Localization of the WD Repeat-Containing Protein 5 to the Virion Assembly Compartment Facilitates Human Cytomegalovirus Assembly

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ABSTRACT We previously reported that human cytomegalovirus (HCMV) utilizes the cellular protein WD repeat-containing protein 5 (WDR5) to facilitate capsid nuclear egress. Here, we further show that HCMV infection results in WDR5 localization in a juxtanuclear region and that its localization to this cellular site is associated with viral replication and late viral gene expression. Furthermore, WDR5 accumulated in the virion assembly compartment (vAC) and colocalized with vAC markers of γ -tubulin, early endosomes, and viral vAC marker proteins pp65, pp28, and glycoprotein B (gB). WDR5 coimmunoprecipitated with multiple virion proteins, including major capsid protein (MCP), pp150, pp65, pIRS1, and pTRS1, which may explain WDR5 accumulation in the vAC during infection. WDR5 fractionated with virions in either the presence or absence of Triton X-100 and was present in purified viral particles, suggesting that WDR5 was incorporated into HCMV virions. Thus, WDR5 localized to the vAC and was incorporated into virions, raising the possibility that in addition to capsid nuclear egress, WDR5 could also participate in cytoplasmic HCMV virion morphogenesis.

IMPORTANCE Human cytomegalovirus (HCMV) has a large (~235-kb) genome that contains over 170 open reading frames (ORFs) and exploits numerous cellular factors to facilitate its replication. In the late phase of HCMV infection, cytoplasmic membranes are reorganized to establish the virion assembly compartment (vAC), which has been shown to be necessary for efficient assembly of progeny virions. We previously reported that WDR5 facilitates HCMV nuclear egress. Here, we show that WDR5 is localized to the vAC and incorporated into virions, perhaps contributing to efficient virion maturation. Thus, findings in this study identified a potential role for WDR5 in HCMV assembly in the cytoplasmic phase of virion morphogenesis.

KEYWORDS human cytomegalovirus, WDR5, virion structure, virion morphogenesis, virion assembly compartment, vAC

Human cytomegalovirus (HCMV) is a ubiquitous pathogen that is highly adapted to its human host. Approximately 50 to 90% of adults are infected globally (1), and in

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China the HCMV seroprevalence is as high as 93.7% (2). About 90% of primary HCMV infections in immunocompetent individuals are either asymptomatic or mildly symptomatic (3, 4). Following primary infection, the virus remains latent and establishes life-long persistence in its host. However, HCMV can be reactivated and cause severe disease in immunosuppressed or immunodeficient individuals, such as recipients of solid organ or bone marrow transplants and AIDS patients (5–9). In these patients, HCMV infection contributes to multiple end-organ diseases, including pneumonia, gastrointestinal disease, retinitis, central nervous system disease, poor engraftment, and severe graft-versus-host reaction, and can result in high mortality rates (10–13). In patients receiving hematopoietic stem cell transplantation, the incidence of HCMV disease has been reported to range between 30 and 70% (14). In addition, congenital HCMV infection is a leading cause of birth defects and is associated with permanent developmental sequelae in a significant number of infants and children (15–18).

A characteristic feature of HCMV infection is profound remodeling of the secretory and endocytic system (Golgi compartment, *trans*-Golgi network, and early endosomes), leading to the formation of nested, cylindrical layers of membranes into a structure that has been termed the virion assembly compartment (vAC) (6, 19–24). Previously, the vAC had been thought to represent a unique structure associated with HCMV infection that was not described in other herpesviruses (25). However, a recent study showed that herpes simplex virus 1 (HSV-1) forms juxtanuclear assembly compartments similar to the HCMV vAC in primary mouse neurons and human neuron-derived cells, but not in fibroblasts or epithelial cells (26). Although not essential for virion production, vAC formation has been shown to be necessary for efficient HCMV virion assembly and maturation. Multiple viral and cellular proteins are reported to participate in vAC formation. Viral protein pUL136 is important for vAC formation and efficient virion assembly in endothelial cells. Virus mutants lacking UL136 produce fewer enveloped virions along with a larger number of dense bodies compared to wild-type virus (27). Other viral proteins pUL48, pUL94, pUL103, and pUL132 are also important for vAC development (23, 28). Interestingly, HCMV-carried microRNAs (miRNAs) UL112-1, US5-1, and US5-2 target host secretory pathway factors to facilitate vAC formation (29). Cellular proteins, including RhoB (24), IFITMs (25), STX5 (30), dynein (31), BiP (31, 32), bicaudal D1 (33), Rab11, and FIP4 (34), have also been shown to localize to vAC.

A number of cellular proteins are also localized to the vAC, and some are incorporated into virions. The initial proteomic analysis of the virion identified over 70 viral and cellular proteins in HCMV virions. These virion-associated cellular proteins are involved in a wide range of processes and functions, including ATP binding, Ca^{2+} signaling, signal transduction, transcription, and translation (35). WD repeat-containing protein 5 (WDR5) is a highly conserved WD-40 repeat protein that has been shown to play a role in the regulation of multiple cellular processes, possibly through its role as a scaffold for protein-protein interactions that can facilitate formation and/or stabilization of multiprotein complexes (36). Examples of this function include MOF (males absent on the first)-containing NSL, KANSL1 and KANSL2, and histone H3 at lysine 4 (H3K4) methyltransferases of the SET1-family (Set1A, Set1B, MLL1, MLL2, MLL3, and MLL4) (37–41). These previous studies have described the role of WDR5 in the epigenetic regulation of cellular processes (42–45); however, WDR5 can also stabilize actin architecture to promote multiciliated cell formation (46) and has been reported to be important for embryonic stem cell reprogramming, self-renewal, and maintenance of pluripotency (47, 48).

A role of WDR5 in RNA virus infections has also been reported (49, 50). Following Sendai virus infection, WDR5 is translocated from the nucleus to the mitochondria and is essential for formation of the virus-induced-signaling adapter (VISA) complex, also known as mitochondrial antiviral-signaling protein (MAVS) (49). Measles virus recruits WDR5 into viral inclusion bodies in the cytoplasm to facilitate virus replication (50). Our previous work showed that WDR5 contributes to HCMV replication by facilitating capsid nuclear egress (51). In the present study, we demonstrate that the WDR5 protein

FIG 1 Juxtanuclear relocalization of WDR5 is specifically induced by HCMV and MCMV infection. HELFs (A and C) or NIH 3T3 cells (B) were infected with the indicated viruses at an MOI of 1. At 48 hpi, cells were fixed and stained with antibodies against WDR5 (red) and with either HCMV IE1 protein (A) (green) or MCMV mIE1 (green) (B). Infected cells (C) were detected using corresponding GFP-expressing recombinant viruses. All antibodies used in IFA were mouse monoclonal antibodies. Nuclei were counterstained with DAPI (white). M, mock-infected cells; scale bars = 10 μ m.

level increases in the cytoplasm in HCMV-infected cells, accumulates in the vAC, and can be incorporated into progeny virions, suggesting that this cellular protein could contribute to virion morphogenesis.

RESULTS

WDR5 accumulates in a juxtanuclear location in HCMV-infected cells. It has been reported that the cellular protein WDR5 is translocated from the nucleus to mitochondria during Sendai virus infection and to viral replication centers during measles virus infection (49, 50). As both are RNA viruses, we initially determined whether WDR5 relocalized in HCMV-infected cells. In mock-infected human embryo lung fibroblasts retrovirally transduced with hTERT (HELFs), WDR5 displayed a diffuse nuclear localization with a single small juxtanuclear punctum (Fig. 1A). In contrast, in HCMV-infected cells, WDR5 was localized to the nucleus and also exhibited increased expression in the juxtanuclear region of the infected cell (Fig. 1A). To exclude the possibility that WDR5 juxtanuclear accumulation is limited to HELFs or infection by HCMV strain Towne, similar experiments were performed with primary cell human embryo lung fibroblasts (HELs) and U251 glioma cells infected with HCMV strain Towne or AD169. Under both conditions, a similar juxtanuclear WDR5 localization was observed (data not shown). In addition, similar juxtanuclear localization was also observed during murine cytomegalovirus (MCMV) infection of mouse NIH 3T3 fibroblasts (Fig. 1B). Interestingly, this effect was not observed during infection of HELFs by other DNA viruses that do not form vAC-like structures during infection, including herpes simplex virus 1 (HSV-1), varicella-zoster virus (VZV), adenovirus (AdV), or *Autographa californica* multiple nucleopolyhedrosis virus (AcMNPV) (Fig. 1C). These data suggest that juxtanuclear accumulation of WDR5 in primary fibroblasts was specifically induced by HCMV or MCMV infection.

Accumulation of WDR5 in the vACs is microtubule dependent. In HCMV-infected cells, the vAC is enriched in viral structural proteins, including both tegument and envelope proteins that accumulate in this juxtanuclear compartment, together with proteins of the secretory and endocytic systems of the cell (19, 20). To determine if the accumulation of WDR5 in juxtanuclear region resulted from recruitment of WDR5 to the vAC, infected cells were costained for WDR5 and viral vAC markers, including the tegument proteins pp28 and pp65 and the envelope glycoprotein glycoprotein B (gB) (20). As shown in Fig. 2A, pp28, pp65, and gB colocalized with WDR5 in the juxtanuclear region of the infected cell. A three-dimensional (3D) reconstruction from z-stack images of the cell shown in the bottom panel of Fig. 2A clearly demonstrated the

FIG 2 WDR5 localizes to vAC and MTOC. (A) HCMV-infected HELFs were stained at 96 hpi for WDR5 (red) and costained for viral proteins pp28, pp65, or gB (green). Images to the right show 3D reconstruction of WDR5 colocalization with gB from the cell shown on the left. (B) HELFs were infected with HCMV at an MOI of 3 and at 72 hpi costained for WDR5 and markers of cytoskeleton (α -tubulin), centrosome (γ -tubulin), Golgi compartment (58K), or early endosome (EEA1). (C) HELFs

(Continued on next page)

colocalization of WDR5 with gB, a well-described component of the mature vAC. Together these data indicated that WDR5 accumulated in the vAC during HCMV infection.

The location and morphology of WDR5 in the infected cell argued that it accumulated in the vAC late in infection (19). To further investigate this possibility, colocalization of WDR5 with previously described cellular components of the vAC was determined in HCMV-infected cells. At 72 h postinfection (hpi), WDR5 accumulated in a juxtanuclear region in cells with kidney-shaped nuclei that also displayed cytoskeletal filaments (α -tubulin) radiating outwards to the rim of the plasma membrane (Fig. 2B, panels a to d). This region also colocalized with γ -tubulin (Fig. 2B, panels e to h), which has been described as a marker of the microtubule organizing center (MTOC) within HCMV-infected cells (52, 53). This juxtanuclear region in the infected cell appeared to be wrapped by a nested ring of Golgi membranes (anti-58K [Fig. 2B, panels i to l]) that appeared to be concentrically distributed around the early endosome protein EEA1 (Fig. 2B, panels m to p). These morphological features have all been described as characteristics of the mature vAC (20). These findings argued that WDR5 accumulated in the vAC of infected cells.

To determine if WDR5 accumulation in the vAC was dependent on microtubule function, as has been described for virion structural proteins such as pp150, replicate cultures to those shown in Fig. 2A were treated with nocodazole (NOC) to disrupt microtubule polymerization and analyzed at 20 min, 4 h, or 6 h of NOC treatment (33, 54). While γ -tubulin was less sensitive to NOC treatment (Fig. 2C, panels c and g), α -tubulin filaments became fragmented and punctate in distribution after 4 to 6 h of NOC treatment (Fig. 2B, panels k and o). Concomitant with the fragmentation of microtubules, WDR5 that had accumulated in the vAC dispersed throughout the cytoplasm of the infected cell (Fig. 2C, panels f, j, and n).

Juxtanuclear accumulation of WDR5 increases via nuclear export during HCMV infection. To gain further insight into the mechanisms that resulted in the subcellular localization of WDR5 during HCMV infection, a time course of the distribution of WDR5 within infected cells was performed from 12 to 96 hpi. Juxtanuclear vAC accumulation of WDR5 was first observed at 24 hpi, and its accumulation in the maturing vAC increased as the infection progressed (Fig. 3A and B). The cytosolic/nuclear distribution of WDR5 in HCMV- and mock-infected cells was further defined by immunoblot analysis (IB) of cytosolic and nuclear fractions from infected cells harvested at 96 hpi (Fig. 3C). The efficiency of nuclear and cytosolic fractionation was confirmed by probing for the cellular markers GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and lamin B1, which localize to cytoplasm and nuclei, respectively, as well as for viral proteins pp28 (pUL99) and IE1, which also localize to the cytosol and nucleus, respectively. In mock-infected cells, WDR5 was distributed almost exclusively in the nuclear fraction, with minimal amounts detected in the cytosol. In contrast, HCMV-infected cells contained less WDR5 protein in the nuclear fraction but a significantly higher level of WDR5 protein in the cytosolic fraction (Fig. 3C). Previous studies have shown that nuclear membranes are significantly remodeled during HCMV infection (31). To exclude a possibility that higher levels of WDR5 in the cytoplasm of infected cells resulted from increased translocation of nuclear protein secondary to loss of the integrity of the nuclear membrane, leptomycin B (LMB) was used to inhibit nuclear export (31). Nuclear retention of WDR5 was clearly observed, and juxtanuclear accumulation of WDR5 was prevented by LMB treatment, as was also noted for the viral tegument protein pp65 (Fig. 3D and E). These data suggested that localization of WDR5 to the cytoplasm and more specifically the juxtanuclear region of the cell during HCMV infection required active translocation from the nucleus and did not represent either loss of nuclear membrane integrity or expression of distinct cytosolic and nuclear pools of WDR5.

FIG 2 Legend (Continued)

were infected for 72 h and then treated with 2 μ M NOC for the times indicated prior to staining for WDR5. All antibodies used in IFA were mouse monoclonal antibodies; nuclei were counterstained with DAPI. Scale bars = 10 μ m.

FIG 3 HCMV infection induces relocation of WDR5. (A) HELFs were mock infected (M) or HCMV infected (V) at an MOI of 3 and then processed for immunofluorescence with antibodies against IE1 (green), WDR5 (red), or nuclei (DAPI, white) at the indicated times postinfection. The images shown are representative of three independent experiments. All antibodies used in IFA were mouse monoclonal antibodies. (B) Over 100 cells in five representative fields from each independent experiment described in panel A were counted to determine the percentages of infected cells exhibiting diffuse nuclear localization of WDR5 (normal) versus accumulation of WDR5 in juxtanuclear foci. Data were analyzed using the chi-square test. *n*, number of counted cells; NS, not significant; ***, *P* < 0.001. (C) HELFs were mock infected (M) or HCMV infected (V) at an MOI of 3. At 96 hpi, cytosolic and nuclear fractions were prepared and analyzed by IB for WDR5, pp28, IE1, GAPDH, or lamin B1. (D) HELFs were mock infected or HCMV infected at an MOI of 1. At 48 hpi, infected cells were treated with dimethyl sulfoxide (DMSO) or nuclear export inhibitor leptomycin B (LMB; 5 μM) for 12 h and were costained for WDR5 (red) and viral protein pp65 (green), and nuclei were counterstained with DAPI (white). All antibodies used in IFA were mouse monoclonal antibodies. (E) Over 100 cells in five representative fields from each experiment described in panel D were counted and used to determine percentages of infected cells exhibiting normal diffuse nuclear localization of WDR5 versus infected cells in which WDR5 accumulated in juxtanuclear foci. Data were analyzed using the chi-square test. *n*, number of counted cells; NS, not significant; ***, *P* < 0.001. Scale bars = 10 μm.

WDR5 juxtanuclear accumulation is viral replication dependent. To determine the kinetic class of viral gene expression required to alter WDR5 localization, HELFs were incubated with HCMV virions that were UV irradiated to block all *de novo* viral gene expression or with infectious virions in the presence of either of the viral DNA polymerase inhibitors ganciclovir (GCV) and phosphonoacetic acid (PAA) to inhibit viral

FIG 4 WDR5 accumulates in the vAC requires viral replication. HELFs were mock infected (M), UV-inactivated HCMV infected (UV), or live HCMV infected (V) at an MOI of 3 in the presence of DMSO, GCV (100 μ g/ml), or PAA (100 μ g/ml). At 72 hpi, samples were collected and analyzed. (A) The upper panel shows protein levels of WDR5 and viral proteins determined by IB with actin as a loading control. The lower panel shows the relative levels of WDR5 protein in infected versus mock-infected cultures normalized with GAPDH. Data from three independent experiments were analyzed by one-way ANOVA, and the results are presented as means $\pm$ SD. *, $P < 0.05$; **, $P < 0.01$; NS, no significance. (B) Relative mRNA levels of WDR5. Samples were harvested at the indicated times, and total RNA was isolated. Relative mRNA levels of WDR5 were determined by qRT-PCR; GAPDH is an internal control. Data were normalized to levels in mock-infected cells to provide fold changes post-HCMV infection. (C) Accumulation of WDR5 in the juxtanuclear region. HELFs were infected and treated under the same condition as described in panels A and B. WDR5 juxtanuclear foci are indicated by white arrows. Scale bars = 10 μ m.

DNA synthesis and efficient expression of virus late genes. Immunoblot analysis of infected cell lysates at 72 hpi confirmed that UV irradiation blocked expression of viral proteins of all kinetic classes, including IE1/IE2, the early/late protein pUL44, and the true late protein glycoprotein B (gB). In contrast, infection in the presence of either GCV or PAA had no effect on IE1 expression, while IE2 and pUL44 levels were reduced but still detectable, and expression of the virion structural protein gB was dramatically inhibited and was undetectable (Fig. 4A). The levels of expression of WDR5 protein and mRNA were compared during HCMV infection with or without GCV or PAA treatment. Compared to the mock-infected group, levels of both WDR5 protein (Fig. 4A) and mRNA (Fig. 4B) were elevated in virus-infected cells in either the presence or absence of either GCV or PAA. Of note, in HCMV-infected cells, WDR5 protein levels were not significantly altered by GCV or PAA treatment.

We further investigated whether WDR5 juxtanuclear accumulation was altered by treatment of GCV or PAA. Characterization of replicate cultures by an immunofluorescence assay (IFA) revealed that WDR5 localization was similar to that in mock-infected

cells when virions were inactivated by exposure to UV. Both GCV and PAA dramatically inhibited juxtanuclear accumulation of WDR5 and juxtanuclear focus formation (Fig. 4C). Thus, even though the levels of expression of both WDR5 protein and mRNA were increased in HCMV-infected cells in the presence of GCV and PAA (Fig. 4A and B), the juxtanuclear localization of WDR5 was inhibited. These results indicated that WDR5 juxtanuclear localization was not explained simply by increased WDR5 expression but more likely secondary to relocation of WDR5 in the infected cell by mechanisms dependent on HCMV DNA synthesis and/or *de novo* expression of late viral proteins.

WDR5 contributes to vAC formation and virion morphogenesis. To determine if WDR5 contributes to formation of vAC, we used a pair of HELF-derived cell lines: one in which the expression of WDR5 was knocked down (KD) by short hairpin RNA (shRNA) and a second cell line expressing an irrelevant control (Ctl) shRNA. Both cell lines have been previously described (51). As seen in Fig. 5A, WDR5 expression in KD cells was significantly decreased. The Ctl and KD cells were then infected with HCMV at a multiplicity of infection (MOI) of 1, and infectious virus titers in the cell culture were determined at different days postinfection (dpi) to generate single growth curves. The virus titers produced by KD cells were 8.2-fold lower than those produced by Ctl cells at 5 dpi at an MOI of 1 (Fig. 5B). In KD cells, the morphogenesis of the vAC, as determined by staining for gB, was markedly altered in some but not all of the cells. Infected cells containing an atypical vAC exhibited multiple juxtanuclear foci, while in others, a distinct vAC was not observed (Fig. 5C). Because the assembly of mature infectious virions has been shown to require the formation of a mature vAC and the KD of WDR5 dramatically decreased the number of cells with a mature vAC, we utilized transmission electron microscopy (TEM) in Ctl and KD cells to define virion morphology (51). As shown in Fig. 5D (panels f to k), knockdown of WDR5 resulted in a decrease of mature virions and distinct defects in virion maturation (white arrows). Quantitation of these data revealed that nearly 39% of viral particles lacked envelope in KD cells, compared to only 6% in Ctl cells (Fig. 5E). Taken together, these data further suggest that WDR5 contributes to both vAC formation and virion morphogenesis.

WDR5 is incorporated into HCMV particles. Since WDR5 accumulated in the vAC and expression of WDR5 was correlated with virion morphogenesis, we next determined if WDR5 is incorporated into viral particles. HFFs were mock infected or HCMV infected, and intra- and extracellular particles were prepared by combining supernatants with sonicated cell lysate followed by centrifugation in an iodixanol density gradient (Fig. 6A). Fractions were collected from top of the gradient and analyzed by immuno-dot blotting for the presence of WDR5, gB, or pUL48 and by quantitative PCR (qPCR) for viral DNA. WDR5 was undetectable in all fractions derived from mock-infected cultures (Fig. 6B). In contrast, WDR5 was detected in fractions derived from HCMV-infected cultures and with peak levels of WDR5 corresponding to those containing peak concentrations of gB, ppUL48, and viral DNA (Fig. 6B and C), raising the possibility that WDR5 is incorporated into HCMV particles.

To determine whether WDR5 is incorporated into the virion, extracellular virions were purified from infected-cell culture supernatants as described previously (55). The purified virions were then either mock treated or treated with 2% Triton X-100 at room temperature for 1 h, layered onto a 10% to 50% iodixanol continuous gradient, and ultracentrifuged. Fractions were collected from the top of the gradient, and the presence of WDR5, structural viral proteins, including major capsid protein (MCP), tegument proteins pp28, pp65, pp71, pIR51, pTRS1, and ppUL48, and the envelope protein glycoprotein B (gB), was determined by immunoblotting. The nonstructural virus-encoded proteins IE1/2 and pUL44 were present in the infected-cell lysates but were not detected in any gradient fractions, indicating that under these conditions, fractions from these gradients were enriched in cell-free virions (Fig. 7A). As shown previously, gradient fractions of mock-treated virions, fractions 9 and 10, contained peak levels of gB, the tegument protein ppUL48, and WDR5 (Fig. 7A, upper panel). In contrast, in gradient fractions derived from virions incubated with Triton X-100 prior to centrifugation, the peak levels of MCP and tegument proteins had shifted to fraction 11 (Fig. 7A).

FIG 5 Knockdown of WDR5 altered vAC formation and virion morphogenesis. HELFs were transduced with shRNA to WDR5 (KD) or a scrambled control shRNA (Ctl) as described previously (51). (A) WDR5 protein level in the established cell lines. WDR5 levels in Ctl cells and KD cells were examined by IB. (B) Growth kinetics of viruses. Ctl and KD cells were infected with HCMV at an MOI of 1. Culture supernatants were collected at the indicated times postinfection, and infectious virus titers were determined by a plaque formation assay. Data were collected from three independent experiments performed in triplicate and analyzed by one-way ANOVA. Results are presented as means $\pm$ SDs. **, $P < 0.01$. (C) Cells were infected with HCMV at an MOI of 0.5, harvested at 96 hpi, and stained for gB with mouse monoclonal antibody, and nuclei were counterstained with DAPI (left panels [scale bars = 10 μ m]). Based on morphology, vACs were classified as regular or irregular, and representative images are shown (bottom left, panels a, b, c, and d). Regular and irregular vACs were quantitated (right panel). n , number of quantitated infected cells. Data were analyzed by chi-square test. ***, $P < 0.001$. (D) Ctl (a to e) and KD (f to k) cells were infected with HCMV at an MOI of 0.5. At 120 hpi, cells were negative stained and analyzed by TEM. Representative images are shown to illustrate the enveloped virions (arrowheads) and nonenveloped capsids (arrows) in the cytoplasm. (E) Quantitation and classification of viral particles. About 300 total cytoplasmic viral particles were counted. The percentages of morphologically normal virions or nonenveloped capsids in each infection are illustrated. Data were analyzed by chi-square test. n , number of viral particles counted in cells. ***, $P < 0.001$.

Importantly, most of the envelope protein gB was removed by Triton X-100 treatment, while the remaining gB was also shifted to lower-density fractions that cofractionated with MCP (Fig. 7A, lower panel). These results are consistent with Triton X-100 removal of the envelope from the virion particles and argued that WDR5 was incorporated into the virion and not copurifying with enveloped virions. Lastly, a significant amount of

FIG 6 WDR5 copurifies with HCMV particles by gradient fractionation. (A) Flow chart illustrating the analysis of particles from mock- or HCMV-infected cultures by iodixanol density gradient centrifugation (see Materials and Methods for details). (B) Gradient fractions derived from mock-infected (M) or HCMV-infected (V) cultures were analyzed by immune-dot blotting for WDR5, gB, or pUL48. (C) Viral genome copies in each fraction were determined by qPCR and plotted together with protein levels quantitated using ImageJ software from the dot blots shown in panel B.

WDR5 remained in the capsid-containing fraction (number 11) following Triton X-100 treatment (Fig. 7A, lower panel). In addition, WDR5 was also associated with the tegument layer, as evidenced by cosedimentation with pp65, pp71, pIR51, pTRS1, and pp28 (Fig. 7A, lower panel). These findings were further confirmed by the demonstration that capsids purified from infected-cell nuclei or supernatant virions both contained

FIG 7 WDR5 is present in HCMV virions. (A) Purified HCMV virions were mock treated or treated with 2% Triton X-100 for 1 h at room temperature then analyzed by iodixanol density gradient centrifugation. Fractions collected from gradients of virions that were mock treated (top) or Triton X-100 treated (bottom), as well as unfractionated lysates from mock-infected (M) or HCMV-infected (V) HELFs, were analyzed by IB using the indicated antibodies. (B) HCMV virions were purified as previously described. Nuclei from infected HELs were prepared at 60 hpi, and intranuclear capsids were purified as in described in Materials and Methods. Purified virions and capsids were visualized by electron microscopy. The components (viral proteins and WDR5) were analyzed by IB.

WDR5 when analyzed by immunoblotting (Fig. 7B). Taken together, these results indicate that WDR5 associated with capsids before nuclear egress, translocated to the vAC, and then was incorporated into enveloped virions.

WDR5 interacts with viral structural proteins that traffic to the vAC. Our findings raised the possibility that WDR5 interacted not only with MCP but also with other virion proteins and these interactions facilitated its localization in the vAC and, ultimately, incorporation into virions. To investigate the latter hypothesis, Flag-tagged WDR5 was overexpressed in HELF cells followed by mock or HCMV infection. After 96 h of infection, cell lysates were immunoprecipitated (IP) with anti-Flag antibody and subjected to SDS-PAGE and Coomassie blue staining. There were four different distinct protein species (Fig. 8A, red arrows) that were detectable in the immunoprecipitates from virus-infected cells that were not present in immunoprecipitates from the mock infection controls. These four species were combined and further analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify and quantify the proteins. The viral structural proteins and the corresponding numbers of peptides derived from each protein, including MCP, pp150, pp65, pIRS1, and pTRS1, are shown in Fig. 8B.

To confirm these WDR5-viral protein interactions, HEK293T cells were cotransfected with plasmids encoding hemagglutinin (HA)-tagged WDR5, as well as each of the candidate viral protein interactors (Myc-MCP, Flag-pp65, Myc-pp150, Flag-pIRS1, or pTRS1-V5). Each viral protein was immunoprecipitated using its respective epitope tag, and the immunoprecipitates were probed for HA-WDR5 by IB. HA-WDR5 was detected in IPs of MCP, pp65, pp150, pIRS1, and pTRS1, but not those of pp28 or the IgG controls (Fig. 8C to H). We further validated these interactions in HCMV-infected HELFs and determined that WDR5 interactions with MCP, pIRS1, and pTRS1 were independent of DNA or RNA binding (Fig. 8I). In contrast, a WDR5-pp65 interaction was lost when lysates were pretreated with a DNase/RNase cocktail, indicating that this WDR5-pp65 interaction could be mediated through nucleic acid binding (Fig. 8I). Taken together, these results suggested that WDR5 interacts with several different viral structural proteins and provides a possible explanation for the accumulation of WDR5 in vAC, as well as a potential mechanism for a role of WDR5 in virion morphogenesis.

DISCUSSION

During viral infections, cellular proteins can be incorporated into viral particles both nonspecifically and secondary to specific interactions with virion structural proteins. In many cases, cellular proteins are incorporated randomly without defined stoichiometry and have not been shown to play a role in the infectivity of the mature virion, whereas in other cases, cellular proteins selectively incorporated into virions have been proposed to play a role in virion assembly and/or have a role in the infectivity of the progeny virion. In this regard to HCMV, Varnum et al. identified over 70 host proteins in virions by LC-MS/MS (35). These cellular proteins function in diverse processes, including ATP and Ca^{2+} binding, chaperones, cytoskeleton, enzymatic activities, glycolysis, protein transport, signal transduction, transcription, and translation (35). Some have been suggested to function during specific stages of the infection process, suggesting that these proteins are incorporated into the virion to facilitate virus infectivity and replication, including evasion of host immune functions (35). Several different mass spectrometry-based strategies have been utilized to define the proteome of virus-encoded proteins and host cellular responses to HCMV infection in primary fibroblasts (56, 57). These studies have identified a vast number of host proteins and a large number of viral proteins at different time points during infection (56, 57). Interestingly, WDR5 was not identified in previous studies, including in the original study of the virion proteome reported by Varnum et al. (35, 56, 57). Although there is no obvious explanation for the differences in the results from our studies and these earlier studies, our studies enriched WDR5-interacting proteins prior to analysis by mass spectrometry and thus may have increased the recovery of the interacting viral proteins.

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prevent the formation of the vAC. Although the morphogenesis of the vAC was inhibited by inhibition of viral genome replication and late gene expression, the expression of WDR5 remained unchanged, arguing that localization of WDR5 to the vAC required HCMV late gene expression and microtubule polymerization and was not a result of increased expression of WDR5. Taken together, these findings suggest that WDR5 accumulation in the vAC results from specific HCMV-induced cellular responses, although this mechanism and effectors of these responses remain undefined.

Previously, we reported that knockdown of WDR5 decreased the infoldings of the inner nuclear membranes (INMs) and the numbers of capsids budding from the nucleoplasm into the enlarged nuclear cisterna, thus affecting nuclear egress (51). During the process of nuclear egress, the subviral particle undergoes temporary primary envelopment and de-envelopment process at the nuclear cisterna (56). The resolution of our electron microscopy (EM) images is not high enough to distinguish whether the primary envelopment in INM is affected by WDR5 depletion. However, the numbers of capsids in INMs significantly decreased in KD cells, which suggested that WDR5 depletion affects the HCMV capsids' nuclear egress (51). Following knockdown of WDR5, approximately 39% of viral particles in the cytoplasm were unenveloped. Thus, depletion of WDR5 appears to affect the secondary envelopment of HCMV. Whether the defect in secondary virus envelopment in infected cells in which the expression of WDR5 is decreased is a consequence of decreased nuclear egress of tegumented capsids cannot be ruled out by findings in this study and requires further investigation.

WDR5 fractionated with viral proteins present in viral particles, purified virions, or capsids, indicating that WDR5 was incorporated into virions. Furthermore, we showed that WDR5 interacted with virion capsid MCP and tegument proteins pp65, pp150, pIRS1, and pTRS1, suggesting that WDR5 resides in an internal layer of the virion and not in the envelope. Findings consistent with this proposed topology of WDR5 in the virion were provided by the finding that WDR5 remained associated with virions following treatment with Triton X-100, which removed the envelope from the mature virion. Although from available data we cannot definitively assign the location of WDR5 in the virion, our results would argue that a likely location would be the inner tegument layer of the virion.

In summary, the data provided in this study suggest several potentially linked roles for WDR5 during HCMV assembly (Fig. 9). During nuclear egress, WDR5 modulates assembly of the nuclear egress complex (NEC) to facilitate capsid nuclear egress. Subsequently, it translocates along with capsids from the nucleus into the cytoplasm secondary to its interactions with MCP and possibly with other virion proteins. Interactions with multiple virion structural proteins facilitate its localization in the vAC, where it could contribute to virion morphogenesis by stabilizing multiprotein complexes through its function as a scaffold for virion structural protein interactions. Although a role of WDR5 as a scaffold for the assembly of the HCMV virion would be consistent with the data presented in this study, at this time, this function for WDR5 in HCMV assembly remains speculative, and further studies are required to define its function. However, these findings add to existing evidence that HCMV manipulates existing cellular mechanisms, including the localization of cellular proteins in infected cells to facilitate the assembly of progeny virions.

MATERIALS AND METHODS

Ethics statement. Human embryonic lung fibroblast cells (HELs) were isolated from postmortem embryo lung tissue. The original source of the anonymized tissues was Zhongnan Hospital of Wuhan University (China). The cell isolation procedures and research plans were approved by the Institutional Review Board (IRB) (WIVH10201202) according to the Guidelines for Biomedical Research Involving Human Subjects at Wuhan Institute of Virology, Chinese Academy of Sciences. The need for written or oral consent was waived by the IRB (57).

Cells and cell culture. HELs were isolated and maintained as described previously (57). HELFs, kindly provided by Jason J. Chen at Columbia University, are human embryonic lung fibroblasts that have been retrovirally transduced with human telomerase (hTERT). Clonal cell lines isolated from HELFs that were transduced with WDR5 shRNA (KD) and scrambled shRNA (Ctl) were described previously (51). Both HELs and HELFs were cultured in minimum essential medium (MEM [catalog no. 41500-034; Thermo

FIG 9 Working model. In the nucleus, WDR5 facilitates capsid nuclear egress by promoting assembly of the NEC as described by Yang et al. (51). WDR5 may egress from the nuclei with the capsids and then be transported to the vAC together with capsids; WDR5 interacts with tegument proteins and accumulates in the vAC. In the vAC, WDR5 is incorporated into maturing virions.

Fisher]) supplemented with 10% fetal bovine serum (FBS [catalog no. 10099-141; Thermo Fisher]) and penicillin-streptomycin (100 U/ml and 100 μ g/ml, respectively [catalog no. 15140-122; Thermo Fisher]). Human embryonic kidney 293T cells (HEK293T) were purchased from ATCC (CRL-11268) and cultured in Dulbecco's modified Eagle's medium (DMEM [catalog no. 11995-123; Thermo Fisher]) supplemented with 10% FBS and penicillin-streptomycin, as described above. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂.

Viruses. HCMV Towne strain (ATCC VR 977) was used in this study. HSV-1 strain H129 expressing green fluorescent protein (GFP) was previously constructed by our laboratory (58). The VZV Oka strain expressing GFP (59) was a gift from Hua Zhu, Rutgers-New Jersey Medical School, and MCMV strain K181 was a gift from Qiyi Tang, Howard University College of Medicine. GFP-expressing AdV and AcMNPV were provided by Zhengli Shi and Zhihong Hu, respectively, Wuhan Institute of Virology, Chinese Academy of Sciences.

Plasmid construction. Primers used for plasmid construction are shown in Table 1. WDR5 cDNA (GenBank accession no. [NM_017588](#)) was derived by reverse transcription as described previously (51). The cDNA was PCR amplified and then cloned into either EcoRI/XhoI-restricted pCMV-HA to generate plasmid HA-WDR5 or into XbaI/EcoRI-restricted pCDH-Flag to generate Flag-WDR5. The UL83 ORF encoding pp65 and IRS1 ORF encoding pIR51 were PCR amplified from the Towne-BAC genome (60) and cloned into BamHI/KpnI-restricted pXJ40-Flag to generate plasmids Flag-pp65 and Flag-pIR51,

TABLE 1 Primers used in this study

Plasmid	Primer ^a
HA-WDR5	F: 5'- <u>GGAATTC</u> GAGTGGCGACGGAGGAGAAGAAGC-3' R: 5'- <u>CCGCTCGAG</u> TAGCAGTCACTCTCCACAGTT-3'
Flag-WDR5	F: 5'- <u>GCTCTAGA</u> ATGGCGACGGAGGAGAAGAAGC-3' R: 5'- <u>GGAATTC</u> GCGCAGTCACTCTCCACAGTTT-3'
Flag-pp65	F: 5'- <u>CGGGATCC</u> ATGGAGTCGCGCGGTGCGCGTTGTCC-3' R: 5'- <u>CGGGGTACCT</u> CAACCTCGGTGCTTTTGGGCG-3'
pp28-6×His	F: 5'- <u>CGGGATCC</u> ATGGGTGCCGAACCTGCAAACG-3' R: 5'- <u>GCTCTAG</u> ACTAAAGGGCAAGGAGGCGGCGG-3'
Flag-IRS1	F: 5'- <u>CGGGATCC</u> ATGGCCCAGCGCAACGGCATGTC-3' R: 5'- <u>GGGTACCT</u> CAATGATGAACGTGGTGAGGGGCG-3'
6Myc-pp150	F: 5'-GGCGCGGCAGCCGCTTCAAGTTTGAGTTTATCGGTCTACAGCG-3' R: 5'-GAGGTTGATTAGGATCTATCGATCTATTCCTCCGTGTTCTTAATCTTC-3'
6Myc-MCP	F: 5'-GGCGCGGCAGCCGCTTCAAGAACTGGTCGCGCTCGAGCTCC-3' R: 5'-GAGGTTGATTAGGATCTATCGATTACGAGTTAAATAACATGGATTG-3'

^a, F, forward; R, reverse. Restriction enzyme sites are underlined.

respectively. The *UL99* ORF encoding pp28 was PCR amplified and cloned into BamHI/XbaI-restricted pcDNA3.1-V5/6×His to generate plasmid pp28-6×His. The *UL86* ORF encoding MCP and *UL32* ORF encoding pp150 were amplified from the Towne-BAC genome (60) and inserted into a vector with 6×Myc by cloning recombination (ClonExpress II One Step Cloning kit, catalog no. C112; Vazyme). The *TRS1* ORF encoding pTRS1 fused with a carboxyl-terminal 6×His epitope tag and V5 tag was a gift from Adam P. Geballe, Fred Hutchinson Cancer Research Center and University of Washington, which had been previously described as pEQ981 (61). All the primers used in this study are listed in Table 1.

Transient transfection. HEK293T cells (1.5×10^6) were seeded into 100-mm dishes. The next day, the medium was changed 2 h prior to transfection via $\text{Ca}_2(\text{PO}_4)_2$ precipitation with 10 μg HA-WDR5 along with 10 μg pp28-His, Myc-MCP, Flag-pp65, Myc-pp150, Flag-pIRS1, or pTRS1-V5, as described previously (57). HELFs (2×10^6) were seeded onto coverslips in 12-well plates. Medium was changed at 2 h prior to transfection the next day. Plasmids (1.2 μg for each well) expressing full-length and truncated WDR5 were transfected into HELFs using Lipofectamine 2000 reagent (catalog no. 11668-019; Thermo Fisher) according to the manufacturer's instructions.

Quantitative reverse transcriptase PCR. For quantitative reverse transcriptase PCR (qRT-PCR), HELFs were infected at an MOI of 3 and harvested at the indicated times postinfection. A total of 1×10^6 cells were used for total RNA extraction using RNAiso Plus reagent (catalog no. 9109; TaKaRa), followed by treatment with 10 U of recombinant DNase I (catalog no. 2270A; TaKaRa) to remove residual DNA. One microgram of RNA of each sample was reverse transcribed with a RevertAid H minus first-strand cDNA synthesis kit (catalog no. K1631; Fermentas) with random primers. Then qPCR was performed on a real-time Connect thermocycler (Bio-Rad) using SYBR green PCR master mix (catalog no. 4309155; Applied Biosystems) in 20- μl reaction mixtures for 40 PCR cycles as described previously (57). The PCR primers for WDR5 were 5'-GGTGGGAAGTGGATTGTGTC-3' and 5'-GCAGCAGAGGCGATGATG-3'. The PCR primers for GAPDH were 5'-GAGTCAACGGATTGGTCGT-3' and 5'-GACAAGCTTCCCGTTCTCAG-3'.

IB. Cells were harvested in cell lysis buffer (catalog no. P0013; Beyotime) containing protease inhibitor cocktail (catalog no. 04693159001; Roche) and homogenized by ultrasonication. Protein concentrations of lysates were determined by Bradford assay (catalog no. 500-0205; Bio-Rad). After boiling with loading buffer, cell lysates containing equal amounts of protein were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (catalog no. ISEQ00010; Millipore). Membranes were sequentially probed with primary antibodies and the appropriate peroxidase-conjugated secondary antibodies. All antibodies used for IB are listed in Table 2. The antibodies used to detect IE1 (62), pUL48 (63), pIRS1 (64), pTRS1 (64), MCP (65), and mIE1 (66) were described previously. Blots were developed using the SuperSignal West Femto chemiluminescent substrate (catalog no. 34095; Thermo Fisher), signals were detected using the FluorChem HD2 system (Alpha Innotech), and quantification was performed using ImageJ software (NIH).

IP. HEL cells were mock infected or infected with HCMV strain Towne at an MOI of 3 and harvested at 72 hpi. Cells were lysed in IP lysis buffer (catalog no. P0013; Beyotime) for 1 h at 4°C and then centrifuged at $120,000 \times g$ for 5 min to remove cell debris. Samples were mock treated or nuclease treated by incubation with 10 mM MgCl_2 , 100 $\mu\text{g}/\text{ml}$ Dnase I (catalog no. 2270A; TaKaRa) and 100 $\mu\text{g}/\text{ml}$ RNase A (catalog no. AC118; Omega) for 1 h at 37°C. IP was performed by incubation of the resulting lysates overnight at 4°C with mouse monoclonal anti-Flag antibody (catalog no. F3165; Sigma) or with normal mouse IgG (catalog no. A7028; Beyotime). Protein A+G agarose beads (catalog no. P2012; Beyotime) were added, incubated for 3 h at 4°C, and then washed five times with lysis buffer. Loading buffer was added, and samples were boiled for 5 min before separation by SDS-PAGE followed by IB for detection of WDR5, pp65, or pp28, as described above.

TABLE 2 Primary and secondary antibodies used in IFA and IB

Antibody	Host (isotype)	Source or reference
Primary antibodies		
Normal mouse IgG	Mouse polyclonal	Beyotime; catalog no. A7028
Epitope tag		
Flag	Mouse monoclonal (IgG1)	Sigma; catalog no. F3165
HA	Mouse monoclonal (IgG1)	Sigma; catalog no. H9658
6×His	Mouse monoclonal (IgG1)	Proteintech; catalog no. 66005-1-Ig
	Rabbit polyclonal	Abcam; catalog no. ab9108
V5	Rabbit polyclonal	Proteintech; catalog no. 14440-1-AP
Myc	Mouse monoclonal (IgG1)	Proteintech; catalog no. 66003-2-Ig
Cellular		
WDR5 (36 kDa)	Rabbit polyclonal	Sigma; catalog no. 07-706
	Mouse monoclonal (IgG2b)	Abcam; catalog no. ab56919
	Mouse monoclonal (IgG2b)	Santa Cruz; catalog no. sc-100895
GAPDH (36 kDa)	Rabbit polyclonal	Proteintech; catalog no. 10494-1-AP
GFP (30 kDa)	Rabbit polyclonal	Proteintech; catalog no. 50430-2-AP
Lamin B1 (66 kDa)	Rabbit polyclonal	Proteintech; catalog no. 12987-1-AP
β-Actin (43 kDa)	Mouse monoclonal (IgG1)	Proteintech; catalog no. 60008-1-Ig
α-Tubulin (50 kDa)	Mouse monoclonal (IgG1)	Beyotime; AT819
γ-Tubulin (48 kDa)	Mouse monoclonal (IgG1)	Gift from Yan Zhou, Wuhan University, China
EEA1 (180 kDa)	Mouse monoclonal (IgG1)	Abcam; catalog no. ab70521
58K (96 kDa)	Mouse monoclonal (IgG1)	Abcam; catalog no. ab27043
HCMV		
IE1 (UL123) (72 kDa)	Mouse monoclonal (IgG2a)	62
IE1/2 (UL123/122) (72/86 kDa)	Mouse monoclonal (IgG1)	Virusys; catalog no. P1215
pUL44 (ICP36) (46 kDa)	Mouse monoclonal (IgG1)	Virusys; catalog no. P1202-1
gB (UL55) (52 and 105 kDa)	Mouse monoclonal (IgG1)	Virusys; catalog no. P1201
pp71 (UL82) (71 kDa)	Goat polyclonal	Santa Cruz; catalog no. sc-33323
pp65 (UL83) (65 kDa)	Mouse monoclonal (IgG1)	Virusys; catalog no. P1205
pp28 (UL99) (28 kDa)	Mouse monoclonal (IgG2a)	Virusys; catalog no. CA004-1
pUL48 (253 kDa)	Rabbit polyclonal	Gift from Wade Gibson, Johns Hopkins University (63)
pIRS1 (110 kDa)	Mouse monoclonal (IgG1)	Gift from Thomas E. Shenk, Princeton University (64)
pTRS1 (90 kDa)	Mouse monoclonal (IgG1)	Gift from Thomas E. Shenk, Princeton University (64)
MCP (150 kDa)	Mouse monoclonal (IgG2a)	65
MCMV		
mIE1 (m123) (89 kDa)	Mouse monoclonal (IgG1)	Gift from Qiyi Tang, Howard University College of Medicine (66)
Secondary antibodies		
Peroxidase–anti-mouse IgG	Goat	Jackson ImmunoResearch Laboratories; catalog no. 115-035-003
Peroxidase–anti-rabbit IgG	Goat	Jackson ImmunoResearch Laboratories; catalog no. 111-035-003
Peroxidase–anti-goat IgG	Donkey	Jackson ImmunoResearch Laboratories; catalog no. 705-035-147
TRITC–anti-mouse IgG2b	Goat	Southern Biotech; catalog no. 1090-03
TRITC–anti-mouse IgG2a	Goat	Southern Biotech; catalog no. 1080-03
AF488–anti-mouse IgG1	Goat	Thermo Fisher; catalog no. A-21121
AF488–anti-mouse IgG2b	Goat	Thermo Fisher; catalog no. A-21141
AF647–anti-mouse IgG1	Goat	Thermo Fisher; catalog no. A-21240
AF647–anti-mouse IgG2a	Goat	Thermo Fisher; catalog no. A-21241
10-nm-gold particle–anti-mouse IgG	Goat	Boster; catalog no. GA1004

IFA. HELFs or HFFs were seeded onto coverslips and after attachment infected with HCMV at an MOI of 3. Coverslips were collected at the indicated times postinfection and fixed with 4% paraformaldehyde. Target proteins were detected by incubation with primary antibodies and appropriate secondary antibodies, as listed in Table 2 and as described previously (51). Nuclei were counterstained with DAPI (4',6-diamidino-2-phenylindole [catalog no. D9542; Sigma]). Images were obtained using a UltraVIEW VoX (Perkin Elmer) spinning-disk laser confocal scanning microscope. 3D z-axis image stacks were acquired with 0.2-μm spacing, and 3D modeling was performed using Volocity 5.5 software (Perkin Elmer). Images were median filtered to reduce noise, and contrast was enhanced to improve resolution.

Cytosolic and nuclear fractionation. HELFs were mock infected or infected with HCMV at an MOI of 3, harvested at 72 hpi, and fractionated using the Qproteome cell compartment kit (catalog no. 37502; Qiagen) as described previously (67). Fractions were analyzed by IB using GAPDH and lamin B1 antibodies to confirm separation of cytosolic and nuclear fractions, respectively.

Quantitation of viral genome copy number. Genomic DNA from each fraction were analyzed by qPCR to quantitate viral DNAs using HCMV UL83 primers as described previously (57). Means and standard deviations (SD) from at least three independent experiments were calculated.

Virus particles and capsid purification. HELs were infected with HCMV at an MOI of 0.02 and harvested after the cytopathic effect (CPE) reached 100%. Culture medium was collected and clarified by centrifugation at $2,000 \times g$ for 10 min. Virus particles were pelleted from the supernatants at $100,000 \times g$ for 2 h at 4°C in an SW 32Ti rotor using a Beckman Optima L-100 XP ultracentrifuge. The pellet was resuspended and layered onto an iodixanol gradient that was prepared by sequentially layering 800- μ l volumes of 50, 40, 30, 20, and 10% iodixanol (catalog no. D1556; Sigma) in phosphate-buffered saline (PBS [vol/vol]) followed by incubation overnight 4°C. Gradients were then centrifuged at $100,000 \times g$ for 2 h at 4°C by using an SW 55Ti rotor and Optima MAX-XP ultracentrifuge. The virion-containing band was observed by light scattering, collected, and stored at -80°C .

For purification of nuclear capsids, infected HELs were harvested at 48 hpi, and cell pellets were washed with $1 \times$ PBS and incubated in NP-40 lysis buffer (0.5% NP-40, 5 M NaCl, 1 M Tris-HCl, pH 7.0) at 4°C for 20 min. Nuclei were centrifuged at $2,000 \times g$ for 10 min, resuspended in TNE (500 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0), sonicated (VCX130; Sonics) at 25% amplitude for 18 s (3 s on and 3 s off), and centrifuged at $2,000 \times g$ for 10 min. This step was repeated once, and combined supernatants were centrifuged at $10,000 \times g$ for 20 min at 4°C to remove cell debris. Supernatants containing capsids were subjected to iodixanol density gradient centrifugation as described above. Capsids were observed as light-scattering bands, collected, and stored at -80°C .

TEM. Control (Ctl) HELFs and the WDR5 knockdown (KD) HELFs were infected with HCMV at an MOI of 0.5 and harvested at 120 hpi. Cells were fixed with 2.5% (wt/vol) glutaraldehyde for 1 h at room temperature and then treated with 1% osmium tetroxide and dehydrated through a graded series of ethanol concentrations (from 30% to 100%) and embedded with an Embed 812 kit (Electron Microscopy Sciences, Fort Washington, PA). Ultrathin sections (60 to 80 nm) of embedded specimens were prepared and deposited onto Formvar-coated copper grids (200 mesh), stained with 2% (wt/vol) phosphotungstic acid (PTA [pH 6.8]), and observed under a Tecnai transmission electron microscope (FEI) operated at 200 kV as described previously (51).

LC-MS/MS analysis. The four distinct bands were excised and combined for LC-MS/MS analysis. LC-MS/MS analysis was performed on a Q Exactive mass spectrometer (Thermo Scientific) coupled to an Easy nLC (Proxeon Biosystems [now Thermo Fisher Scientific]) for 60 min. The mass spectrometer was operated in positive-ion mode. MS data were acquired using a data-dependent top 10 method dynamically choosing the most abundant precursor ions from the survey scan (m/z of 300 to 1,800) for high-energy collisional dissociation (HCD) fragmentation. The automatic gain control (AGC) target was set to 3e6 and maximum inject time to 10 ms. Dynamic exclusion duration was 40.0 s. Survey scans were acquired at a resolution of 70,000 at an m/z of 200, and resolution for HCD spectra was set to 17,500 at an m/z of 200. The isolation width was an m/z of 2. Normalized collision energy was 30 eV, and the underfill ratio, which specifies the minimum percentage of the target value likely to be reached at maximum fill time, was defined as 0.1%. The instrument was run with peptide recognition mode enabled.

Statistical analyses. Data were analyzed by chi-square test or one-way analysis of variance (ANOVA), as appropriate, using SPSS software (version 18.0; SPSS). Results are shown as means $\pm$ 1 standard deviation from three independent experiments. A P value of <0.05 was considered significant.

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The authors declare that they have no conflicts of interest for this work.

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