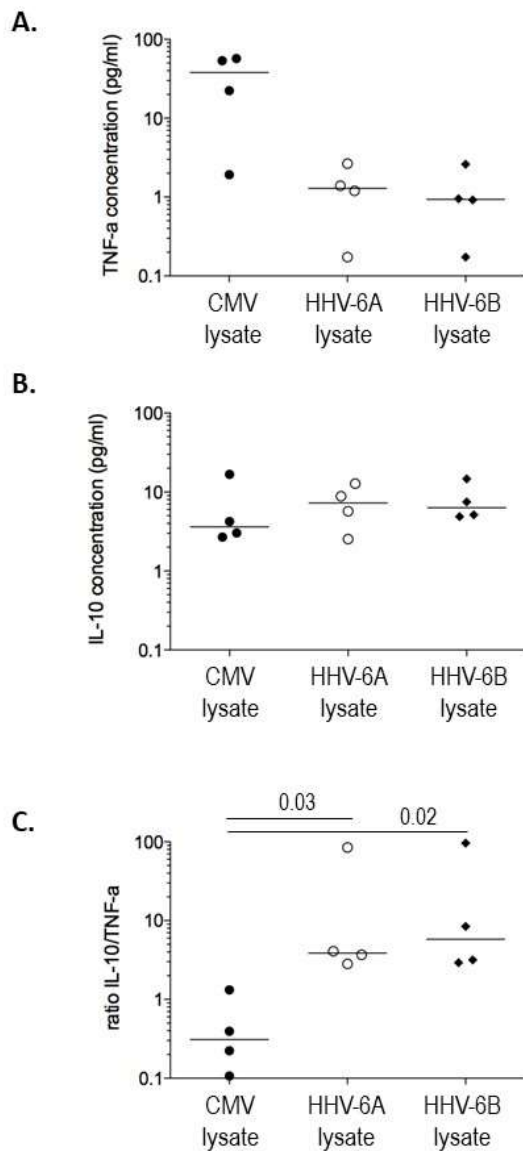
| ID donors | Unstimulated | Mock HHV-6A | Mock HHV-6B | CMV lysate | HHV-6A lysate | HHV-6B lysate | pp65 | IE1 | U54 | U90 |
|-----------|--------------|-------------|-------------|------------|---------------|---------------|-------|-------|-------|-------|
| #1 | 0.005 | 0.021 | 0.019 | 0.684 | 0.022 | 0.020 | 0.701 | 0.267 | 0.006 | 0.009 |
| #2 | 0.002 | 0.019 | 0.023 | 0.166 | 0.033 | 0.050 | 0.056 | 0.005 | 0.003 | 0.016 |
| #3 | 0.006 | 0.040 | 0.022 | 1.071 | 0.080 | 0.023 | 0.942 | 0.230 | 0.074 | 0.066 |
| #4 | 0.006 | 0.031 | 0.025 | 1.615 | 0.052 | 0.045 | 1.073 | 0.201 | 0.013 | 0.024 |
| #5 | 0.003 | 0.035 | 0.026 | 0.585 | 0.036 | 0.027 | 0.412 | 0.010 | 0.088 | 0.004 |
| #6 | 0.002 | 0.024 | 0.046 | 0.194 | 0.025 | 0.047 | 0.061 | 0.025 | 0.021 | 0.010 |
| #7 | 0.003 | 0.013 | 0.017 | 0.555 | 0.019 | 0.018 | 0.637 | 0.239 | 0.012 | 0.031 |
| #8 | 0.001 | 0.021 | 0.025 | 0.362 | 0.022 | 0.045 | 0.560 | 0.008 | 0.002 | 0.033 |
| #9 | 0.002 | 0.019 | 0.023 | 0.519 | 0.111 | 0.023 | 0.777 | 0.168 | 0.012 | 0.039 |
| #10 | 0.017 | 0.009 | 0.008 | 0.202 | 0.051 | 0.009 | 0.132 | 0.034 | 0.023 | 0.018 |
| #11 | 0.003 | 0.007 | 0.006 | 0.018 | 0.014 | 0.007 | 0.026 | 0.004 | 0.004 | 0.006 |

Supplementary Table 1B: Frequency of IFN-g secreting CD4+ T cells obtained for 11 donors post PBMCs stimulation with either Mock for HHV-6A or HHV-6B or in unstimulated conditions. Virus-specific stimulations were performed with lysates (CMV, HHV-6A, HHV-6B) or peptides (pp65, IE1, U54, U90). Data are expressed in percentage.

Supplementary Figure 1: Cytokines release

TNF- α (A) and IL-10 (B) productions were measured in supernatant of PBMCs stimulated during 45 hours with HCMV (black circles) or HHH-6A (white circles) and HHV-6B (black diamonds) lysates. The concentrations (in pg/mL) shown here are calculated after deduction of the levels measured in respective Mock conditions. (C) Ratio of anti-inflammatory (IL-10) vs pro-inflammatory (TNF- α) cytokines. p values were calculated using Wilcoxon pair test for group comparisons.

Supplementary Figure 1

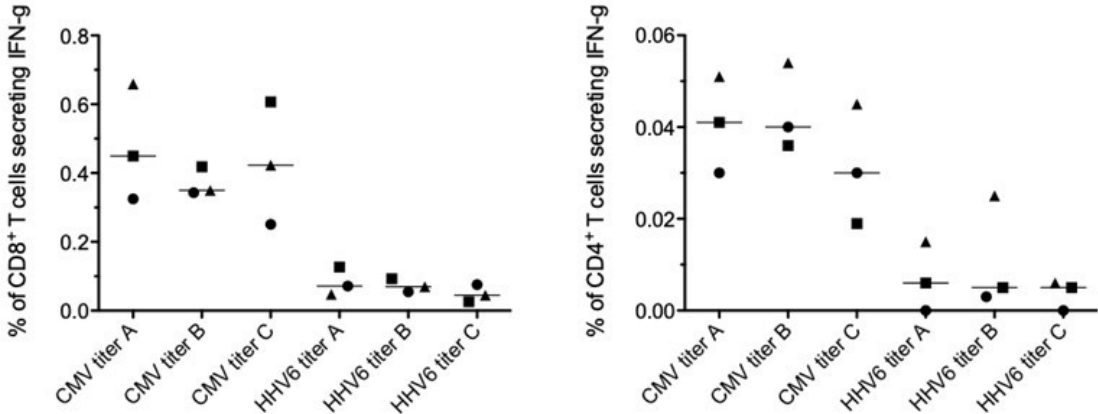


Supplementary Figure 2: Titration of viral T-cell responsiveness

Frequencies of IFN- γ (A) or TNF- α (B) secreting HCMV or HHV-6 specific T-cells are depicted, both for CD4 (right panels) and for CD8 (left panels). Overnight stimulation was performed using 3 doses of overlapping peptides specific for HCMV or HHV-6 (titer A= 1 μ M; titer B=3.3 μ M and titer C=10 μ M).

Supplementary Figure 2

A.



B.

