Supplementary Materials and Methods

Cells and cell culture. All cell lines were maintained at 37°C, 5% CO_2 in a humidified incubator according to the guidelines provided by the vendors. Adult retinal pigment epithelial cells (ARPE-19) were obtained from American Type Culture Collection (ATCC) (CRL-2302) and propagated in Dulbecco's Modified Eagle Medium (DMEM)/F-12 supplemented with 10% fetal bovine serum (FBS) and 1× penicillin/streptomycin/glutamine. Neo-natal normal human dermal fibroblast cells (NHDF) were purchased from ATCC (PCS-201-010) and propagated in DMEM supplemented with 10% FBS and 1× penicillin/streptomycin. HepG2 and Huh7 cells are human hepatocellular carcinoma-derived cell lines that were obtained from ATCC (HB-8065) and Japanese Collection of Research Bioresources (JCRB; JCRB0403), respectively. Human foreskin fibroblast cells (HFF-1) were obtained from ATCC (SCRC-1041) and grown in DMEM supplemented with 15% FBS, 4 mM GlutaMax, and 1× penicillin/streptomycin. HepG2 cells were passaged in Eagle's Minimal Essential Medium (EMEM) with 10% FBS, 1× penicillin-streptomycin, and 2 mM L-glutamine (optional); Huh7 cells were grown in DMEM with 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, and 10% FBS. MT-4 cells are a human T cell suspension line that was obtained from JCRB (JCRB0135); they were passaged in RPMI 1640, 10% FBS, 1× penicillin-streptomycin, and 2 mM L-glutamine (optional).

For routine maintenance of adherent cells, monolayers at 90-95% confluence were rinsed gently with 10 mL Dulbecco's phosphate buffered saline (DPBS) and detached by incubation with 3 mL trypsin (0.25%) at 37°C, 5% CO_2 for 2-5 min. Cells were resuspended in 10 mL medium and an appropriate volume was then transferred to a fresh T150 flask with additional fresh media as necessary. Cells were split by 1:3 to 1:5 dilution factors once a week with media exchange three to four days after cells were put to a fresh flask. For routine maintenance of MT-4 suspension cells, cultures were gently pipetted up-and-down three times to ensure dispersal of clumps. Cells were seeded into a new T75 flask with fresh medium to a

final density of 2×10^5 cells/mL in 30 mL. MT-4 cells were split every three or four days and discarded after 20 passages.

Primary cell lines were maintained in the media suggested by the manufacturers, supplemented with 1× penicillin/streptomycin. Uterine micro vascular endothelial cells were obtained from Lonza (CC-2564); renal proximal tubule epithelial (RPTE) cells (FC-0013), human coronary artery endothelial cells (FC-0032), and human umbilical vein endothelial cells (HUVEC) (FC-0044) were purchased from Lifeline Cell Technologies; placental epithelial cells (C-12662), placental fibroblast cells (C-12390), renal cortical epithelial cells (C-12662), and renal medullary epithelial cells (C-12662) were purchased from Promocell.

Cloning of gB and UL130 mutations in HCMV strain AD169. To generate HCMV expressing gB and UL130 mutations, Bacterial Artificial Chromosome (BAC) recombination technology was employed (1) using the HCMV AD169 genome cloned into a self-excising BAC (pAD/Cre—a generous gift from the laboratory of Thomas Shenk) (2). In the first step, pAD/Cre was mutagenized via homologous recombination in the bacterial strain SW102 (NIH). The GalK gene (GalK plasmid obtained from the NIH) was inserted in place of the sequence of interest, followed by selection on minimal medium with galactose. The GalK gene was then replaced by the mutagenized gB or UL130 sequence, with selection on the toxic galactose analog 2-d-deoxy-galactose. Recombinant BAC DNA was verified by restriction digest and sequencing of the region of interest. To reconstitute the virus, mutagenized pAD/Cre was electroporated into normal human dermal fibroblast NHDF cells. The following BAC-derived HCMV mutants were generated: UL130 Q191K, gB E361K, gB D362N, gB E361KD362N, and a gB E381 deletion. Primer sequences are available upon request.

HCMV titering. HCMV was titered on ARPE-19 or NHDF cells that were seeded the day prior to the assay (1×10^4 cells per well, 100uL growth medium) in a tissue culture treated 96-well plate. On the day of infection, monolayers (80 – 90% confluent) were rinsed once with 100 µL serum-

free medium (DMEM/F12 for ARPE-19 and DMEM for NHDF cells) and inoculated in triplicate with 50 µL serially diluted virus. Cells were incubated for three hours at 37°C, 5% CO_2 followed by removal of the inoculum and rinsing twice with 100 µL serum-free medium. Finally, growth medium (100 µL per well) was added and plates were incubated for 18 hours at 37°C, 5% CO_2. Infection was detected by immunofluorescence staining of HCMV immediate early (IE) 1/2 proteins and quantified using the Cellomics ArrayScan® VTI HCS reader as described below.

Recombinant adenovirus and transduction. Recombinant adenoviruses were generated encoding individual HCMV glycoproteins (strain VR1814) known to be important for entry (gB, gH, gL, UL128, UL130, UL131a). Each viral gene was subcloned into the Clontech Adeno-X expression vector, which was subsequently linearized by PacI and transfected into HEK293 cells using Fugene6 (Roche) according to the manufacturer's instructions. Recombinant virus was harvested according to the Clontech Adeno-X expression System 1 kit instructions. Adenovirus stocks were produced by infecting (MOI of 5) HEK293 cells for five days, followed by cell lysis using three freeze thaw cycles to collect the virus. To transduce ARPE-19 cells, monolayers (80 – 90% confluent) in T25 flasks were washed once with 2 mL of serum-free DMEM/F-12 followed by infection with recombinant adenovirus (MOI of 25, 50, 75, and 100) in 2 mL serum-free DMEM/F-12. Cells were incubated at 37°C, 5% CO_2 for 1 hour and before the addition of 3 mL growth medium, without removing the inoculum. Cells were then incubated at 37°C, 5% CO_2 overnight.

Syncytia formation assay. ARPE-19 cells (1.2×10^4 cells/well) in a 96-well plate were infected with recombinant adenovirus (MOI of 100) in 50 µL serum-free DMEM/F12. Two hours post-infection, 50 µL of a 10-fold serial dilution of antibody prepared in growth medium was added to each well to give final antibody concentrations of 100, 10, 1, 0.1, 0.01, and 0.001 µg/mL. Cells were incubated for 30 or 48 hours to allow for syncytia formation. Medium was then removed and cells were fixed with 50 µL 4% paraformaldehyde (PFA) for 15 min at room temperature.

Cells were washed twice with PBS and permeabilized for 15 min at room temperature with 50 µL 0.1% Triton X-100. Fifty microliters DAPI (1:5000 in PBS) were added for 30 min at room temperature in the dark, followed by 50 µL Cellmask stain red (Life Technologies) (1:5000 in PBS) with incubation for an additional 45 minutes. Finally the cells were washed twice with 50 µL PBS. The final wash was left on the cells and the plates were imaged on Cellomics ArrayScan® VTI HCS reader as described below.

Immunofluorescence. Immunofluorescence detection of HCMV immediate early (IE) 1/2 or gB proteins was used to monitor infection. For IE1/2, growth medium was removed at ~18 hours post-infection (hpi) (or up to 48 hpi for low titer clinical isolates) and cells washed twice with 100 µL 1× PBS. Cells were fixed with 100 µL/well of 4% PFA for 15 min at room temperature followed by washing three times with 100 µL/well 1× PBS. Primary antibody (50 µL/well of mouse anti-HCMV IE 1/2 antibody (EMD Millipore, mab8131) diluted 1:3000 in PBS containing 0.2% gelatin (PBS-GC) and 0.1% Triton X-100) was added and incubated at room temperature for 1 hour. Cells were washed three times and secondary antibody was added (50 µL/well of 1:3000 anti-mouse Alexa Fluor 594 antibody [Life Technologies] and 1:5000 DAPI diluted in PBS-GC containing 0.1% Triton X-100). Plates were incubated at room temperature for 1 hour, after which cells were washed three times and stored at 4°C in 1× PBS until ready for high content image analysis.

For gB, growth medium was aspirated and cells were fixed at room temperature for 40 min with 1 mL methanol per well followed by three washes with 2 mL 1X PBS. Cells were permeabilized and blocked for 20 min at room temperature with 1 mL DMEM/F12 containing 2% FBS and 0.5% Triton X-100 (blocking/permeabilization (BP) buffer) and then incubated overnight at 4° C with 500 µL per well anti-HCMV gB antibody (US Biological, C9100-21B,1:500 diluted in BP buffer), followed by three washes with 1X PBS. Cells were then incubated for 2 hrs at room temperature with 500 µL per well goat anti-mouse AP conjugate secondary antibody

(KPL, 05-18-06, 1:250 diluted in BP buffer) followed by three washes with 1X PBS. Subsequently, 0.5 mL per well BCIP/NBT solution (KPL, 50-81-18) was added and plates were incubated at RT for 15 min followed by three washes with ddH_2O. Plates were dried for 2 hours at room temperature and imaged on Gel-Doc XR+ with default setting for ethidium bromide.

High content imaging and analysis. HCMV infection was quantified by high content image analysis using the Cellomics ArrayScan®VTI HCS Reader. In this method, detection of the DAPI signal in Channel 1 was used to identify at least 1000 cells per well. Imaging of Alexa Fluor 594-labeled HCMV IE 1/2 protein in Channel 2 then indicated the proportion of cells that were infected.

For neutralization assays, the rate of infection in the absence of antibody was calculated from the average of three to twelve replicates. Infection in the presence of antibody was then normalized to that of the untreated virus. To calculate EC_{50} values, normalized infectivity was plotted against antibody concentration and analyzed with the four parameter logistic equation: y = (A+((B-A)/(1+((C/x)^D)))) where A is the minimum y value, B is the maximum y value, C is the logEC_{50} value, and D is the slope factor; EC_{90} values were calculated similarly.

For syncytia analysis, five fields per well were captured at 10× object magnification. Entire cell bodies were first detected using Cellmask stain red and syncytia were then defined as objects that had a size greater than 350 μm^2 and more than 3 nuclei as determined by DAPI staining. Rate of syncytia for a given field was determined as (number of nuclei present in fused cell bodies)/ (total number of nuclei [DAPI stain in channel 2]).

Cytotoxicity Assay. Cytotoxicity was monitored using proliferating (50% confluent on the day of the assay) or stationary-phase (90% confluent on a day of assay) cells in 96-well plates. Puromycin was used as a positive control for cytotoxicity. Growth medium from adherent cells was removed and diluted antibody or puromycin was added. For MT-4 cells, which grow in

suspension, diluted antibody or puromycin was added directly to the cells without removing the medium. LJP538, LJP539 and puromycin were tested in triplicate using 12-point serial dilutions starting at 500, 50 and 75 µg/mL, respectively. LJP538 and LJP539 were also tested in combination starting at top concentrations of 500 µg/mL and 50 µg/mL, respectively. The total volume was adjusted to 100 µL per well with assay medium and plates were incubated for 48 hours (ARPE-19 cells) or 72 hours (all other cells types) at 37°C, 5% CO_2. Cytotoxicity was quantified using the Cell Titer-Glo® Luminescent Cell Viability Assay system (Promega) following the manufacturer's instructions and relative luminescence units (RLU) were measured using a microplate reader (Omega POLARstar, BMG Labtech). CC_{50} values were calculated as EC_{50} above.

Synergy Analysis. Synergy analysis was conducted and interpreted using MacSynergy II(3). Synergy volumes were calculated from eight replicates for each cell line tested. Synergy volumes at the 95% confidence level were reported.

Measurement of ADCC. Antibody-dependent cell-mediated cytotoxicity (ADCC) was tested in ARPE-19 cells, which were plated in 96-well plates (at 1×10^4 cells per well) the day before infection. Cells were infected with HCMV strain VR1814 (MOI of 0.05, 0.1 and 0.5) in serum free DMEM/F-12 and incubated for 3 hours at 37°C before replacement with growth medium. Cells were incubated at 37°C for 72 hours. Infected cells were washed with serum free Phenol Red Free-RPMI-1640 (PRF-RPMI) and then treated for 30 min at room temperature with antibody diluted in the same medium. Cytotect® (175 µg/mL), LJP538 (12.0 µg/mL) and LJP539 (0.07 µg/mL) antibodies were used at ~10× EC_{90} concentrations. Cetuximab and IgG1 isotype control (human anti-chicken lysozyme) antibodies were used at 100 µg/mL. Cytotoxicity in the presence of peripheral blood mononuclear cells (PBMC, isolated from fresh HCMV-seronegative human blood acquired from AllCells, LLC using Ficoll-Paque Premium [GE Healthcare] gradient according to the manufacturer's instructions) was measured by lactate dehydrogenase (LDH)

release. Infected, antibody-treated ARPE-19 cells were washed with serum free PRF-RPMI and co-cultured with PBMC diluted in the same medium (final PBMC to ARPE-19 cells ratio of 100:1) for 4 hours at 37°C. Thirty minutes before the end of co-incubation, 4% Triton X-100 was added to lyse cells in six wells per plate; an additional six wells per plate were left with medium alone. After four hours, cell culture supernatants from all wells were transferred to 96-well, U-bottom plates, followed by centrifugation at 280 ×g for 10 min at room temperature to pellet cells and debris. Cleared supernatant was used to quantify LDH using the LDH Cytotoxicity Detection Kit (Roche) according to manufacturer recommendations where background-corrected optical densities were used to determine LDH release as a measurement of cytotoxicity. Cytotoxicity was calculated as ($OD_{\text{test sample}} - OD_{\text{media only}}$)/($OD_{\text{triton lysed cells}} - OD_{\text{media only}}$), and relative cytotoxicity was determined as (Cytotoxicity of Sample – Cytotoxicity of Isotype Control)/Cytotoxicity of Cetuximab.

Generation of HCMV with reduced susceptibility to ganciclovir. HCMV with reduced susceptibility to ganciclovir (GCV) was generated by infecting cells in the presence of increasing concentrations of the drug. Briefly, HFF-1 cells were washed with serum free media and infected with AD169 (MOI of 0.01) in the presence of 2.5 µM ganciclovir (~2.5× EC_{50}) for 3 hrs. The inoculum was then removed and replaced with growth media in the presence of drug. After complete CPE was observed (2-3 weeks), cells and supernatant were harvested by scraping and the virus was titered. Further rounds of infection were performed in the presence of 5 µM, 10 µM, and 20 µM ganciclovir using the same procedure. The virus harvested after 20 µM ganciclovir selection was titered and tested for susceptibility to the drug.

Quantitative (q)PCR replication assay. Nine-thousand NHDF cells per well were seeded in a 96 well-plate and infected at MOI of 1 with wild-type or resistant virus in presence of serial dilutions of GCV. At 3 days post infection (dpi) supernatant was removed, cells washed once with sterile PBS and cell lysates prepared using prepGEM tissue (ZyGEM, USA) according to

manufacturer's guidelines. HCMV DNA replication in infected cells was measured using QuantiFast PCR kit (Qiagen, USA) according to manufacturer's guidelines using primers and probe specific to the immediate early (IE) region of the virus. Data were normalized to cellular DNA detected using primers and probe specific to aspartoacylase (ACY) housekeeping gene.

Sequencing of HCMV glycoproteins. Viral genomic DNA was isolated using the QIAamp DNA Mini Kit (Qiagen) according to manufacturer's protocol. gB, gH, gL, UL128, UL130, and UL131a open reading frames were then PCR amplified using the Expand High Fidelity PCR System (Roche) and C1000 Touch Thermal Cycler (Bio-Rad) and gel purified using QIAquick PCR Purification Kit (Qiagen) according to manufacturers' protocols. Sanger sequencing was carried out by ELIM biopharmaceuticals. PCR and sequencing primers are available upon request. Sequence alignments were performed using Align X in Vector NTI 11.5 (Invitrogen). *In silico* restriction fragment length polymorphism (RFLP) analyses of the gB cleavage region were performed using Vector NTI 11.5 to determine gB genotypes.

Virus purification by sorbitol pelleting centrifugation. To harvest virus from cells showing complete CPE, the side of the flask was tapped to loosen the monolayer and supernatant and cells were collected in 50 mL conical tubes. The mixture was sonicated on high (169 watts) for 5 min in an ice-water bath and centrifuged at 1000 ×g, 4°C for 15 min to pellet cell debris. The supernatant was carefully layered on top of 7-10 mL sterile 20% sorbitol in 1× Tris buffered saline in a sterile 38.5 mL polyallomer tube. The tube was then filled to 37.5 mL with serum free-medium and centrifuged for 1 hour at 4°C at 43000 ×g in Sorval SW32Ti rotor. After centrifugation, the supernatant was discarded and the pellet was resuspended in appropriate volume (5-10 mL) of serum free-medium, aliquoted and stored at -80 °C.

Protein purification for electron microscopy assay. HCMV gB (spanning residues 88-698; missing the N-terminal flexible region and the C-terminal transmembrane and cytoplasmic domains) was codon-optimized for expression in mammalian cells and subcloned into the

eukaryotic expression vector pCMVKm2 (4). This construct along with LJP538 Fab were transfected into 293EBNA cells (0.75×10^6 cells/mL) for transient expression. Proteins were affinity purified using NiNTA or Strep-Tactin Superflow Plus resin (Qiagen, Valencia, CA) and eluted in 25 mM Tris pH 7.6, 150 mM NaCl, with 250 mM imidazole or 5 mM desthiobiotin. The proteins were immediately subjected to size exclusion chromatography (SEC) on a calibrated Superose 6 PC 3.2/30 column (GE Healthcare, Uppsala, Sweden) equilibrated in elution buffer. To obtain antibody-protein complexes, individual Fabs and gB were incubated with a 1.5:1 molar ratio for 2 h on ice and purified by SEC as described above.

Electron microscopy data collection and data processing. EM grids were prepared by depositing a thin layer of continuous carbon over a holey carbon layer on a 400-mesh copper grid (Electron Microscopy Sciences). Five microliters of purified sample (approximately 50 ng) were pipetted onto a freshly glow discharged grid. After 30 s of incubation, the grid was stirred gently for five subsequent 10-s staining steps over 75 µL drops of a freshly prepared 2% (w/v) uranyl formate solution. Samples were imaged using a Tecnai F20 transmission electron microscope operating at 120 keV at a nominal magnification of 68,000× (3.14 Å/pixel at the detector level) using a defocus range of −0.8 to −1.2 µm. Images were recorded under low-dose conditions on a Gatan 4096 × 4096 pixel CCD camera. Particle picking was done semi-automatically using e2boxer (EMAN2, (5)). A 288 × 288-pixel particle window was used for all datasets. For reference free 2D classification, all datasets were band-pass filtered with a 200-Å high-pass cutoff and either a 20-Å low-pass cutoff. Iterative multivariate statistical analysis (MSA) and multi-reference alignment (MRA) in Imagic (6) of the extracted particles provided representative 2D views of the HCMV complexes. On average, ~20 particles were included per class average. To determine the negative staining structure of gB-LJP538 we collected a data set of ~15,000 particles, extracted using a box size of 288 × 288 pixels, decimated by a factor of 2 and normalized and phase flipped. Iterative projection matching was performed using libraries from the SPARX and EMAN2 image processing packages (5). During the projection matching,

a model of postfusion glycoprotein B, obtained from HSV gB crystal structure and low–pass filtered to 80Å, was used as a starting model to allow sorting of the particles and Euler angle assignment. Angular increments for projection matching started at 25 degrees and were reduced stepwise to 6 degrees. The model was refined respectively to ~15 Å resolution according to the 0.5 cut–off in the FSC curve. A clear additional density coming from the bound antibody Fab fragments was observed. Comparative models for HCMV gB and Fabs were obtained using the Modeler Software Package (7) using as starting model HSV glycoprotein B structure (PDB 2GUM) and the neutralizing Fab SM5 structure (PDB 4OSU). The obtained models were docked into the density using the tool “Fit in Map” in Chimera with default parameters (8).

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Supplemental Results

Supplemental Table 1. Neutralization of geographically and temporally distinct HCMV isolates on ARPE-19 cells

HCMV isolate	Origin	Year of isolation	EC_{50} (std dev) in µg/mL			EC_{90} (std dev) in µg/mL		
			LJP538	LJP539	Cytotect	LJP538	LJP539	Cytotect
8816	Massachusetts, USA	2006	0.08 (0.04)	0.0011 (0.0007)	4.8 (3.9)	0.16 (0.03)	0.0037 (0.0030)	15.0 (6.8)
8817	Massachusetts, USA	2006	0.23 (0.04)	0.0025 (0.0003)	12.3 (13.6)	0.37 (0.12)	0.0114 (0.0105)	17.7 (14.4)
8818	Massachusetts, USA	2006	1.06 (0.51)	0.0183 (0.0133)	24.4 (15.5)	1.44 (0.59)	0.0225 (0.0132)	39.7 (19.8)
8819	Massachusetts, USA	2007	0.23 (0.07)	0.0025 (0.0019)	3.1 (1.9)	0.53 (0.21)	0.0114 (0.0080)	22.7 (15.4)
8821	Massachusetts, USA	2007	0.42 (0.19)	0.0039 (0.0021)	5.0 (3.3)	0.75 (0.23)	0.0107 (0.0080)	13.7 (7.4)
8822	Massachusetts, USA	2007	0.30 (0.15)	0.0005 (0.0003)	4.5 (1.7)	0.70 (0.36)	0.0406 (0.0255)	25.4 (16.4)
8824	Massachusetts, USA	2007	0.11 (0.04)	0.0112 (0.0127)	20.6 (16.2)	0.25 (0.13)	0.0167 (0.0118)	38.4 (18.8)
MP-LW-1802	Alabama, USA	2013	0.15 (0.06)	0.0013 (0.0014)	20.6 (13.3)	0.90 (0.69)	0.0113 (0.0019)	29.7 (14.0)
MP-LTN-101	Alabama, USA	2013	0.07 (0.07)	0.0007 (0.0006)	8.2 (9.1)	0.26 (0.24)	0.0215 (0.0057)	11.2 (11.3)
TR	California, USA	1998	0.73 (0.59)	0.0054 (0.0029)	18.7 (13.9)	1.61 (1.00)	0.0114 (0.0054)	135.8 (157.6)
TM-E5271	Germany	2000	0.13 (0.05)	0.0026 (0.0007)	12.6 (12.9)	0.21 (0.03)	0.0070 (0.0054)	20.2 (17.4)
TM-E20744	Germany	2012	0.18 (0.11)	0.0034 (0.0010)	12.0 (14.3)	0.42 (0.16)	0.0113 (0.0050)	69.0 (73.9)
TM-E28175	Germany	2003	0.08 (0.09)	0.0009 (0.0009)	10.7 (14.8)	0.40 (0.19)	0.0055 (0.0029)	34.0 (11.4)
TM-E19664	Germany	2002	0.25 (0.03)	0.0018 (0.0009)	11.1 (11.7)	0.52 (0.20)	0.0202 (0.0221)	17.7 (11.2)
TM-31354	Germany	2005	1.00 (0.44)	0.0078 (0.0030)	13.5 (8.2)	1.47 (0.52)	0.0128 (0.0023)	21.0 (9.3)
TM-E9361	Germany	2001	0.28 (0.04)	0.0019 (0.0011)	11.8 (15.0)	0.50 (0.11)	0.0258 (0.0330)	14.8 (16.6)
TM-E28257	Germany	2011	0.21 (0.02)	0.0011 (0.0010)	11.2 (12.8)	0.33 (0.02)	0.0211 (0.0104)	15.8 (15.7)
TM-E14953	Germany	2002	0.20 (0.13)	0.0020 (0.0006)	26.2 (2.5)	0.38 (0.18)	0.0086 (0.0068)	31.9 (2.9)
TM-20749	Germany	1999	0.21 (0.02)	0.0007 (0.0005)	3.6 (0.4)	0.35 (0.02)	0.0335 (0.0165)	8.0 (3.4)
VR1814	Italy	2001	0.85 (0.32)	0.0024 (0.0009)	4.0 (1.8)	1.39 (0.33)	0.0102 (0.0079)	8.6 (2.0)

Data are an average of three experiments with triplicates samples for each experiment.

Supplemental Table 2. Neutralization of geographically and temporally distinct HCMV isolates on HUVEC cells

HCMV isolate	Origin	Year of isolation	EC_{50} (std dev) in µg/mL			EC_{90} (std dev) in µg/mL		
			LJP538	LJP539	Cytotect	LJP538	LJP539	Cytotect
MP-LW-1802	Alabama, USA	2013	0.16 (0.10)	0.0021 (0.0003)	3.6 (0.7)	0.83 (0.53)	0.0121 (0.0028)	35.3 (23.4)
MP-LTN-101	Alabama, USA	2013	0.15 (0.08)	0.0023 (0.0005)	8.7 (3.5)	0.39 (0.16)	0.0061 (0.0012)	44.5 (30.7)
TR	California, USA	1998	0.22 (0.03)	0.0017 (0.0011)	3.8 (1.9)	1.09 (1.29)	0.0288 (0.0234)	13.9 (6.8)
TM-E9361	Germany	2001	0.28 (0.05)	0.0020 (0.0002)	6.7 (2.3)	0.44 (0.14)	0.0123 (0.0041)	32.5 (25.3)
TM-E28257	Germany	2011	0.11 (0.08)	0.0015 (0.0004)	7.7 (4.4)	0.46 (0.13)	0.0120 (0.0044)	21.1 (2.7)
TM-E14953	Germany	2002	0.14 (0.06)	0.0023 (0.0006)	5.1 (2.4)	0.31 (0.06)	0.0109 (0.0019)	14.2 (10.0)
TM-20749	Germany	1999	0.36 (0.11)	0.0017 (0.0011)	14.8 (16.1)	0.96 (0.48)	0.0123 (0.0044)	26.8 (13.9)
TM-E34165*	Germany	2011	0.11 (0.02)	0.0072 (0.0092)	5.5 (3.3)	2.15 (1.58)	0.0499 (0.0478)	8.5 (4.2)
TM-31354	Germany	2005	0.20 (0.05)	0.0016 (0.0009)	13.3 (9.9)	0.36 (0.11)	0.0145 (0.0060)	27.9 (7.3)
TM-E5271*	Germany	2000	0.12 (0.12)	0.0018 (0.0016)	17.4 (20.9)	0.18 (0.11)	0.0219 (0.0086)	25.1 (30.0)
TM-E20744	Germany	2012	0.11 (0.08)	0.0026 (0.0017)	12.5 (12.9)	1.39 (1.73)	0.0068 (0.0014)	21.1 (14.3)
TM-E19664	Germany	2002	0.21 (0.03)	0.0056 (0.0072)	4.1 (0.9)	1.31 (1.76)	0.0262 (0.0210)	15.6 (7.9)
VR1814	Italy	2001	0.29 (0.06)	0.0017 (0.0008)	6.3 (2.9)	1.02 (0.75)	0.0244 (0.0214)	16.0 (8.9)

Data are an average of three experiments with triplicates samples for each experiment, except for isolates noted with an asterisk (*), which are an average of two experiments with triplicate samples.

Supplemental Table 3. Neutralization of geographically and temporally distinct HCMV isolates on NHDF cells

			EC_{50} (std dev) in µg/mL		EC_{90} (std dev) in µg/mL	
HCMV isolate	Origin	Year of isolation	LJP538	Cytotect	LJP538	Cytotect
8816	Massachusetts, USA	2006	0.13 (0.03)	17.0 (5.3)	0.45 (0.44)	672.4 (321.3)
8817	Massachusetts, USA	2006	0.37 (0.17)	48.3 (57.6)	1.26 (0.20)	430.6 (153.4)
8818	Massachusetts, USA	2006	1.25 (0.53)	136.5 (141.2)	2.68 (0.26)	418.0 (401.9)
8819	Massachusetts, USA	2007	0.61 (0.14)	98.8 (128.1)	3.10 (1.57)	268.2 (302.8)
8821	Massachusetts, USA	2007	0.51 (0.26)	73.1 (27.8)	2.06 (1.15)	315.3 (214.6)
8822	Massachusetts, USA	2007	0.71 (0.31)	57.1 (34.5)	4.72 (1.24)	882.5 (482.0)
8824	Massachusetts, USA	2007	0.20 (0.06)	38.7 (33.4)	0.59 (0.07)	266.2 (51.3)
MP-LW-1802	Alabama, USA	2013	2.44 (0.71)	1153.8 (388.3)	8.45 (1.81)	12686.0 (8487.1)
MP-LTN-101	Alabama, USA	2013	0.17 (0.05)	21.0 (14.9)	1.10 (1.37)	95.5 (41.7)
TR	California, USA	1998	1.27 (0.59)	90.9 (60.3)	4.38 (1.99)	455.6 (640.7)
TM-E5271	Germany	2000	0.05 (0.03)	10.6 (4.0)	1.15 (0.78)	138.2 (91.4)
TM-E20744	Germany	2012	0.23 (0.07)	44.8 (14.7)	1.02 (0.16)	891.9 (1024.8)
TM-E28175	Germany	2003	0.28 (0.03)	129.2 (151.2)	0.80 (0.13)	400.5 (356.5)
TM-E19664	Germany	2002	0.31 (0.09)	41.7 (46.4)	1.10 (0.61)	296.0 (111.1)
TM-31354	Germany	2005	1.29 (0.30)	159.5 (124.4)	3.24 (1.52)	586.8 (463.1)
TM-E9361	Germany	2001	0.12 (0.09)	12.1 (8.2)	15.22 (18.83)	266.4 (257.4)
TM-E28257	Germany	2011	0.28 (0.09)	44.5 (19.3)	0.91 (0.94)	337.9 (243.3)
TM-E14953	Germany	2002	0.14 (0.08)	22.4 (16.5)	1.66 (1.74)	118.9 (49.3)
TM-20749	Germany	1999	0.33 (0.07)	48.2 (15.5)	1.38 (1.54)	514.4 (183.5)
VR1814	Italy	2001	1.34 (0.69)	181.6 (202.0)	7.27 (3.35)	785.9 (650.8)

Data are an average of three experiments with triplicates samples for each experiment.

Supplemental Table 4. LJP538 and LJP539 are not cytotoxic

	CC_{50} (µg/mL)			
Cell type	**LJP538**	**LJP539**	**(LJP538+LJP539)**	**Puromycin CC_{50} (standard deviation)**
Proliferating cells				
ARPE-19	> 500	> 50	> (500 + 50)	0.105 (0.064)
NHDF	> 500	> 50	> (500 + 50)	0.656 (0.688)
RPTE	> 500	> 50	> (500 + 50)	0.845 (1.039)
HepG2	> 500	> 50	> (500 + 50)	2.053 (0.199)
MT-4	> 500	> 50	> (500 + 50)	0.767 (1.105)
Huh7	> 500	> 50	> (500 + 50)	0.287 (0.445)
Stationary cells				
ARPE-19	> 500	> 50	> (500 + 50)	0.400 (0.014)
NHDF	> 500	> 50	> (500 + 50)	1.766 (1.832)
RPTE	> 500	> 50	> (500 + 50)	0.270 (0.050)
HepG2	> 500	> 50	> (500 + 50)	1.077 (0.772)
MT-4	> 500	> 50	> (500 + 50)	1.387 (1.089)
Huh7	> 500	> 50	> (500 + 50)	2.373 (0.104)

Supplemental Table 5. Absence of cross-resistance between LJP538, LJP539 and ganciclovir

Activity of ganciclovir against HCMV with reduced sensitivity to LJP538 and LJP539			
Antibody used for selection	Cell type for viral selection	Ganciclovir EC_{50}-fold shift [a]	
		NHDF cells	ARPE-19 cells
LJP538	NHDF	1.73	0.68
LJP538	ARPE-19	0.87	1.16
LJP539	ARPE-19	0.66	1.22
Activity of antibodies against HCMV with reduced sensitivity to ganciclovir in NHDF cells			
		EC_{50} (µM) (SD) [b]	
Virus		LJP538	Cytotect®
Wild type AD169		0.805 (0.15)	852.97 (337.62)
Ganciclovir resistant		0.830 (0.25)	680.765 (538.4)

[a] Values shown in the table are expressed as EC_{50} fold shift over wtVR1418 EC_{50} in qPCR assay. Data are the average of two independent experiments, with two replicates per experiment.
[b] EC_{50} values are averages of two independent virus neutralization experiments with three replicates each.
SD, standard deviation.

Supplemental Table 6. Reduced susceptibility to LJP538 is not acquired in a single passage in fibroblast cells

Virus	Antibody used during selection	Virus titer (IE positive cells/mL)[a]	Neutralization by MSL-109[b]	
			EC_{50} (µg/mL)	EC_{90} (µg/mL)
wtVR1814	-	7.75×10^5	0.97	15.93
wtVR1814	MSL-109	7.84×10^4	>50	>50
wtVR1814	LJP538	6×10^2	ND	ND

[a] Virus is titered in the absence of antibody. [b] EC_{50} and EC_{90} values of wtVR1814 and MSL-109^R (f) viruses. ND, not done

Supplemental Table 7. Potency of MSL-109 on wild type and passaged HCMV

	Neutralization by MSL-109 (NHDF cells)	
Virus	EC_{50} (µg/mL)	EC_{90} (µg/mL)
MSL-109^R (f)	> 50	> 50
MSL-109^R (f), single passage without Ab	0.57	8.85

EC_{50} and EC_{90} values of wtVR1814 and MSL109^R(f) that had been grown without MSL-109.