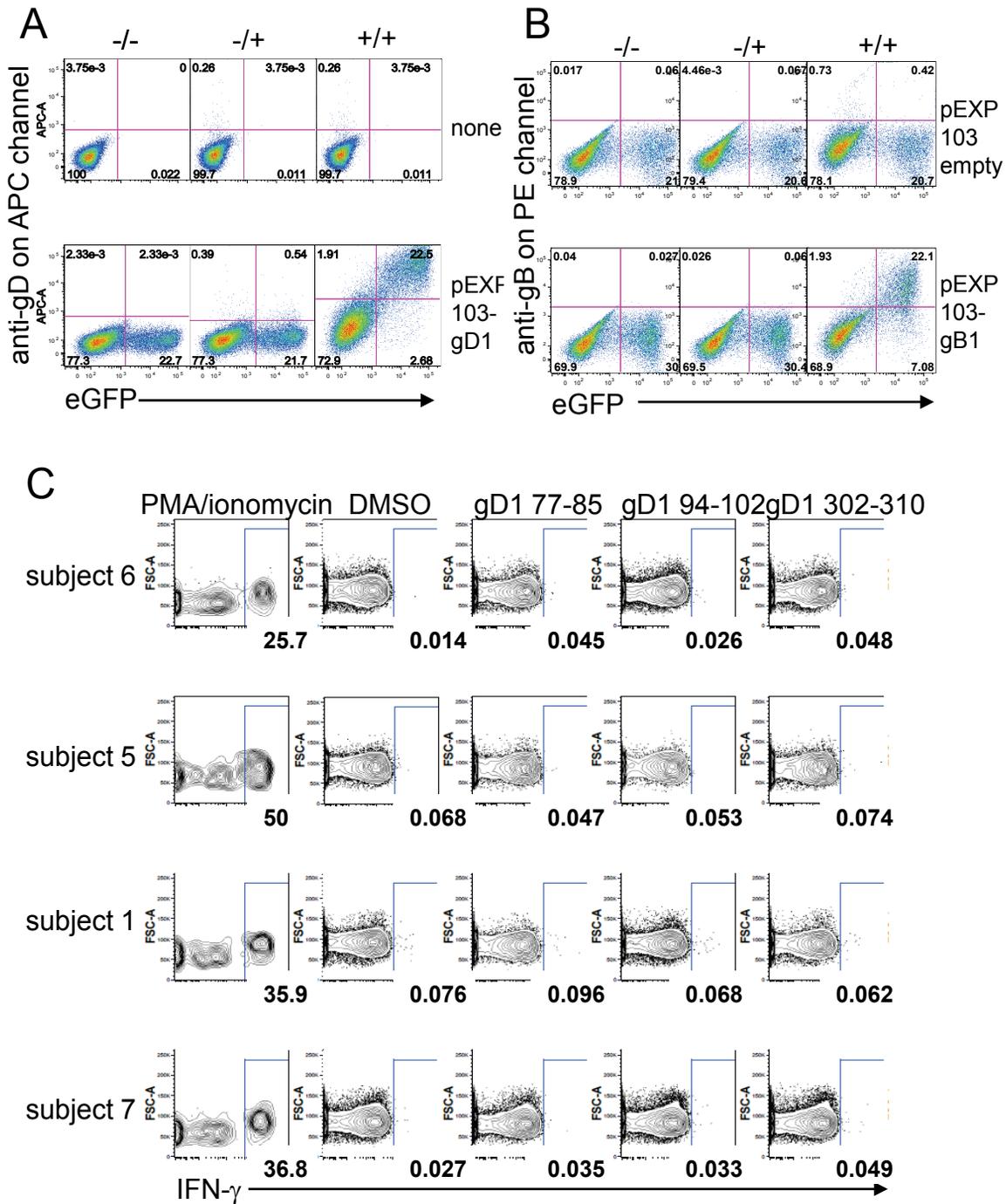
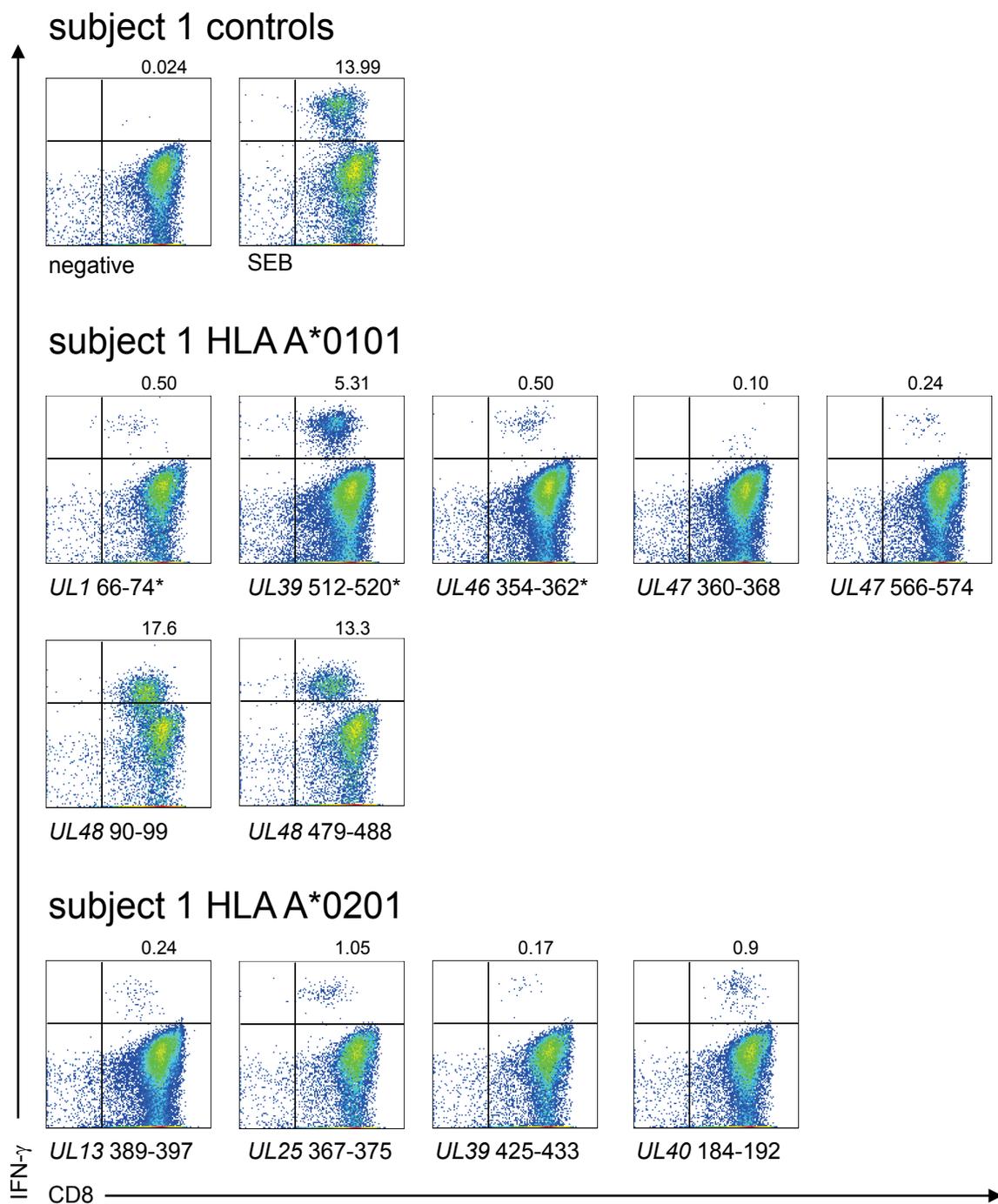


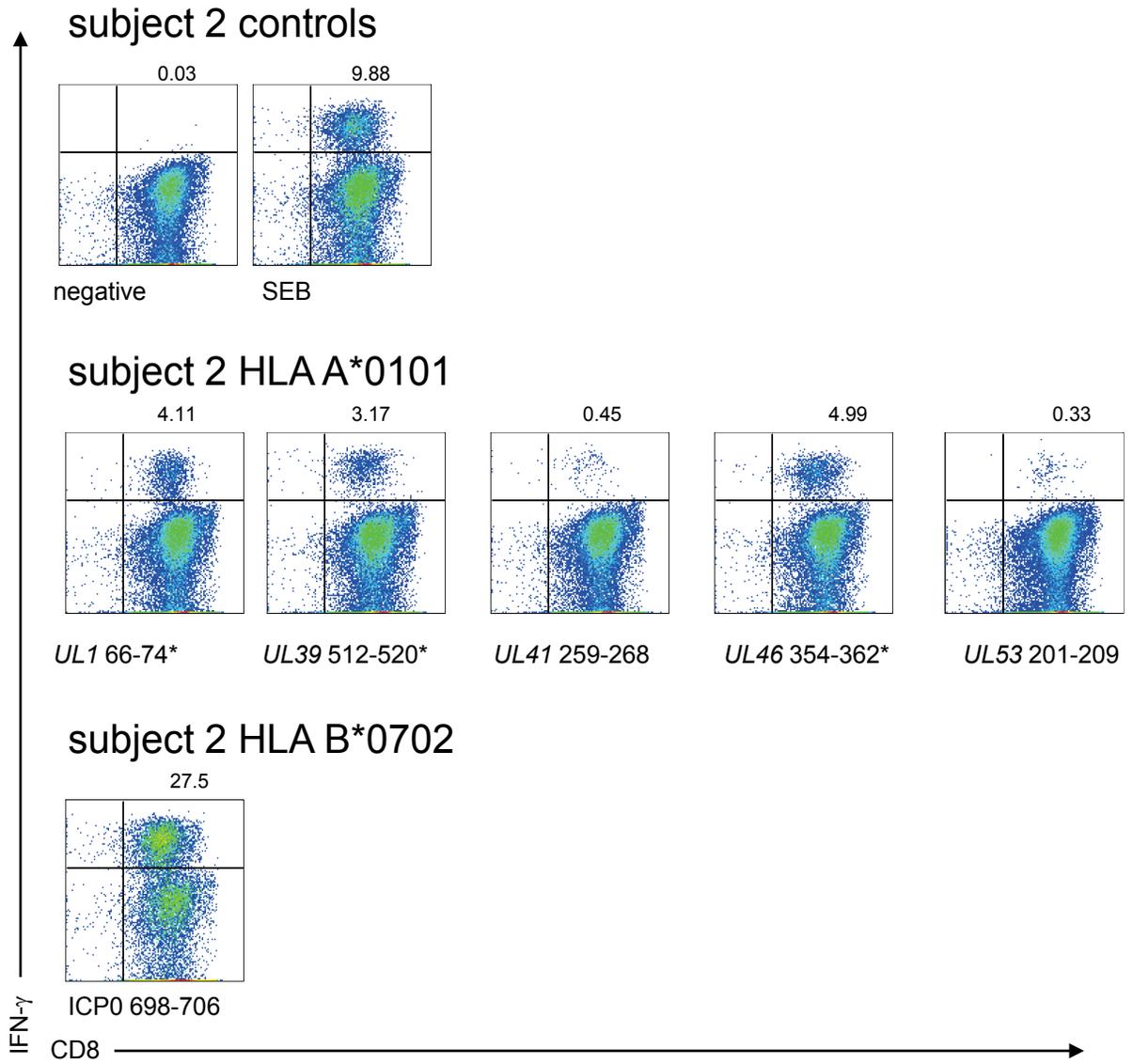
Supplementary Figure 1. Expression of HSV-1 proteins or protein fragments as fusions with EGFP at the N-terminal. HSV-1 clones are arranged with protein names arrayed on the X-axis in their nominal order in the HSV-1 genome per Genbank NC\_001806.1. Proteins are full length except those expressed as fragments as detailed in Supplementary Table 1. Cos-7 cells were recovered by trypsinization 48 h after transfection with the indicated plasmid pEXP103-HSV-1 gene DNA as detailed in Materials and Methods, stained with Violet live/dead, washed free of cell culture medium and analyzed by flow cytometry using 488 nm excitation. Live cells in the appropriate forward and side scatter regions were analyzed for EGFP fluorescence and the percent positive indicated with bars. At least 5000 live cells were analyzed per plasmid.



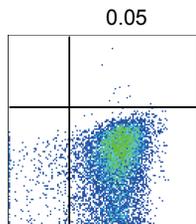
Supplementary Figure 2. Confirmation of expression of selected HSV-1 proteins. A: Expression of HSV-1 envelope glycoprotein gD1, encoded by gene US6, after no transfection (top) or transfection of Cos-7 cells with pEXP103-US6 (bottom). Cells were stained with no antibody (left), secondary APC- labeled anti-mouse antibody only (middle), or primary anti-gD mAb followed by secondary antibody (right) and analyzed for EGFP and APC fluorescence. Numbers are percentages of cells in indicated quadrants. B: Similar data for HSV-1 envelope glycoprotein B1, encoded by gene UL27. Cos-7 were transfected with either empty pEXP103 expressing EGFP (top), or the construct containing the cloned UL27 gene (bottom). Primary antibody was anti-gB, and secondary antibody used PE. C: Polyclonal expanded CD8 cell lines derived from CD3+ CD137<sup>high</sup> cells show low reactivity with the indicated candidate HLA A\*0201-restricted peptides from gD1 at 1  $\mu$ g/ml. Participants were selected for expression of HLA A\*0201 or a related HLA A\*02 allele (Table 1). APC were autologous PBMC that are dump-gated for CFSE label (not shown). After gating on live CD3+ CD8+, dot-plots of forward scatter vs. intracellular IFN- $\gamma$  are shown with numbers indicating percentages of cells in the indicated positive region. Positive control at left followed by DMSO negative control.



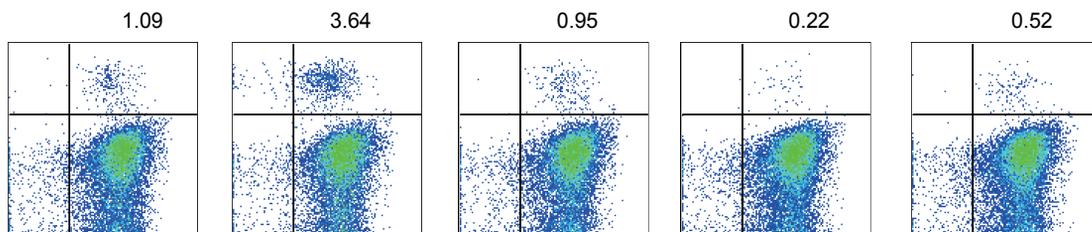
Supplementary Figure 3. Five pages. Reactivity of polyclonal expanded CD8 cell lines derived from CD3<sup>+</sup> CD137<sup>high</sup> cells with synthetic HSV-1 peptides at 1  $\mu$ g/ml. These data are the basis for Table 4. Autologous PBMC used as APC were CFSE-labeled and dump-gated (not shown). Each page shows results from a distinct participant from Table 1, and begins with negative control DMSO stimulation and positive control SEB stimulation, followed by peptides. Each dot-plot shows expression of CD8 and intracellular IFN- $\gamma$ . The identity of the peptides is indicated below each dot-plot, with asterisks after three HLA A\*0101-restricted peptides studied in both participants 1 and 2. The identity of the HLA allele used to assign restriction is indicated above the relevant dot-plots. The numbers are the percentages of cells in the upper right quadrants of each dot-plot.



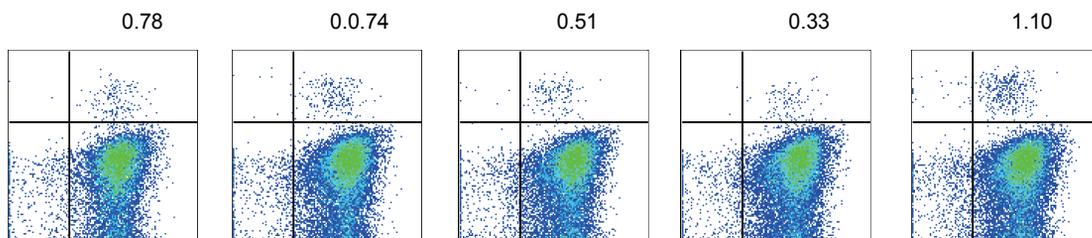
### subject 3 control



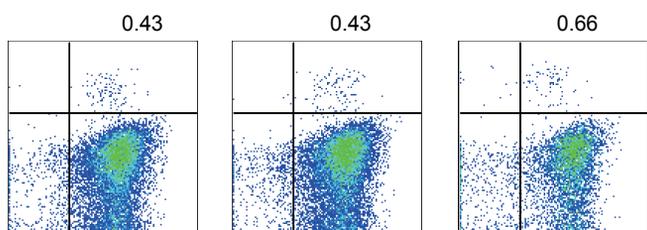
### subject 3 HLA A\*2902



*UL25* 170-179      *UL25* 235-243      *UL26* 22-30      *UL26* 326-334      *UL27* 295-303

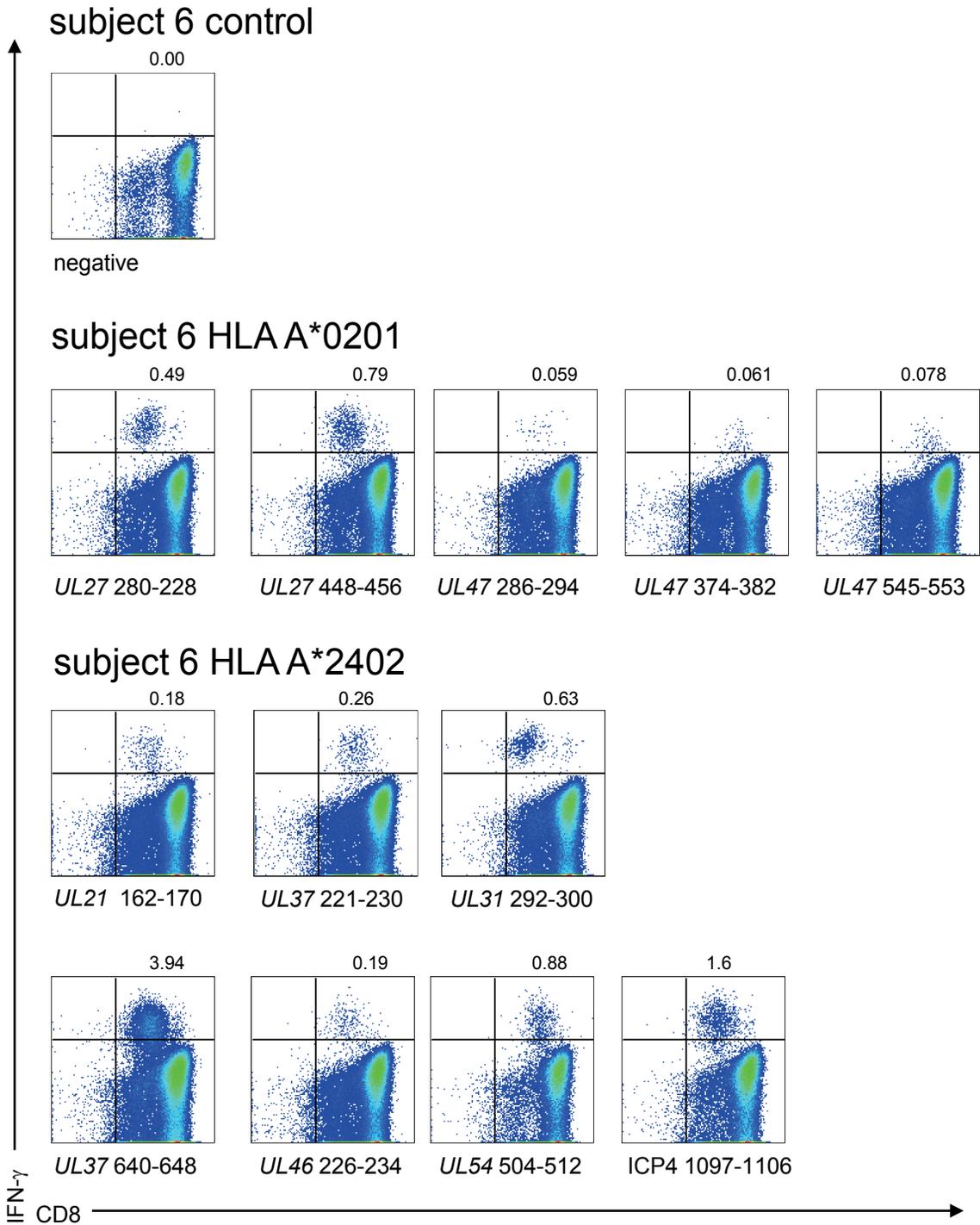


*UL27* 641-649      *UL29* 460-468      *UL29* 895-903      *UL46* 93-101      *UL46* 126-134

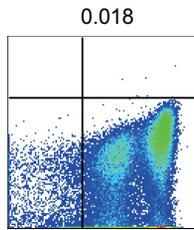


*UL46* 224-232      *UL46* 333-341      *UL47* 508-516

IFN- $\gamma$   $\uparrow$   
CD8  $\rightarrow$

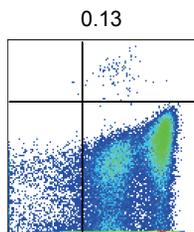


subject 7 control

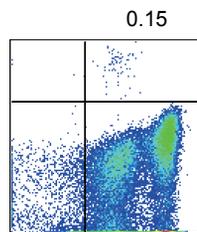


negative

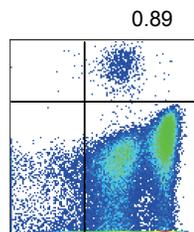
subject 7 HLA B\*0702



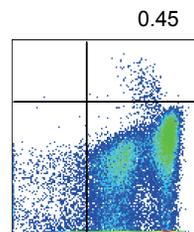
*UL21* 382-390



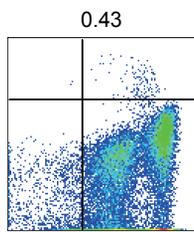
*UL49* 281-290



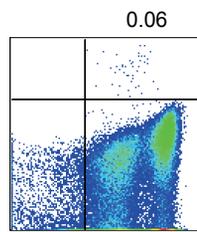
*US1* 236-244



*US7* 22-30



*US7* 97-105

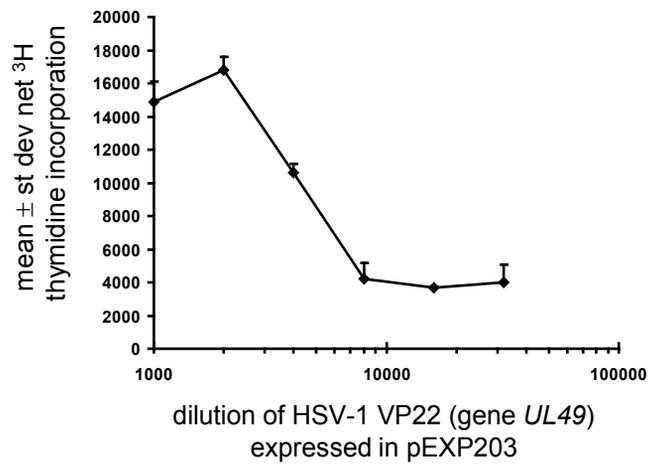


*US7* 195-203

IFN- $\gamma$

CD8

## Jing et al. Supplementary Fig. 4



Supplementary Figure 4. Expression of full-length HSV-1 protein VP22 (gene *UL49*) in the custom pEXP203 vector. Protein derived by in vitro transcription/translation was tested in triplicate  $^3\text{H}$  thymidine incorporation assays using a CD4 T-cell clone known to be specific for VP22, and autologous irradiated PBMC as APC, as detailed in the text. Y axis is mean  $\pm$  standard deviation of net CPM  $^3\text{H}$  thymidine incorporation after subtracting media control.

## Supplementary Tables

Supplementary Table 1. Primers used to amplify predicted HSV-1 protein coding regions.

clone name <sup>a</sup>	5' primer <sup>b</sup>	5' primer position <sup>c</sup>	3' primer <sup>b</sup>	3' primer position <sup>c</sup>
<i>RL1</i> ( $\gamma$ 34.5) full	GGGGACAAGTTTGTACAAAAAAGCGAGGCTTCacgctctctctccatggc	<i>RL1</i> , (-)15-5	GGGGACCACTTTGTACAAGAAAGCTGGGTcagcttagaccgagttcgccg	<i>RL1</i> , 731-747(+) <sup>3</sup>
<i>RL2</i> (ICP0) frag A	GGGGACAAGTTTGTACAAAAAAGCGAGGCTTCatggagccccccccggagcgag	<i>RL2</i> , 1-23	GGGGACCACTTTGTACAAGAAAGCTGGGTcgcgccgccccctcacctcg	<i>RL2</i> , 54-57(+) <sup>16</sup>
<i>RL2</i> (ICP0) frag B	GGGGACAAGTTTGTACAAAAAAGCGAGGCTTccccccccgatgctctgggtg	<i>RL2</i> , 58-80	GGGGACCACTTTGTACAAGAAAGCTGGGTcttagctccaggtctgtcatcctcg	<i>RL2</i> , 699-723(+) <sup>3</sup>
<i>RL2</i> (ICP0) frag C	GGGGACAAGTTTGTACAAAAAAGCGAGGCTTCgcagactacglaccgccc	<i>RL2</i> , (-)1-724-741	GGGGACCACTTTGTACAAGAAAGCTGGGTcttattgtttccctctccccgggtcg	<i>RL2</i> , 2303-2328
<i>UL1</i> full	<b>AAAAAGCGAGGCT</b> ccgccatggggatlttgggtgggtc	<i>UL1</i> , 1-21	<b>AGAAAGCTGGGT</b> ctagagggccgggagtg	<i>UL1</i> , 657-672
<i>UL2</i> full	GGGGACAAGTTTGTACAAAAAAGCGAGGCTTCatgaagcggcctgcagccc	<i>UL2</i> , 1-20	GGGGACCACTTTGTACAAGAAAGCTGGGTcTcaaccgaccagtcgatgggtgaa	<i>UL2</i> , 981-1005
<i>UL3</i> full	<b>AAAAAGCGAGGCT</b> ccgccatggttaacctctgtctcatac	<i>UL3</i> , 1-24	<b>AGAAAGCTGGGT</b> ctactcgcccccgaggc	<i>UL3</i> , 691-705
<i>UL4</i> full	<b>AAAAAGCGAGGCT</b> ccgccatgccaatccacagacgacc	<i>UL4</i> , 1-21	<b>AGAAAGCTGGGT</b> ctaggacccccaaagtgtctgc	<i>UL4</i> , 577-597
<i>UL5</i> full	GGGGACAAGTTTGTACAAAAAAGCGAGGCTTCatggcggcggccggcgggga	<i>UL5</i> , 1-20	GGGGACCACTTTGTACAAGAAAGCTGGGTctaatagacaatgaccacgt	<i>UL5</i> , 2630-2649
<i>UL6</i> full	<b>AAAAAGCGAGGCT</b> ccgccatgaccgaccacgctcg	<i>UL6</i> , 1-18	<b>AGAAAGCTGGGT</b> ctatctgcccggctcgccg	<i>UL6</i> , 2013-2028
<i>UL7</i> full	<b>AAAAAGCGAGGCT</b> ccgccatggccgcccgcgacgg	<i>UL7</i> , 1-16	<b>AGAAAGCTGGGT</b> ctaacaaaactgataaaacagcgacg	<i>UL7</i> , 866-888
<i>UL8</i> full	GGGGACAAGTTTGTACAAAAAAGCGAGGCTTCatggacaccgacagatcgt	<i>UL8</i> , 1-20	GGGGACCACTTTGTACAAGAAAGCTGGGTctcaggcaaacgaaacgaca	<i>UL8</i> , 2234-2253
<i>UL9</i> frag A	<b>AAAAAGCGAGGCT</b> ccgccatgcctttgtggggggc	<i>UL9</i> , 1-18	<b>AGAAAGCTGGGT</b> ctacatgtaaatcagtagctcccc	<i>UL9</i> , 1180-1200
<i>UL9</i> frag B	<b>AAAAAGCGAGGCT</b> ccgccatggagcctccggggcg	<i>UL9</i> , 1201-1215	<b>AGAAAGCTGGGT</b> ctatagggtgctaaagtccaccg	<i>UL9</i> , 2533-2553
<i>UL9</i> frag C	GGGGACAAGTTTGTACAAAAAAGCGAGGCTTCatgaactacggaccggacatggtg	<i>UL9</i> , 1114-1137	GGGGACCACTTTGTACAAGAAAGCTGGGTcagcgtgcatctctcaaagtagt	<i>UL9</i> , 1402-1425
<i>UL10</i> full	<b>AAAAAGCGAGGCT</b> ccgccatgggacgccccgccc	<i>UL10</i> , 1-17	<b>AGAAAGCTGGGT</b> ctaacacggcggagcgtg	<i>UL10</i> , 1404-1419
<i>UL11</i> full	GGGGACAAGTTTGTACAAAAAAGCGAGGCTTCatggcctctctgtctccgg	<i>UL11</i> , 1-20	GGGGACCACTTTGTACAAGAAAGCTGGGTcttattgctatcgacatggggg	<i>UL11</i> , 269-291
<i>UL12</i> full	<b>AAAAAGCGAGGCT</b> ccgccatggagtcacgtagggcc	<i>UL12</i> , 1-19	<b>AGAAAGCTGGGT</b> ctagcagacgacctccccg	<i>UL12</i> , 1862-1878
<i>UL13</i> full	<b>AAAAAGCGAGGCT</b> ccgccatggatgagtcgccgacagag	<i>UL13</i> , 1-21	<b>AGAAAGCTGGGT</b> ctacgacagcgcgtgccc	<i>UL13</i> , 1540-1554
<i>UL14</i> full	<b>AAAAAGCGAGGCT</b> ccgccatggaccgagatccgccc	<i>UL14</i> , 1-18	<b>AGAAAGCTGGGT</b> ctattgcccctgggatagcc	<i>UL14</i> , 639-657
<i>UL15</i> full	<b>AAAAAGCGAGGCT</b> ccgccatggttgtagcagctggc	<i>UL15</i> , 1-20	<b>AGAAAGCTGGGT</b> ctacgagacgcgtgtagatag	<i>UL15</i> , 2189-2205
<i>UL16</i> full	<b>AAAAAGCGAGGCT</b> ccgccatggcgcagctgggacc	<i>UL16</i> , 1-17	<b>AGAAAGCTGGGT</b> ctattgggatcgtctgaggag	<i>UL16</i> , 1101-1119
<i>UL17</i> full	GGGGACAAGTTTGTACAAAAAAGCGAGGCTTCatgaacgcgacctggccaacg	<i>UL17</i> , 1-22	GGGGACCACTTTGTACAAGAAAGCTGGGTcctagcagacggccgttcc	<i>UL17</i> , 2093-2112

UL18 full	<b>AAAAAGCGAGGCT</b> ccgcatgctggcggaacgctttg	UL18, 1-19	<b>AGAAAGCTGGGT</b> ctaggatagcgtataacggg	UL18, 936-954
UL19 full	<b>AAAAAGCGAGGCT</b> ccgcatggccgctcccaaccg	UL19, 1-17	<b>AGAAAGCTGGGT</b> ctacagagcagtccttgagc	UL19, 4104-4122
UL20 full	<b>AAAAAGCGAGGCT</b> ccgcatgaccatgcggaatgacc	UL20, 1-19	<b>AGAAAGCTGGGT</b> ctagaacgcgacgggtcattc	UL20, 648-666
UL20 frag A	<b>AAAAAGCGAGGCT</b> ccgcatgaccatgcggaatgacc	UL20, 1-19	<b>AGAAAGCTGGGT</b> ctagcctttgaaagaccggc	UL20, 171-189
UL21 full	<b>AAAAAGCGAGGCT</b> ccgcatggagcttagctacgccac	UL21, 1-20	<b>AGAAAGCTGGGT</b> ctacacagactgctcglttgg	UL21, 1588-1605
UL22 full	<b>AAAAAGCGAGGCT</b> ccgcatggggaatggttatggtcg	UL22, 1-22	<b>AGAAAGCTGGGT</b> ctattcgctctcaaaaaacgg	UL22, 2494-2514
UL23 full	<b>AAAAAGCGAGGCT</b> ccgcatggcttctgtaaccctgac	UL23, 1-19	<b>AGAAAGCTGGGT</b> ctagttagctccccatctcc	UL23, 1110-1128
UL24 full	<b>AAAAAGCGAGGCT</b> ccgcatggccgcgagaaacgcg	UL24, 1-17	<b>AGAAAGCTGGGT</b> ctattcgaggcgctcggg	UL24, 791-807
UL25 full	<b>AAAAAGCGAGGCT</b> ccgcatggaccgactgcccatttg	UL25, 1-22	<b>AGAAAGCTGGGT</b> ctaaaccgacagactgctgtg	UL25, 1721-1740
UL26 full	<b>AAAAAGCGAGGCT</b> ccgcatggcagccgatgcccc	UL26, 1-17	<b>AGAAAGCTGGGT</b> ctagcgggccccatcatctg	UL26, 1888-1905
UL26 frag A	<b>AAAAAGCGAGGCT</b> ccgcatggcagccgatgcccc	UL26, 1-17	<b>AGAAAGCTGGGT</b> ctagtaatgggagccgggatc	UL26, 984-1002
UL26 frag B	<b>AAAAAGCGAGGCT</b> ccgcatgaaccagctgctgcggcg	UL26, 1003-1019	<b>AGAAAGCTGGGT</b> ctagcgggccccatcatctg	UL26, 1888-1905
UL26.5 full	<b>AAAAAGCGAGGCT</b> ccgcatgaaccgcttccgacatc	UL26.5, 1-20	<b>AGAAAGCTGGGT</b> ctagcgggccccatcatctg	UL26, 1888-1905
UL27 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCgtagtcccgcgatgccaag	UL27, (-)12-10	GGGGACCACCTTTGTACAAGAAAGCTGGGTCAAacccccatcacaggtcgtcc	UL27, 2703-2715 (+)10
UL27 frag A	<b>AAAAAGCGAGGCT</b> ccgcatgcccgtgagcaaccc	UL27, 2398-2414	<b>AGAAAGCTGGGT</b> ctacaggtgctcctctgctg	UL27, 2696-2712
UL28 frag A	<b>AAAAAGCGAGGCT</b> ccgcatggccgccccggtgctc	UL28, 1-17	<b>AGAAAGCTGGGT</b> ctaccgacgcccggctcagg	UL28, 995-1011
UL28 frag B	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCgactgtttcaggccaccacgc	UL28, 886-907	GGGGACCACCTTTGTACAAGAAAGCTGGGTCTctggcgcagtcgggactac	UL28, 2355-2358(+)+16
UL29 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCatggagacaagcccaagac	UL29, 1-20	GGGGACCACCTTTGTACAAGAAAGCTGGGTCTcacagatalccaacgtca	UL29, 3572-3591
UL30 full	<b>AAAAAGCGAGGCT</b> ccgcatggtttccggtggcgcg	UL30, 1-19	<b>AGAAAGCTGGGT</b> ctagctagagatcaaggctctatg	UL30, 3681-3705
UL31 full	<b>AAAAAGCGAGGCT</b> ccgcatgtatgacaccgacccccatc	UL31, 1-22	<b>AGAAAGCTGGGT</b> ctaccgcccggagaaactgctc	UL31, 901-918
UL32 full	<b>AAAAAGCGAGGCT</b> ccgcatggcaactgcccccc	UL32, 1-18	<b>AGAAAGCTGGGT</b> ctacataggtacacaggggtgctc	UL32, 1767-1788
UL33 full	<b>AAAAAGCGAGGCT</b> ccgcatggctggcgggagggg	UL33, 1-18	<b>AGAAAGCTGGGT</b> ctagcccgcgagaatctggtg	UL33, 373-390
UL34 full	<b>AAAAAGCGAGGCT</b> ccgcatggcgggactgggcaag	UL34, 1-18	<b>AGAAAGCTGGGT</b> ctataggcgcgcccagcac	UL34, 809-825
UL34 frag A	<b>AAAAAGCGAGGCT</b> ccgcatggcgggactgggcaag	UL34, 1-18	<b>AGAAAGCTGGGT</b> ctaccgtaggtgcttaagacc	UL34, 723-741
UL35 full	<b>AAAAAGCGAGGCT</b> ccgcatggcctcccgaatttc	UL35, 1-19	<b>AGAAAGCTGGGT</b> ctaccgggtcccggcgctc	UL35, 321-336
UL36 frag A	<b>AAAAAGCGAGGCT</b> ccgcatgggtggcggaacaacac	UL36, 1-20	<b>AGAAAGCTGGGT</b> ctattctcgccatgcgcg	UL36, 2984-3000
UL36 frag B	<b>AAAAAGCGAGGCT</b> ccgcatggcgctgcgagggcg	UL36, 3001-3015	<b>AGAAAGCTGGGT</b> ctagaagcagagcgcgtactgc	UL36, 5983-6000
UL36 frag C	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCaagaggccgaccgagatgctgc	UL36, 5833-5857	GGGGACCACCTTTGTACAAGAAAGCTGGGTCTcgggtggcgcgcccagggagcgtg	UL36, 8063-8085
UL36 frag D	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCgttccgcccggcgctgctgc	UL36, 2851-2873	GGGGACCACCTTTGTACAAGAAAGCTGGGTCTcaagaacttcccggcgctgc	UL36, 3065-3087
UL37 frag A	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCatggcagaccgcttcccgtc	UL37, 1-23	GGGGACCACCTTTGTACAAGAAAGCTGGGTCTgaccatgatagcgcagcgtc	UL37, 1533-1555
UL37 frag B	<b>AAAAAGCGAGGCT</b> ccgcatggcggctgctgctgctg	UL37, 1501-1516	<b>AGAAAGCTGGGT</b> ctattgtaactagtaacggcaag	UL37, 3348-3369
UL38 full	<b>AAAAAGCGAGGCT</b> ccgcatgaagaccaatccgctacc	UL38, 1-21	<b>AGAAAGCTGGGT</b> ctaccgcatgcccacc	UL38, 1381-1395

UL39 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTctctgtgaaatgccagcgc	UL39, (-)9-12	GGGGACCACCTTTGTACAAGAAAGCTGGGTcttctggtcacagcgcga	UL39, 3403-3414(+) <sup>7</sup>
UL40 full	<b>AAAAAGCGAGGCT</b> ccgcatgattccgcccag	UL40, 1-19	<b>AGAAAGCTGGGT</b> tcacagatcgttgacgaccg	UL40, 1004-1020
UL40 frag A	<b>AAAAAGCGAGGCT</b> ccgcatgattccgcccag	UL40, 1-19	<b>AGAAAGCTGGGT</b> ctactttccggaacggaggcg	UL40, 519-537
UL40 frag B	<b>AAAAAGCGAGGCT</b> ccgcatgaccaacaacctctcgagg	UL40, 607-625	<b>AGAAAGCTGGGT</b> tcacagatcgttgacgaccg	UL40, 1004-1020
UL41 full	<b>AAAAAGCGAGGCT</b> ccgcatgggtttgtcgatgatg	UL41, 1-21	<b>AGAAAGCTGGGT</b> ctactcgtcccgaatttggcc	UL41, 1449-1467
UL42 full	<b>AAAAAGCGAGGCT</b> ccgcatgacgattccccggcg	UL42, 1-19	<b>AGAAAGCTGGGT</b> ctaggggaatccaaaaccatacgg	UL42, 1444-1464
UL43 full	<b>AAAAAGCGAGGCT</b> ccgcatggtggcgtagcgaac	UL43, 1-19	<b>AGAAAGCTGGGT</b> ctaatacgtcccaccgccc	UL43, 1287-1302
UL43 frag A	<b>AAAAAGCGAGGCT</b> ccgcatggtggcgtagcgaac	UL43, 1-19	<b>AGAAAGCTGGGT</b> ctaccgcgcgacataacgac	UL43, 205-222
UL43 frag B	<b>AAAAAGCGAGGCT</b> ccgcatggtggcgtagcgaac	UL43, 643-660	<b>AGAAAGCTGGGT</b> ctaaacctggaaaaccgaaacac	UL43, 1075-1095
UL44 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCatggccccgggggggtggcct	UL44, 1-23	GGGGACCACCTTTGTACAAGAAAGCTGGGTctaccgcatgacgctgcc	UL44, 1517-1536
UL45 full	<b>AAAAAGCGAGGCT</b> ccgcatgctctcgggcatcg	UL45, 1-18	<b>AGAAAGCTGGGT</b> ctacggcagccccagcgc	UL45, 502-516
UL46 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCgtcaccatgacgcccggac	UL46, (-)6-14	GGGGACCACCTTTGTACAAGAAAGCTGGGTctaccggctcgggctcctt	UL46, 2144-2163
UL47 full	<b>AAAAAGCGAGGCT</b> ccgcatgctggctcgcgaacc	UL47, 1-18	<b>AGAAAGCTGGGT</b> ctatggcgtagggggcc	UL47, 2065-2079
UL48 full	<b>AAAAAGCGAGGCT</b> ccgcatgacctctgtgtagcg	UL48, 1-19	<b>AGAAAGCTGGGT</b> ctaccaccgtactcgtcaattc	UL48, 1451-1470
UL49 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCatgacctctgccgctcct	UL49, 1-20	GGGGACCACCTTTGTACAAGAAAGCTGGGTcttactcagcgggctcct	UL49, 889-906(+) <sup>2</sup>
UL49.5 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCatggcccccccagaagggt	UL49A, 1-20	GGGGACCACCTTTGTACAAGAAAGCTGGGTcaccggcggttcaggcgtg	UL49A, 268-276(+) <sup>11</sup>
UL50 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCaacaggaagatgagtcagtg	UL50, (-)9-12	GGGGACCACCTTTGTACAAGAAAGCTGGGTcctaataaccgtagaaccaa	UL50, 1097-1116
UL51 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCatggctctctcggggc	UL51, 1-20	GGGGACCACCTTTGTACAAGAAAGCTGGGTcttattgacccaaaacacagc	UL51, 716-735
UL52 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCatggggcaggaagcgggaa	UL52, 1-20	GGGGACCACCTTTGTACAAGAAAGCTGGGTcctaagacgacggttagagg	UL52, 3158-3177
UL53 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCatgctgcgctcgttcct	UL53, 1-20	GGGGACCACCTTTGTACAAGAAAGCTGGGTctacatacacaacaggcgc	UL53, 998-1017
UL53 frag A	<b>AAAAAGCGAGGCT</b> ccgcatgcccgtgcaccgatgtattac	UL53, 97-117	<b>AGAAAGCTGGGT</b> ctaccgtgttaccatagggtc	UL53, 348-366
UL54 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCatggcgactgacattgat	UL54, 1-20	GGGGACCACCTTTGTACAAGAAAGCTGGGTcctaatacaggagttgcaat	UL54, 1520-1539
UL55 full	<b>AAAAAGCGAGGCT</b> ccgcatgacagcagccccctc	UL55, 1-18	<b>AGAAAGCTGGGT</b> ctacgcctaattttaacttgacgc	UL55, 536-558
UL56 full	<b>AAAAAGCGAGGCT</b> ccgcatggcttcggaggcgc	UL56, 1-17	<b>AGAAAGCTGGGT</b> ctaccgccacaggaataaccag	UL56, 685-702
US1 full	<b>AAAAAGCGAGGCT</b> ccgcatggccgacattccccag	US1, 1-19	<b>AGAAAGCTGGGT</b> ctaccgccgagaaacgtgc	US1, 1243-1260
US2 full	<b>AAAAAGCGAGGCT</b> ccgcatggcggttgtgtgcaac	US2, 1-21	<b>AGAAAGCTGGGT</b> ctaaagggtgtaaccgatagc	US2, 854-873
US3 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTTCatggcctgtgtaagtttg	US3, 1-20	GGGGACCACCTTTGTACAAGAAAGCTGGGTctatttctgtgaaacagcg	US3, 1427-1446
US4 full	<b>AAAAAGCGAGGCT</b> ccgcatgctgcagggcccatg	US4, 1-18	<b>AGAAAGCTGGGT</b> ctaccgcgttcggacggc	US4, 699-714
US4 frag A	<b>AAAAAGCGAGGCT</b> ccgcatgctgcagggcccatg	US4, 1-18	<b>AGAAAGCTGGGT</b> ctaggttcaggcgggg	US4, 555-570
US5 full	<b>AAAAAGCGAGGCT</b> ccgcatgtctctgcccagtc	US5, 1-18	<b>AGAAAGCTGGGT</b> ctatacgaactgggtccatgtag	US5, 255-276
US6 full	<b>AAAAAGCGAGGCT</b> ccgcatgggggggctgccc	US6, 1-16	<b>AGAAAGCTGGGT</b> ctagtaaacagggtggtg	US6, 1163-1182
US7 full	<b>AAAAAGCGAGGCT</b> ccgcatgcccgtgcccggcttg	US7, 1-18	<b>AGAAAGCTGGGT</b> ctataccaacaggggagggcgtg	US7, 1151-1170

US7 frag B	<b>AAAAAGCGAGGCT</b> <i>ccgccatgcacagatgcaacgccgc</i>	US7, 889-906	<b>AGAAAGCTGGGT</b> <i>ctataccaacagggaggcggtg</i>	US7, 1151-1170
US8 full	<b>AAAAAGCGAGGCT</b> <i>ccgccatggatcgcgggcg</i>	US8, 1-16	<b>AGAAAGCTGGGT</b> <i>ctaccagaagacggacgaatcg</i>	US8, 1632-1650
US8 frag A	<b>AAAAAGCGAGGCT</b> <i>ccgccatggatcgcgggcg</i>	US8, 1-16	<b>AGAAAGCTGGGT</b> <i>ctaccctaaccgagggcg</i>	US8, 1247-1263
US8 frag B	<b>AAAAAGCGAGGCT</b> <i>ccgccatgcgcagcgctgcctggc</i>	US8, 1333-1348	<b>AGAAAGCTGGGT</b> <i>ctaccagaagacggacgaatcg</i>	US8, 1632-1650
US8.5 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTT <i>Catggatccggccttgagatc</i>	US8A, 1-20	GGGGACCACTTTGTACAAGAAAGCTGGGTC <i>ttagcgcctcgggcaattg</i>	US8A, 461-480
US9 full	<b>AAAAAGCGAGGCT</b> <i>ccgccatgacgtcccgcctctcc</i>	US9, 1-18	<b>AGAAAGCTGGGT</b> <i>ctagcggagcagccacatcag</i>	US9, 253-270
US10 full	GGGGACAAGTTTGTACAAAAAGCGAGGCTT <i>Catgatcaagcggcgggca</i>	US10, 20	GGGGACCACTTTGTACAAGAAAGCTGGGTC <i>ctagcacaggggtgggta</i>	US10, 940-939
US11 full	<b>AAAAAGCGAGGCT</b> <i>ccgccatgagccagaccaacccc</i>	US11, 1-19	<b>AGAAAGCTGGGT</b> <i>ctatacagaccgcgagcgcg</i>	US11, 467-483
US12 full	<b>AAAAAGCGAGGCT</b> <i>ccgccatgctgtggccctggaaatg</i>	US12, 1-21	<b>AGAAAGCTGGGT</b> <i>ctaaccgggtaccggattacgcg</i>	US12, 252-264
RS1 (ICP4) full	GGGGACAAGTTTGTACAAAAAGCGAGGCTT <i>Catggcctcggagacaagcagc</i>	RS1, 1-23	GGGGACCACTTTGTACAAGAAAGCTGGGTC <i>ttagcagcaccctgccccctcg</i>	RS1, 3876-3897
RS1 (ICP4) frag A	GGGGACAAGTTTGTACAAAAAGCGAGGCTT <i>Catggcctcggagacaagcagc</i>	RS1, 1-23	GGGGACCACTTTGTACAAGAAAGCTGGGTC <i>gctgacgtaccgcgcatag</i>	RS1, 915-936
RS1 (ICP4) frag B	GGGGACAAGTTTGTACAAAAAGCGAGGCTT <i>Cctgagctggacccgacgcgac</i>	RS1, 871-893	GGGGACCACTTTGTACAAGAAAGCTGGGTC <i>caggcgcagcaccagcgcgctc</i>	RS1, 1904-1926
RS1 (ICP4) frag D	GGGGACAAGTTTGTACAAAAAGCGAGGCTT <i>Catggcgcctggatgcgccagatc</i>	RS1, 2743-2767	GGGGACCACTTTGTACAAGAAAGCTGGGTC <i>ttagcagcaccctgccccctcg</i>	RS1, 3876-3897

<sup>a</sup> Name of molecular clone. Full indicates full length clone. Frag indicates clone has a portion of the full length ORF. ORF names and characteristics from Genbank NC\_001806.

<sup>b</sup> Nucleotide sequences of primers. Primers starting with bolded regions are from the Haas lab. Bolded regions have *att* homology for Gateway<sup>TM</sup> cloning, adjacent italic regions are *ccgcc* linkers in 5' primers only, and regular font regions are from the Genbank NC\_001806 HSV-1 genome. Primers without bolding are from the Koelle lab. These have capitalized *att* homology regions followed by lower case regions from the HSV-1 genome.

<sup>c</sup> Name of HSV-1 ORF to be amplified from Genbank NC\_001806, followed by nucleotides within the ORF coding region included in the primer. Integers refer to the nominal isomer of the HSV-1 genome and diploid HSV-1 gene nucleotide numbers correspond to first (leftward) allele along the linear genome of the nominal isomer. Minus or plus signs in parentheses refer the number of nucleotides offset 5' or 3' to the canonical ATG start or last coding nucleotide, respectively.

Supplementary Table 2. HSV-1 ORFs scoring positive for HLA class I-restricted IFN- $\gamma$  responses for responder cells created by cross-presentation of HSV-1-infected cells by moDC to CD8+ T-cells, sort-enrichment of CD137<sup>high</sup>, and non-specific expansion.

gene <sup>a</sup>	protein <sup>a</sup>	kinetics <sup>b</sup>	E/NE <sup>b</sup>	virion <sup>c</sup>	function <sup>b</sup>
<i>UL1</i>	gL	$\gamma$	E	Y	Modulates gH, fusion
<i>UL9</i>		$\beta$	E	N	Helicase/ATPase, DNA binding/synthesis
<i>UL10</i>	gM	$\gamma$ 2	NE	Y	Membrane domains, complexes with UL49.5
<i>UL12</i>		$\beta$	NE	N	Alkaline nuclease
<i>UL13</i>		$\gamma$	NE	Y	Tegument, protein kinase
<i>UL15</i>	terminase	$\gamma$	E	N	DNA cleavage and packaging
<i>UL16</i>		$\gamma$ 1	E	Y	Unknown
<i>UL17</i>		$\gamma$	NE	Y	Tegument, DNA cleavage and packaging
<i>UL18</i>	VP23	$\gamma$ 1	E	Y	Capsid
<i>UL19</i>	VP5/ICP5	$\gamma$	E	Y	Capsid
<i>UL21</i>		$\gamma$	NE	Y	Tegument, microtubule binding
<i>UL23</i>	TK	$\beta$	NE	Y	Thymidine kinase, neurovirulence
<i>UL25</i>		$\gamma$ 2	E	Y	Capsid
<i>UL27</i>	gB	$\gamma$ 1	E	Y	Major envelope glycoprotein, entry/fusion
<i>UL29</i>	ICP8	$\beta$	E	N	ssDNA binding
<i>UL30</i>		$\beta$	E	N	DNA polymerase
<i>UL31</i>		$\gamma$	E/NE	N	Nuclear phosphoprotein, binds lamins
<i>UL34</i>		$\gamma$ 1	E	N	Nuclear egress
<i>UL37</i>	ICP32	$\gamma$ 1	E	Y	Tegument
<i>UL38</i>	VP19C	$\gamma$ 2	E	Y	Capsid
<i>UL39</i>	ICP6/10	$\beta$	NE	N	Ribonucleotide reductase
<i>UL40</i>		$\beta$	U	N	Ribonucleotide reductase
<i>UL41</i>	vhs	$\gamma$ 1	NE	Y	Tegument, RNase, reduces host protein synthesis
<i>UL46</i>	VP11/12	$\gamma$ 1	NE	Y	Tegument
<i>UL47</i>	VP13/14	$\gamma$ 1	NE	Y	Tegument
<i>UL48</i>	VP16/ICP25	$\gamma$	E	Y	Tegument, pre-formed transactivator
<i>UL49</i>	VP22	$\gamma$ ?	NE/E	Y	Tegument

UL50		$\beta$	NE	Y	dUTPase
UL52		$\beta$	E	N	Helicase/primase subunit
UL53	gK	$\gamma$	NE	N	Virion exocytosis, syncytia
UL54	ICP27	$\alpha$	E	N	RNA processing
US1/1.5	ICP22	$\alpha$	NE	N	Cell cycle
US3		$\gamma$ 1	NE	Y	Serine threonine kinase
US6	gD	$\gamma$	E	Y	Major envelope glycoprotein, binding/entry
US7	gI	$\gamma$ 1	NE	Y	Component of viral Fc receptor
US8	gE	$\gamma$ 1	NE	Y	Component of viral Fc receptor
US9		$\gamma$	NE	Y	Unknown
RL2	ICP0	$\alpha$	NE	Y	E3 ligase, IFN evasion, multiple functions
RL1	ICP $\gamma$ fs34.5	$\gamma$ 1	NE	Y	Evasion of type I IFN response
RS1	ICP4	$\alpha$	E	Y	Repressor/transactivator

<sup>a</sup> Gene and protein names from [1] and Genbank NC\_001806. Not all gene products have separate names.

<sup>b</sup> Expression kinetics (pre- $\alpha$ =hypothetical synthesis during latency,  $\alpha$ =immediate early expression dependent on UL48 transactivation,  $\beta$ =early expression but after  $\alpha$  proteins,  $\gamma$ 1=some expression prior to DNA replication,  $\gamma$ 2=expression only after DNA replication,  $\gamma$ =differentiation into  $\gamma$ 1 or  $\gamma$ 2 not reported; requirement for replication *in vitro* (E=essential, NE=not essential, E/NE=cell type dependent, U=unknown); brief functional summary [1].

<sup>c</sup> Presence of absence in highly purified virions [2]; Y = yes, N= no.

## References: Supplementary Tables

- [1] Roizman B, Knipe, D.M., Whitley, R.J. Herpes simplex viruses. In: Knipe DM, Howley, P.M., editor. *Fields Virology*. Fifth ed. Philadelphia: Lippincott, Williams, and Wilkins, 2007: 2501-602.
- [2] Loret S, Guay G, Lippe R. Comprehensive characterization of extracellular herpes simplex virus type 1 virions. *J Virol* 2008 Sep;82(17):8605-18.