OR14I1 is a receptor for the human cytomegalovirus pentameric complex and defines viral epithelial cell tropism

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Supplementary Materials and Methods.

Cell Lines. ARPE-19 epithelial cells (ATCC, Manassas, VA, USA) were grown in DMEM-F12 (Gibco) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Life Technologies). Human embryonic lung fibroblasts (HEL), A549 epithelial cells, and HEK293T cells (all from ATCC) were grown in DMEM (Gibco) supplemented with 10% FBS and 1% penicillin-streptomycin (Life Technologies). H1HeLa cells (ATCC) were grown in minimal essential medium (MEM, Gibco) supplemented with 10% fetal calf serum (FCS), 1% penicillin-streptomycin, and 2 mM L-glutamine. Human embryonic lung fibroblasts (MRC5) (ATCC) were cultured at 37 °C in DMEM supplemented with 10% FBS, 10 mM HEPES, 1 mM sodium pyruvate, 2 mM L-Glutamine, 0.1 mM Non-essential amino acids, 100 U/ml penicillin and 100 µg/ml streptomycin. Sf9 insect cells (ATCC) were cultured in suspension in a rotary shaker at 27ºC in Sf-900^{TM} II SFM medium (ThermoFisher).

CRISPR Screens. ARPE-19-Cas9 cells expressing the GeCKO v.2 library were infected with TB40E-GFP (MOI=5.0). In a parallel screen, HEL-Cas9 fibroblasts expressing the GeCKO v2 library were infected with AD169 (MOI=5.0). GFP signal and cytopathic effect were monitored using microscopy. Control plates were run in parallel using the respective parent cell populations, which did not contain the GeCKO v.2 library. When >95% of the respective control cells had died, the surviving cells were washed to remove debris, re-plated and allowed to expand. These expanded cells underwent a second round of infection with TB40E-GFP (ARPE-19 cells) or AD169 (HEL fibroblasts). The surviving cells were expanded and genomic DNA prepared. The host genome integrated sgRNAs were amplified from genomic DNA using PCR and the amplicons subjected to NGS sequencing using an Ion Proton Sequencer (see below).

sgRNA Sequencing and Analysis. Genomic DNA was prepared (DNeasy Blood & Tissue Kit, QIAGEN) and PCR performed using Herculase II Phusion DNA polymerase (Agilent), and primers flanking the sgRNA:

lentiGP-1_F: 5'-AATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCG-3'
lentiGP-3_R: 5'-ATGAATACTGCCATTTGTCTCAAGATCTAGTTACGC-3'.

The gel-purified PCR product was 5'-phosphorylated and ligated to adapters and barcodes, gel-purified and NGS was performed on an Ion Proton Sequencing platform (Life Technologies). The FASTQ files were trimmed using the Cutadapt (https://cutadapt.readthedocs.io/en/stable/guide.html) program, and then mapped to the sgRNA library key using the Bowtie2 program. Read counts for each sgRNA were calculated using Samtools (http://samtools.sourceforge.net/) and custom Matlab scripts. Selected candidate genes were chosen for further study if they had ≥20 reads per sgRNA across ≥3 independent sgRNAs.

ARPE-19$^{-/-}$ and HEL$^{-/-}$ Cells. The following sgRNA sequences were cloned into LentiGuide-Puro (Addgene, Plasmid #84752):

GACCTTCAATGGACTTACCC (sg*PDGFRA_1*; SEQ ID: 5156),
AGCTATGGGGACTTCCCATC (sg*PDGFRA_2*; SEQ ID: 5156),
TCCTACGCAGCCGTCCACAC (sg*OR14I1_1*; SEQ ID: 401994),
TGAGCACCGTTGAGAAGATT (sg*OR14I1_2*; SEQ ID: 401994),
CGGGATGCAGCTGGAGAGGA (sg*CON*_1; SEQ ID: CP020802.1),

CCAGTTGCTCTGGGGGAACA (sg*CON*_2; SEQ ID: CP020782.1).

ARPE-19-Cas9 or HEL-Cas9 fibroblasts were transduced with lentiviruses and selected with puromycin (2.5μg/ml). Clonal *OR14I1*$^{-/-}$ cells were generated by serial dilution. DNA was isolated from cells representing clones and both alleles were confirmed as successfully mutated by sequencing.

ARPE-19 and HEL-shRNA Cells. shRNAs were cloned into the pLKO.1-blast lentiviral vector (Addgene) followed by viral packaging. The lentiviruses were used to transduce the ARPE-19 or HEL fibroblasts followed by selection with blasticidin (Life Technologies, 10μg/ml).
shRNA sequences:

shOR14I1: GCAGAAGCTCCATCTCTTATC (SEQ ID: 401994)
shPDGFRA: GCCTTTGTACCTCTAGGAATG (SEQ ID: 5156)
shCON: GATGCTGCATATAAGCAGC (SEQ ID: MF944225.1)

Immunoblotting. Detection of proteins was performed with antibodies specific for HCMV pp65 (Virusys Corporation, CA003-100); OR14I1 (Aviva Systems Biology, ARP71293-P050); PDGFR-α (EMD Millipore, 07-276); Flag (Sigma, F-1804); p-AKT (Cell Signaling, 4060S); p-PKA (Cell Signaling, 5661S); Calnexin (Santa Cruz Biotechnology, sc-23954) and β-actin (Sigma, A5316). Horseradish peroxidase (HRP)-conjugated secondary antibodies (GE Healthcare Life Sciences) were used for binding to the primary antibody. Protein bands were visualized in G:Box (Syngene) using ECL (Perkin-Elmer)

Imaging. For quantitation of fixed cells, cells were washed with PBS (Life Technologies) and fixed with 4% formalin in PBS (Sigma). DNA was stained with Hoechst 33342 dye (Life Technologies). The fixed cells were imaged using an Image Xpress Micro (IXM, Molecular Devices) at x4 magnification. Images were analyzed with MetaXpress imaging software (Molecular Devices) to determine the total cells per well and the percentage of infected cells (GFP positive) in each well. For IE immunostaining, cells were permeabilized *with* 0.2*%* Triton X-100 (Sigma) in PBS. Blocking was performed with 1% BSA (Calbiochem, 2930) in PBS with 0.3M glycine (Sigma). Cells were then incubated in antibody targeting the major IE proteins (IE, EMD Millipore, MAB8131, 1:500) diluted in 1% BSA in PBS. Cells were then incubated in secondary antibody (Texas Red-conjugated goat anti-mouse IgG2A, Southern Biotech, 1080-07) in PBS containing 1% BSA. For pp71 immunostaining, cells were permeabilized with 0.2% Triton X-100 (Sigma) in PBS. Blocking was performed with 1% BSA (Calbiochem, 2930) in PBS with 0.3M glycine (Sigma). Cells were incubated in antibody targeting the pp71 proteins (pp71 antibody, a kind gift from Robert Kalejta, University of Wisconsin-Madison) diluted in 1% BSA in PBS. Cells were then incubated in secondary antibody (donkey anti-mouse rhodamine-X, Jackson ImmunoResearch, 715-295-151).

Plasmids. PQCXIN-OR14I1 was constructed using the PQCXIN retroviral vector (Clontech) and Not1 and BamH1 sites. Cloning of human OR14I1 cDNA was straightforward as OR14I1 is a monoexonic gene whose full-length coding sequence was amplified from ARPE-19 genomic DNA. Forward primer contains a Not1 site and reverse primer contain a BamH1 site. Primers sequences are:

Forward primer: TAAGCGGCCGCCGCCACCATGGACAATCTCACAAAAGTGACAG

Reverse primer: ACAGGATCCCTACTTTTGCAGAAAATATATCTTCAC

After PCR amplification, reaction products were digested with Not1 and BamH1, and cloned into the Not1 and BamH1 sites of the vector. Pseudotyped viruses were produced using pCG-VSV-g and pCG-GagPol vectors. PQCXIN-Flag-OR14I1 was constructed using the same vector and same digestion sites as above. An amino-terminal FLAG epitope was added in the forward primer.

For PQCXIN-OR14I1-rescue, a three-step PCR amplification procedure was used to generate silent mutation fragments in the guide RNA targeted region.

Original sequence: TCCTACGCAGCCGTCCACACT
Silent mutation sequence: AGCTATGCTGCTGTGCATACC

The final plasmid was generated using the same vector and same digestion sites as above and sequence verified.

Transient Transfections and Confocal Imaging of CD71 and Flag-OR14I1. Cells were plated on glass coverslips pretreated with 40% HCl for 2min followed by a 5min wash in 70% ethanol. ARPE-19 cells were seeded at 70% confluency. On the second day, Flag-OR14I1 plasmid was transiently transfected into ARPE-19 cells using Lipofectamine 2000 (Life Technologies). 36h after transfection, cells were fixed in 4% formalin followed by permeabilization with 0.2% Triton X-100 in PBS. Blocking was performed with 1% BSA in PBS containing 0.3M glycine. Cells were next incubated with anti-Flag (Sigma, F1804, 1:100) and anti-CD71 (BD Pharmingen, #555534, 1:100) antibodies diluted in 1% BSA in PBS. Secondary antibodies used were Texas Red-conjugated goat anti-mouse IgG1 (Southern Biotech, 1070-07) or FITC-conjugated goat anti-mouse IgG2a (Southern Biotech, 1080-02). Cells were washed 3 times with PBS before mounting to slides using Vectashield with DAPI (4', 6'-diamidino-2-phenylindole) (Vector Laboratories, H-1500). Samples were imaged using a Nikon A1 inverted confocal microscopy. All microscope and image capture settings were kept constant throughout the entirety of a given experiment. 3D Projections were created using a 2µm z stack consisting of 28 steps in the NIS-Elements Software from Nikon. The pinhole used on the Nikon A1 microscope is 0.9AU (airy units).

Cell Surface Protein Detection. The Pierce® Cell Surface Protein Isolation Kit (Thermo Scientific) was used for isolation and collection of surface proteins, generally following the manufacturer's protocol. Per biological replicate, $3x10^7$ cells were cultured in four $75cm^2$ flasks. Cells were washed four times with ice-cold phosphate buffered saline (PBS) followed by incubation with 0.25mg/mL Sulfo-NHS-SS-Biotin in 48 mL ice-cold PBS per flask on a rocking platform for 30 minutes at 4ºC. The biotinylation reaction was quenched by adding 500µl of Quenching Solution. Cells were washed with ice-cold PBS, harvested by gentle scraping and pelleted by centrifugation. The cells were lysed using the Lysis Buffer containing a protease inhibitor cocktail (Sigma) for 30 minutes on ice with intermittent vortexing. The lysates were centrifuged at 10,000x*g* for 2min at 4ºC to remove cell remnants. The clarified supernatant was used for purification of biotinylated proteins on NeutrAvidin Agarose. The unbound proteins, representing the intracellular fraction, were collected by centrifugation of the columns at 1,000x*g* for 2 minutes and stored at -80ºC and served as an internal control for the surface protein isolation process. Any remaining unbound proteins were removed by repetitive washing; The captured surface proteins were eluted from the biotin-NeutrAvidin Agarose by incubation with 400µL of sample buffer containing 50mM dithiothreitol (DTT) for 1h at room temperature (RT) in an end-

over-end mixing on a rotator. The eluted proteins, representing the cell surface proteins, were collected by column centrifugation at 1,000x*g* for 2min. The lysates were stored at -80ºC until use. Immunoblotting was performed with antibodies specific for OR14I1 (Aviva Systems Biology, ARP71293-P050) and Calnexin (Santa Cruz Biotechnology, sc-23954). Horseradish peroxidase (HRP)-conjugated secondary antibodies (GE Healthcare Life Sciences) were used for binding to the primary antibody. Protein bands were visualized in G: Box (Syngene) using ECL (Perkin-Elmer).

Flow Cytometry. ARPE-19 cells were infected with GFP-expressing virus. At 2dpi, cells were treated with enzyme free cell dissociation buffer (Gibco). Cells were fixed with cold 2% paraformaldehyde in PBS. Flow cytometry was performed on a MACSQuant analyzer (Miltenyi Biotec) using a 488nm blue laser and 525/50nm filter. Data were analyzed using FlowJo V9.9 software (www.flowjo.com). Forward and side scatter gates were used to enrich lives cells, and singlets were selected by forward scatter area versus height. The gate for GFP-positive cells was set using a negative control so that the percent GFP positive cells in the control was <1.0%.

Expression of Human OR14I1 in Sf9 Cells. Human OR14I1 cDNA was Flag-tagged at the N-terminus and subcloned into BamHI and XhoI sites of the pFasBac/CT-Topo baculovirus vector (ThermoFisher). The pFasBac-OR14I1 construct was then transformed into DH10Bac E. coli for transposition into a bacmid. The recombinant bacmid was transfected into Sf9 cells using Cellfectin II Reagent (Invitrogen) to produce recombinant baculovirus following the manufacturer's instructions. The resulting baculovirus was amplified and titrated. Sf9 cells were infected with the recombinant baculovirus (MOI=0.1). Expression of Flag-OR14I1 protein was confirmed by immunoblotting.

Membrane Vesicle Preparation. Sf9 cells were harvested at 4dpi with the recombinant OR14I1-expressing baculovirus. Pellets were washed twice with PBS, resuspended in hypotonic lysis buffer (20mM Tris, pH 8.0, 25mM NaCl, 2mM MgCl2.6H2O; 1mM EDTA.Na2(H2O)2, 2mM TCEP-HCl, protease inhibitor cocktail (complete EDTA-free tablets, Roche, 1 table/50ml) and cells were allowed to swell on ice for 15min. The cell suspension was subjected to 100 strokes with a type B (tight) Dounce homogenizer. Cell debris was removed by low speed centrifugation (1,000rpm). The membrane vesicles containing supernatant were then subjected to ultracentrifugation in a SW28 rotor at 28,000rpm for 1h to yield a crude membrane pellet that was resuspended in wash buffer (20mM Tris, pH 8.0, 25mM NaCl, 1mM MgCl2.6H2O, 2mM TCEP-HCl, with protease inhibitor cocktail. After a second ultracentrifugation, the membrane pellet was resuspended in freezing medium (wash buffer with 2mM MgCl2, and 10% glycerol) and stored at -80°C.

Membrane Flotation Assay. Purified TB40E-GFP virus, or virus incubated with the antibodies noted above (neutralization assay), was mixed with either Sf9-control or Sf9-Flag-OR14I1 membrane vesicles at 37°C for 30min at 700rpm in a thermomixer. A 26µl aliquot of the membrane vesicles/virus sample was then mixed with 59µl of a 70% sucrose solution in PBS. This mixture was then placed at the bottom of a centrifuge tube (7mm x 20mm, Beckman), and covered gently with 100µl of a 40% sucrose solution in PBS, which in turn was layered with 25µl of a 20% sucrose solution in PBS. The samples were then centrifuged for 60min at 390,000xg at 4°C with a TLA-100 rotor (Beckman). Twenty-three µl fractions were collected from top to bottom and analyzed by immunoblotting. An anti-pp65 antibody (Virusys, CA003-100, 1:1000) was used to identify

fractions containing virions, and an anti-Flag antibody (Sigma, F1804, 1:100 was used to identify OR14I1-containing fractions.

AC/PKA Signaling Pathway Modulation. H-89, SQ22536, and Foskolin were purchased from Sigma-Aldrich Chemicals. ARPE-19 cells expressing the indicated shRNAs were treated with protein kinase A (PKA) inhibitor H-89 (20μM), adenylate cyclase antagonist, SQ22536 (150μM), or adenylate cyclase activator, forskolin (FSK; 20μM) for 2h prior to infection with TB40E-GFP (MOI=2.0). After 2h, the medium was then replaced by the standard medium that also contained the noted small molecules. Images were taken at 2dpi.

Viral Entry Assays. Cells were pretreated with PKA inhibitor H-89 (20μM), adenylate cyclase (AC) antagonist SQ22536 (150μM), AC agonist forskolin (FSK; 20μM) or DMSO solvent for 2hr prior to TB40E-GFP infection. Cells were cooled on ice for 30mins and then incubated with cold TB40E virus (MOI=2.0) together with the noted small molecules for 1h on ice. Cells were then transferred to 37ºC for 2h. At 2hpi, cells were washed and treated with a 1 min incubation with 0.25% trypsin to remove surface-bound virus. Cells were subsequently washed with cold complete media twice and then PBS. Cells were then fixed with 4% paraformaldehyde in PBS, permeabilized, stained with anti-pp71 (red) and imaged (x63). The scale bar represents 10μm.

PKA/AKT Signaling Detection. Cells were serum starved overnight. After cells were cooled on ice for 30mins they were infected with cold TB40E virus for 1h on ice. After incubation, cells were transferred to 37ºC for 0, 5, or 10 mins. At the indicated time points, cells were harvested and the levels of p-AKT (S473), total AKT, p-PKA (T197) and actin were detected by immunoblotting from whole-cell lysates.

Endocytosis Assays. A published protocol was employed that uses labelled transferrin to monitor uptake. Cells were first incubated on ice for 30 mins, then incubated with 50ug/ml FITC (green) transferrin (Life Technologies T2871) with TB40E virus (MOI=2.0) in 2% fetal bovine serum DMEM-F12 on ice for 1h before they were transferred to 37 °C. At the end of the 37°C incubation period at the indicated time points, the cells were cooled to 4°C, removed the media and washed with ice-cold PBS to remove unbound transferrin and virus, and then incubated for 2min at 4°C with ice-cold stripping buffer (150mM NaCl, 20mM HEPES, 5mM KCl, 1mM CaCl2, 1 mM MgCl2, pH 5.5) twice to remove transferrin bound to the cell surface. The cells were then washed with cold PBS and fixed with cold 4% paraformaldehyde in PBS and permeabilized, then co-stained with anti-pp71 (red) and imaged (x63).

Cells were pretreated with ammonium chloride (30 mM) for 2hr prior to TB40E-GFP infection. Cells were cooled on ice for 30mins and then incubated with cold TB40E virus (MOI=2.0) together with ammonium chloride (30 mM) for 1h on ice. Cells were then transferred to 37ºC for 2h. At 2hpi, cells were washed and treated with a 1 min incubation with 0.25% trypsin to remove surface-bound virus. Cells were subsequently washed with cold complete media twice and then PBS. Cells were then fixed with 4% paraformaldehyde in PBS, permeabilized, stained with anti-pp71 (red) and imaged (x63). The scale bar represents 10μm.

Table S1. Enriched Pathways Identified in ARPE19/CRISPR/Cas9 Screen Using ConsensusPath DB

Enriched Pathway	Number of Proteins (% of Pathway)	P-Value	Pathway Source
Signaling Pathway from G-protein Families	17 (65.4)	1.12E-05	BiooCarta
Olfactory Transduction-Homo Sapiens (Human)	130 (32.3)	1.67E-03	KEGG
Neuronal System	92 (33.6)	3.31E-03	Reactome
G Protein Signaling Pathways	35 (39.8)	9.72E-03	Wikipathways
PKA-mediated Phosphorylation of CREB	9 (52.9)	0.0104	Reactome
CREB Phosphorylation Through the Activation of Adenylate Cyclase	5 (71.4)	0.0115	Reactome
DNA Damage Reversal	3 (100.0)	0.0145	Reactome
PKA Activation	9 (56.2)	6.25E-02	Reactome
Calcium Signaling Pathway-Homo Sapiens (Human)	56 (33.5)	4.62E-02	KEGG
P53 Dependent G1/S DNA Damage Checkpoint	13 (100)	0.0336	Reactome
PI3K/AKT Activation	67 (52.3)	0.0148	Reactome

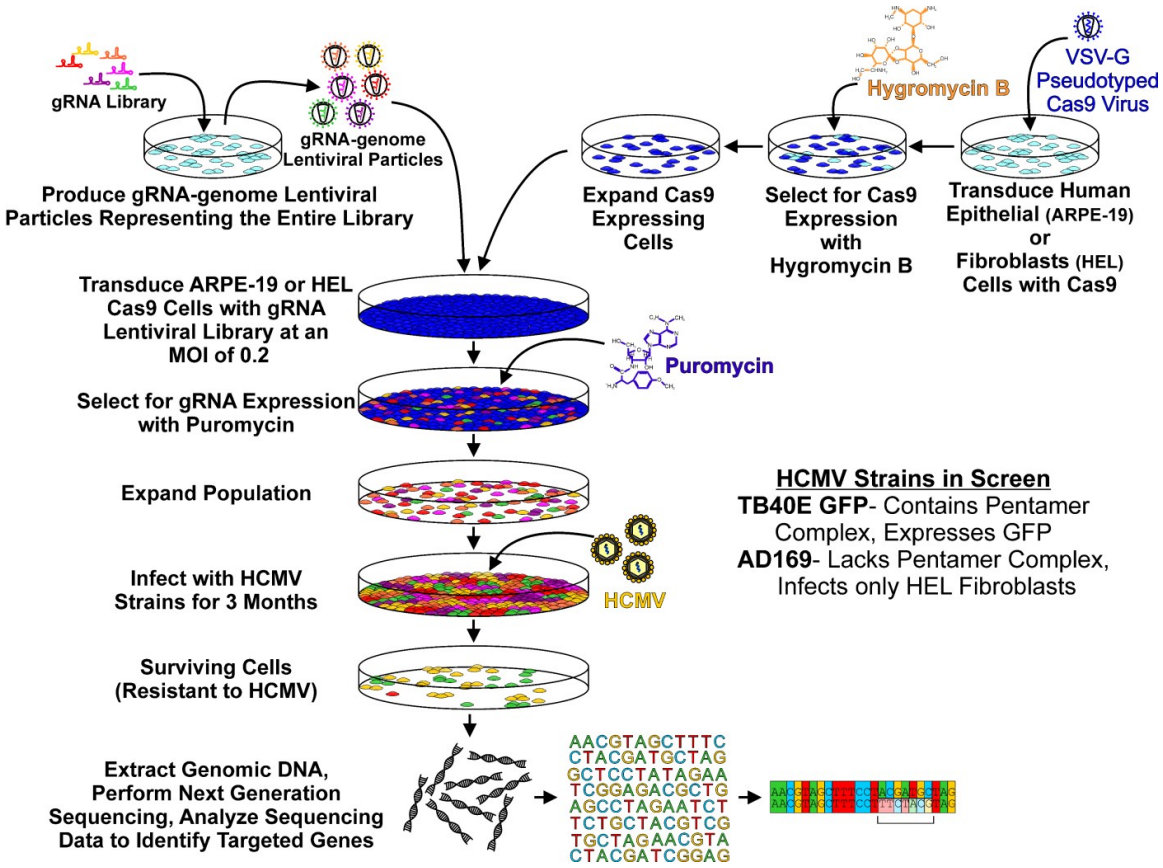

Fig. S1. Schematic diagram of the HCMV-host factor CRISPR screens. To identify host factors required for HCMV replication, two parallel genome-wide CRISPR screens were performed using either TB40E-GFP infection of ARPE-19 epithelial cells, or AD169 infection of HEL fibroblasts. Cells expressing Cas9 and the GeCKO v.2 sgRNA library (19,050 genes targeted with 6 sgRNAs per gene) were exposed to TB40E-GFP infection for three months or AD169 infection for 2-3 weeks. Cells with sgRNA-mediated resistance to HCMV survived additional viral challenges and expanded in number. To identify enriched sgRNAs in the resistant cell population, genomic DNA was subjected to NGS and sequences representing sgRNA were identified and quantified. The reagent redundancy principle was used to select high confidence candidates that scored with $\geq$3 unique sgRNAs, each with $\geq$20 NGS reads.

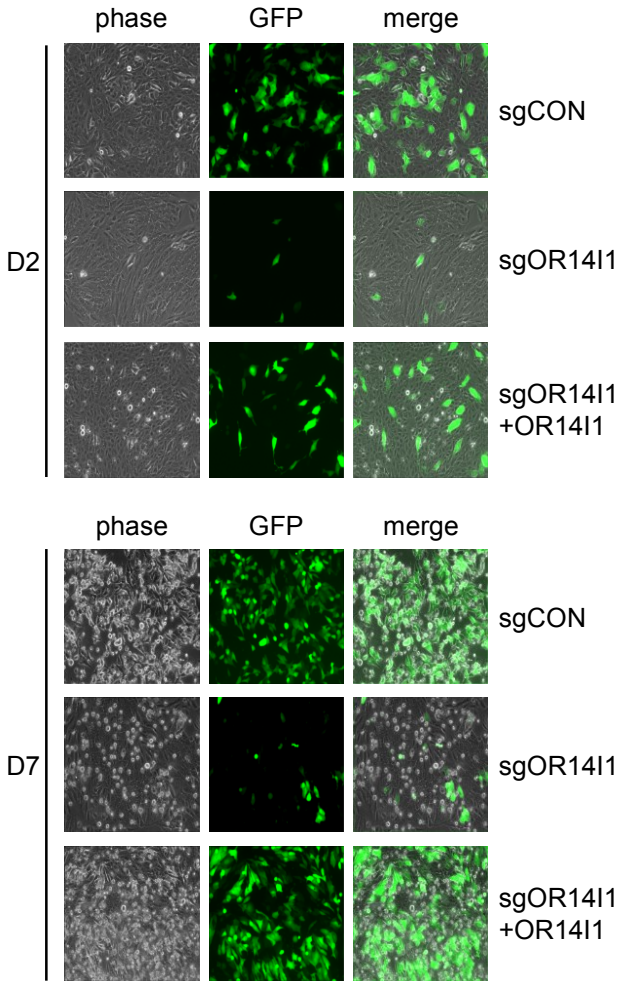

Fig. S2. HCMV infection of sgOR14I1 cells is restored with sgRNA-resistant OR14I1. ARPE-19 cells expressing the indicated sgRNAs were infected with TB40E-GFP (MOI=2.0). A clonal sgOR14I1 cell line (sgOR14I1) was stably transduced with a sgRNA-resistant OR14I1 cDNA (sgOR14I1+OR14I1) prior to TB40E-GFP infection. Cells were imaged (x10) for GFP expression at 2dpi (D2) and 7dpi (D7). Representative images of 3 independent experiments are shown.

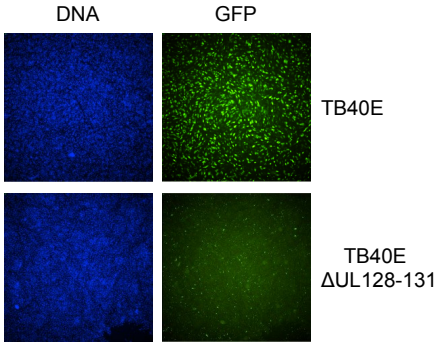

Fig. S3. Viral PC is required for epithelial cell infection. ARPE-19 cells infected with TB40E-GFP or a PC-deleted TB40E-GFP (TB40EΔUL128-131, MOI=3.0). Cells were fixed at 2dpi and imaged (x4).

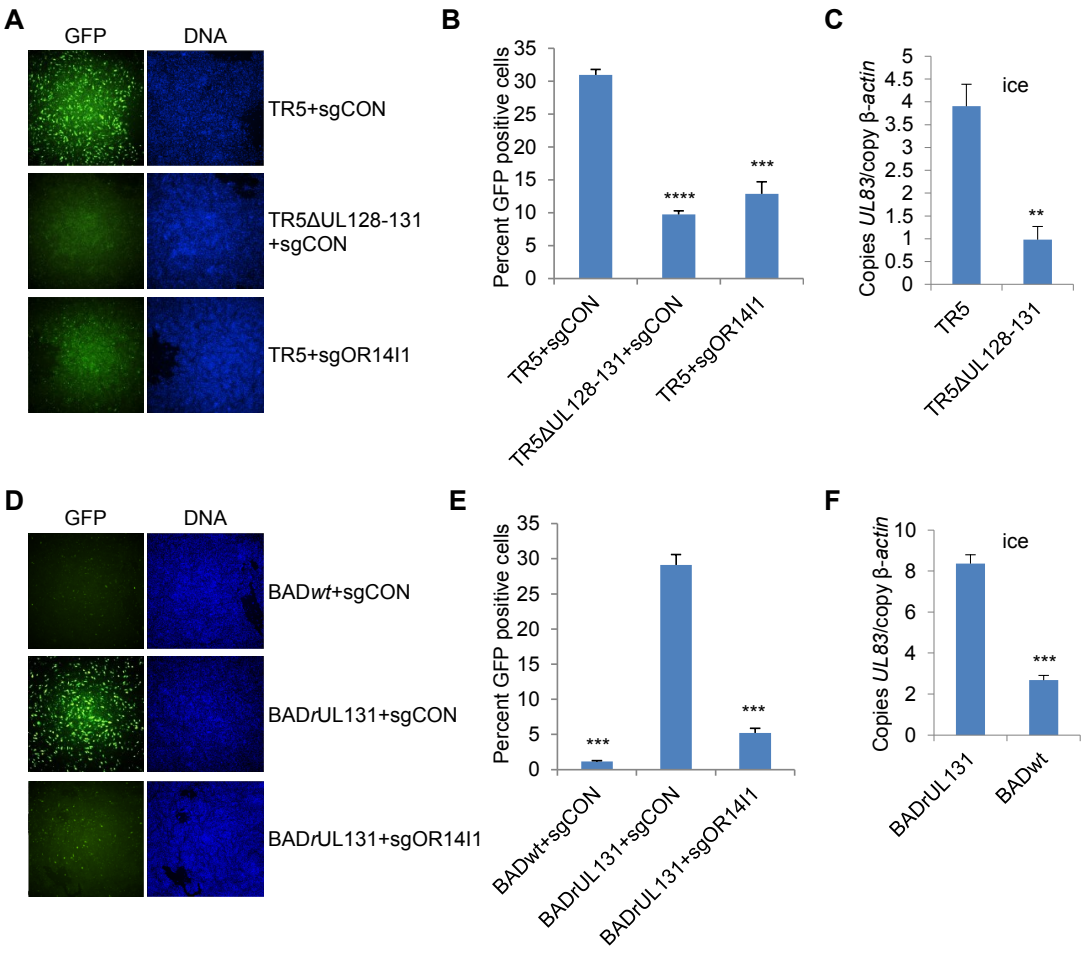

Fig. S4. Additional HCMV strains also require the viral PC and host OR14I1 to bind to and infect ARPE-19 epithelial cells. (A) ARPE-19 cells expressing the indicated sgRNAs were infected with TR5-GFP virus (TR5) or a TR5-GFP virus with the PC deleted (TR5ΔUL128-131) at an MOI=3.0. Cells were fixed at 2dpi and imaged (x4) for GFP (green) and DNA (blue). (B) Quantitation of data in (A) indicating the percent GFP positive cells. (C) ARPE-19 cells were incubated with TR5-GFP or TR5ΔUL128-131 for 1h on ice (MOI=2.0). After washing, cell-surface bound viral DNA (UL83) was quantified by qPCR and normalized to cellular DNA (β-actin). (D) ARPE-19 cells expressing the indicated sgRNAs were infected with either bacteria artificial chromosome-derived AD169-GFP (BAD*wt*) virus which lacks the PC, or UL131-repaired AD169-GFP virus (BAD*r*UL131), which expresses the PC. Cells were fixed at 2dpi and imaged (x4) for GFP (green) and DNA (blue). (E) Quantitation of data in (D) indicating the percent GFP positive cells. (F) ARPE-19 cells were incubated with either BAD*wt* virus or BAD*r*UL131 for 1h on ice (MOI=3.0). After washing, cell-surface bound viral DNA (UL83) was quantified by qPCR and normalized to cellular DNA (β-actin). Data represent the mean of n=3 experiments ± SD. **p<0.01; ***p<0.001; ****p<0.0001.

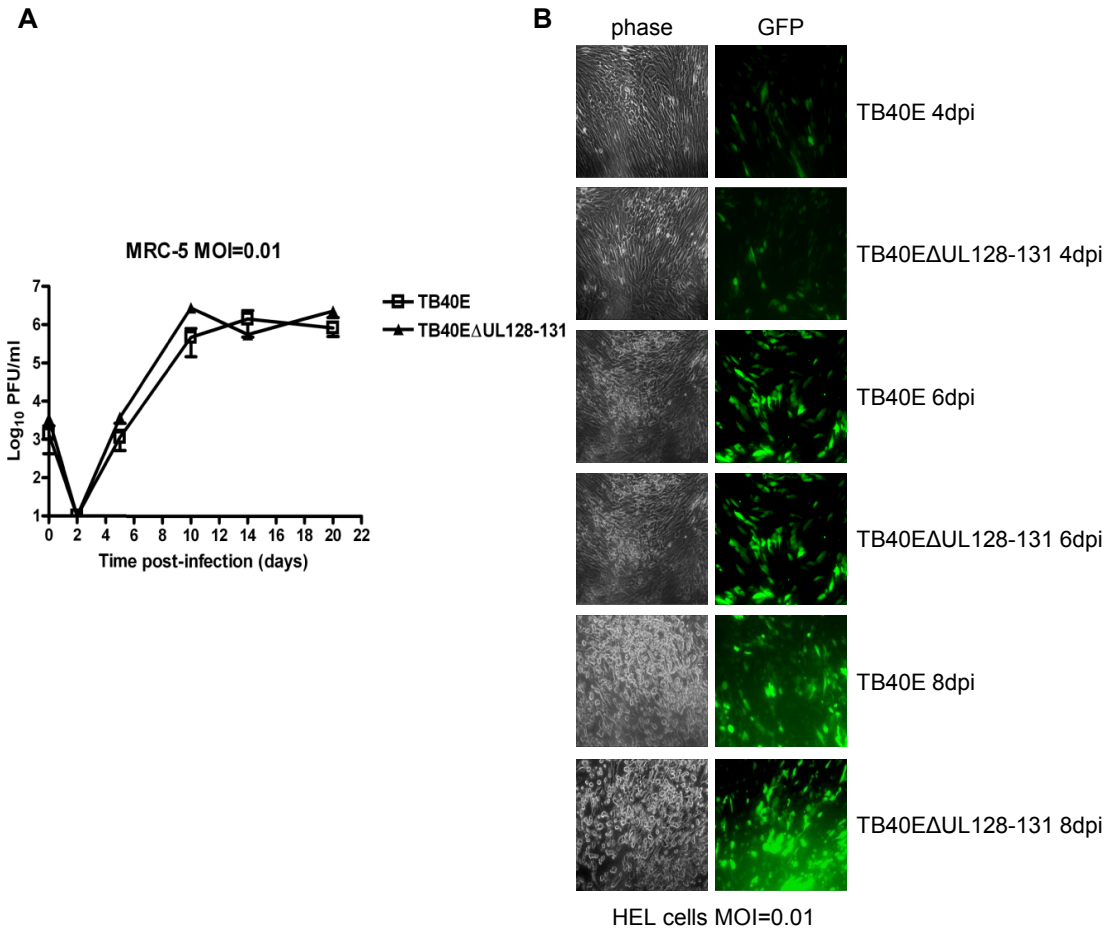

Fig. S5. TB40EΔUL128-131 mutant virus growth is similar to its WT parental in fibroblasts. (A) Growth kinetics of TB40EΔUL128-131 mutant virus relative to the TB40E-WT virus. MRC-5 cells were infected with the parental TB40E and TB40EΔUL128-131 mutant viruses (MOI=0.01). The extent of viral replication was measured at the indicated days pi by titrating the infectivity of cell suspension supernatants on MRC-5 cells using the IE antigen indirect immunoperoxidase staining technique (1). (B) HEL fibroblasts were infected with TB40E-GFP and TB40EΔUL128-131 mutant viruses (MOI=0.01). Cells were imaged (x10) for GFP expression at 4, 6, and 8dpi.

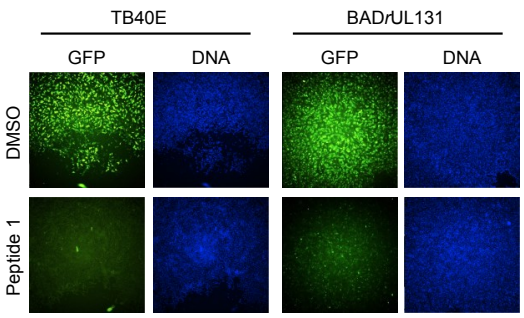

Fig. S6. Synthetic N-terminal peptide of OR14I1 suppresses PC+ HCMV TB40E, BAD*r*UL131 strains infection of epithelial cells. TB40E-GFP or BAD*r*UL131-GFP virus, both expressing PC, were preincubated with peptide 1 (100μg/ml), or DMSO prior to infection of ARPE-19 cells (MOI=2.0). Cells were fixed and imaged (x4) for GFP at 2dpi.

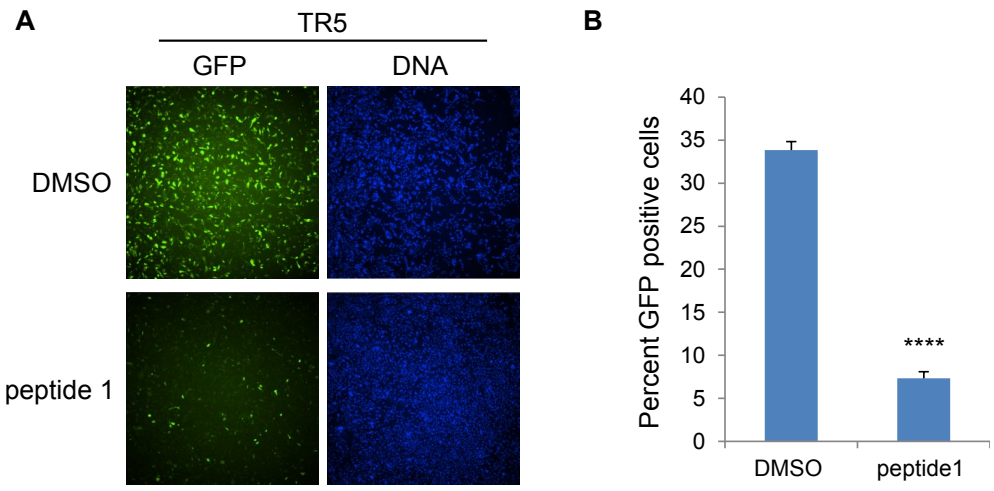

Fig. S7. Synthetic N-terminal peptide of OR14I1 suppresses PC+ HCMV TR5 strain infection of epithelial cells. (A) Peptide 1 (100µg/ml) was preincubated with TR5 virus, which expresses the viral PC and GFP, followed by the infection of ARPE-19 cells (MOI=2.0). Cells were fixed and imaged (x4) for GFP and DNA (cell nuclei) at 2dpi. (B) Quantitation of data in (A) indicating the percent GFP positive cells. Data represent the mean of n=3 experiments ± SD. ****p<0.0001.

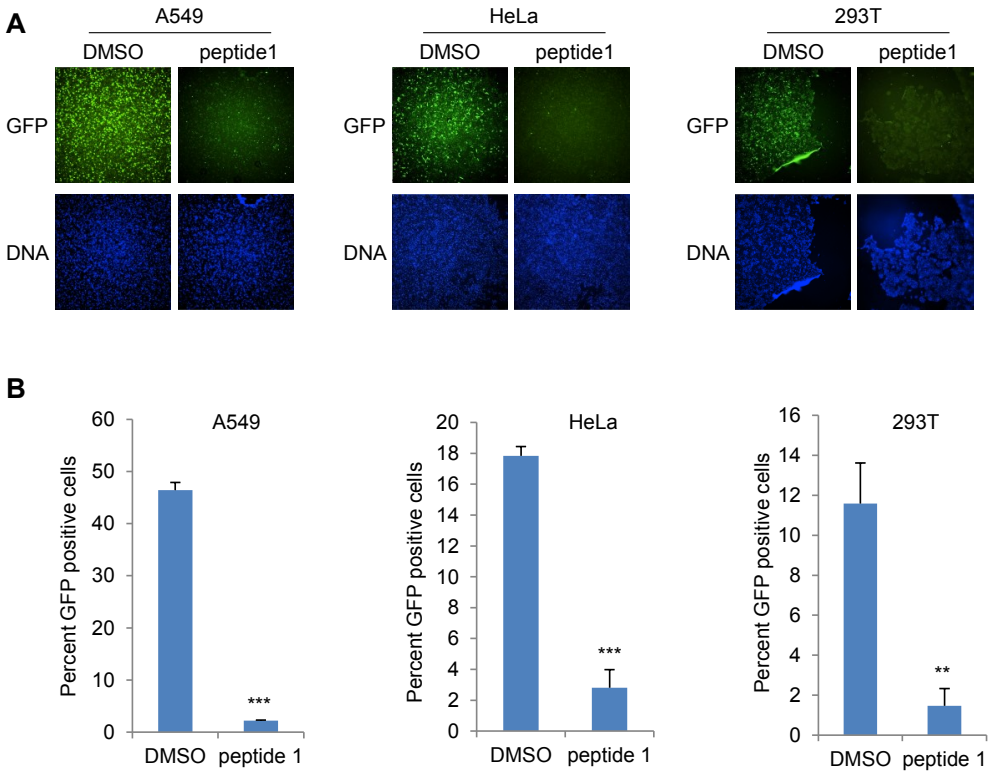

Fig. S8. Synthetic N-terminal peptide of OR14I1 inhibits HCMV infection of several epithelial cell lines. (A) Peptide 1 (100µg/ml), or DMSO alone was preincubated with TB40E-GFP virus, followed by infection of the indicated epithelial cell lines (MOI=2.0). Cells were fixed and imaged (x4) for GFP (green) and DNA (blue) at 2dpi. (B) Quantitation of data in (A) indicating the percent GFP positive cells. Data represent the mean of n=3 experiments ± SD. $^{**}p<0.01$; $^{***}p<0.001$.

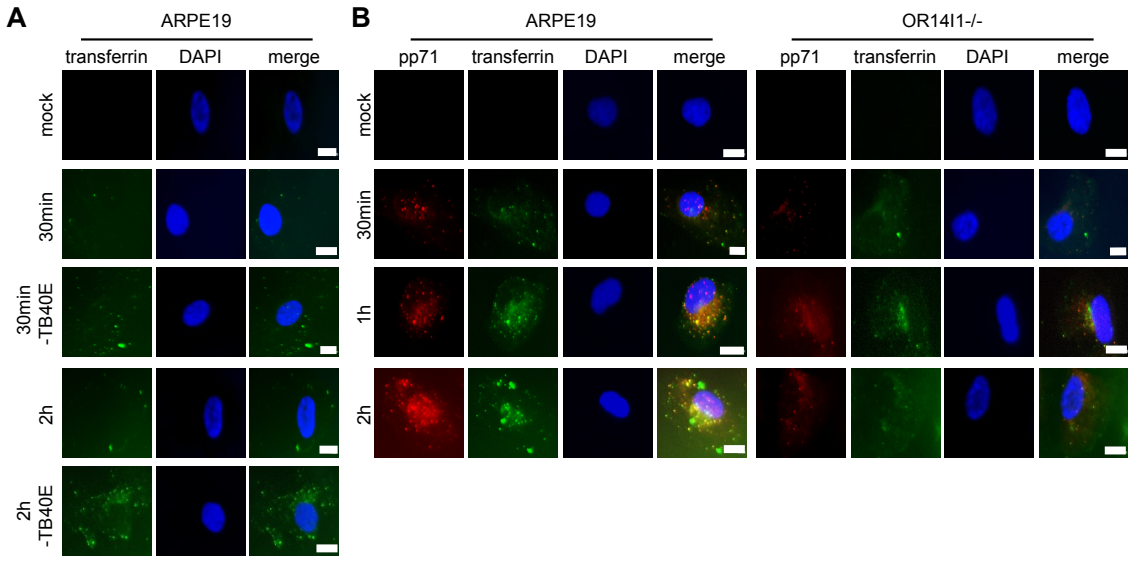

Fig. S9. OR14I1 mediates endocytosis for entry of HCMV. (A) ARPE19 cells were incubated with transferrin-FITC (green) with or without TB40E virus (MOI=2.0) for 1h on ice. Cells were transferred to 37ºC. At 30min or 2hpi, cells were washed with stripping buffer, fixed, permeabilized and imaged (x63). (B) HCMV inner tegument protein pp71 colocalizes with the endocytosis uptake marker, transferrin, during the entry of HCMV. ARPE19 and OR14I1-/- ARPE19 cells were incubated with transferrin-FITC (green) and TB40E virus (MOI 2.0) for 1h on ice and then transferred to 37ºC. At the indicated time points, cells were washed with stripping buffer, fixed, permeabilized and immunostained with pp71 antibody, then imaged (x63).

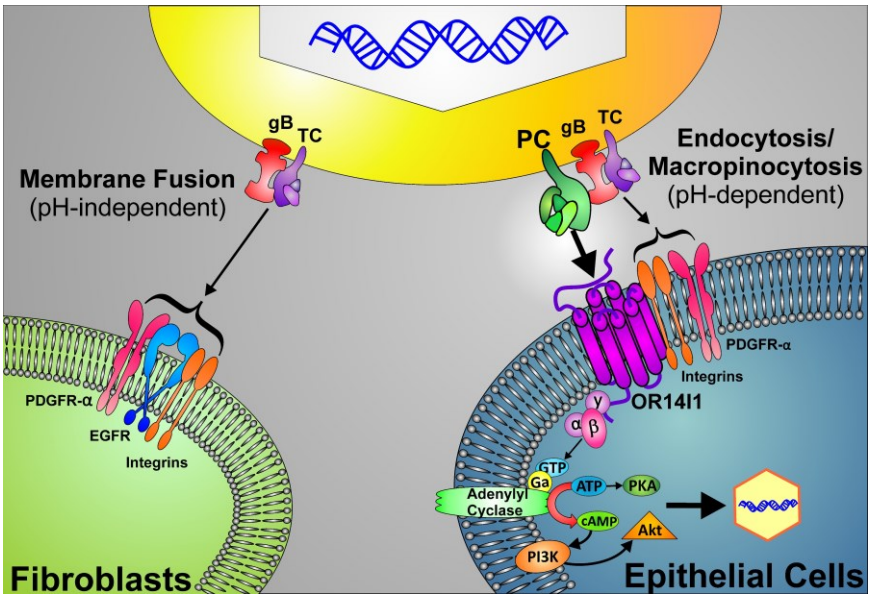

Fig. S10. Working model for HCMV entry into cells. HCMV entry into different cell types involves different gH/gL complexes: gH/gL/gO trimeric complex (TC) and gH/gL/UL128-131 pentameric complex (PC). Entry into fibroblasts entails direct fusion with the plasma membrane whereas entry into epithelial cells uses endocytosis followed by low pH-dependent fusion. HCMV binding of both fibroblasts and epithelial cells depends on envelope glycoprotein complexes composed of gB and TC. HCMV entry into epithelial additionally requires the PC. gB and/or TC interact with the epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor-α (PDGFR-α) and integrins on fibroblasts; gB and/or TC interact with PDGFR-α and probably integrins on epithelial cells. Additionally, the PC interacts with N-terminus of OR14I1, its newly identified receptor. The PC-OR14I1 interaction triggers an adenylyl cyclase/PKA/AKT signaling pathway that facilitates virus entry into epithelial cells.

References

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