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Human cytomegalovirus modulates mTORC1 to redirect mRNA translation within quiescently infected monocytes

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ABSTRACT Human cytomegalovirus (HCMV) utilizes peripheral blood monocytes as a means to systemically disseminate throughout the host. Following viral entry, HCMV stimulates non-canonical Akt signaling leading to the activation of mTORC1 and the subsequent translation of select antiapoptotic proteins within infected monocytes. However, the full extent to which the HCMV-initiated Akt/mTORC1 signaling axis reshapes the monocyte translatome is unclear. We found HCMV entry alone was able to stimulate widescale changes to mRNA translation levels and that inhibition of mTOR, a component of mTORC1, dramatically attenuated HCMV-induced protein synthesis. Although monocytes treated with normal myeloid growth factors also exhibited increased levels of translation, mTOR inhibition had no effect, suggesting HCMV activation of mTOR stimulates the acquisition of a unique translatome within infected monocytes. Indeed, polyribosomal profiling of HCMV-infected monocytes identified distinct prosurvival transcripts that were preferentially loaded with ribosomes when compared to growth factor-treated cells. Sirtuin 1 (SIRT1), a deacetylase that exerts prosurvival effects through regulation of the PI3K/Akt pathway, was found to be highly enriched following HCMV infection in an mTOR-dependent manner. Importantly, SIRT1 inhibition led to the death of HCMV-infected monocytes while having minimal effect on uninfected cells. SIRT1 also supported a positive feedback loop to sustain Akt/mTORC1 signaling following viral entry. Taken together, HCMV profoundly reshapes mRNA translation in an mTOR-dependent manner to enhance the synthesis of select factors necessary for the survival of infected monocytes.

IMPORTANCE Human cytomegalovirus (HCMV) infection is a significant cause of morbidity and mortality among the immunonaïve and immunocompromised. Peripheral blood monocytes are a major cell type responsible for disseminating the virus from the initial site of infection. In order for monocytes to mediate viral spread within the host, HCMV must subvert the naturally short lifespan of these cells. In this study, we performed polysomal profiling analysis, which demonstrated HCMV to globally redirect mRNA translation toward the synthesis of cellular prosurvival factors within infected monocytes. Specifically, HCMV entry into monocytes induced the translation of cellular SIRT1 to generate an antiapoptotic state. Defining the precise mechanisms through which HCMV stimulates survival will provide insight into novel anti-HCMV drugs able to target infected monocytes.

KEYWORDS cytomegalovirus, monocytes, translation

Human cytomegalovirus (HCMV) is an endemic betaherpesvirus with seroprevalence rates ranging between 40% and 90% globally (1). HCMV infection of healthy immunocompetent individuals is generally self-limiting but may present as acute mononucleosis-like symptoms (2, 3). However, for patients with a compromised immune status, including HIV/AIDS, chemotherapy, or organ transplant patients, HCMV remains

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as a significant cause of morbidity and mortality (4–6). Furthermore, HCMV is the most common congenital infection often leading to deafness, blindness, and long-term neurologic sequelae in infected neonates (7–9). Acute HCMV disease is hallmarked by systemic viral dissemination and widespread inflammation leading to severe end-organ damage (10–12).

Peripheral blood monocytes are a critical cell type involved in the HCMV dissemination strategy (13–17). However, monocytes are inherently short-lived cells with an average lifespan of 48 h in the absence of differentiation stimuli following release from the bone marrow. Furthermore, monocytes are not fully permissive for HCMV replication (quiescent infection) unless differentiated into long-lived macrophages (14, 18). To overcome these obstacles, we have shown HCMV binding and entry generates a unique signalsome that promotes the survival and differentiation of short-lived monocytes into long-lived replication permissive macrophages (19–23). A major outcome of the HCMV-specific signalsome is the translation of select antiapoptotic proteins not upregulated during monocyte-to-macrophage differentiation initiated by normal myeloid growth factors (24–26).

During HCMV entry, viral glycoproteins gB and gH simultaneously engage epidermal growth factor receptor (EGFR) and integrin β1, respectively, to trigger signaling necessary for the survival and differentiation of HCMV-infected monocytes (27–30). Specifically, HCMV stimulates a persistent Akt activation following infection of monocytes, which was in contrast to the transient activation of Akt induced by EGF (24, 31). Furthermore, the synchronized activation of EGFR and integrin β1 stimulates a non-canonical activation of the PI3K/AKT signaling pathway leading to a site-specific phosphorylation at residue serine 473 (S473) on Akt rather than the canonical threonine 308 (T308) and S473 activation profile observed with normal myeloid growth factors (25). As the substrate specificity is dependent on the phosphorylation ratio between T308 and S473, the chronic site-specific phosphorylation at S473 indicates a distinct biological activity exhibited by HCMV-activated Akt (32–34). In support, Akt-dependent antiapoptotic factors Mcl-1 and HSP27 were translated at higher rates in HCMV-infected monocytes relative to growth factor-treated cells (24, 35).

mTOR is a downstream target of Akt and a component of the mTORC1 complex, which serves as a critical regulatory signaling hub mediating cap-dependent translation. HCMV-mediated signaling through mTORC1 leads to the phosphorylation of key translation initiation factors, including eIF4E, eIF4G, 4E-BP1, and S6K1 (36–38). These initiation factors work in concert to assemble the eIF4F translation initiation complex and facilitate recruitment of mRNAs. During times of cellular stress, such as viral infections, AMP-regulated kinase (AMPK) hinders mTORC1 through the tuberous sclerosis complex to limit cap-dependent translation (39–41). Simultaneously, AMPK upregulates the expression of protective cap-independent and internal ribosome entry site (IRES)-mediated translation, which are designed to be cytoprotective during stress events (42). However, HCMV drives the simultaneous activity of AMPK and mTORC1, allowing for both cap-independent and cap-dependent mRNA translation (43, 44). During HCMV lytic infection of fibroblasts, the viral protein pUL38 maintains cap-dependent translation by blocking the AMPK-mediated inhibition of mTORC1 (45, 46). In quiescently infected monocytes, HCMV usurps heat shock actor 1, a stress response factor, to uncouple AMPK and mTORC1 activities to allow for the simultaneous expression of both stress and non-stress survival factors (47). These studies suggest HCMV manipulates the PI3K/AKT/mTORC1 signaling axis to drive a highly unique translational landscape by simultaneously increasing both cap-dependent and cap-independent translation to promote the viability and differentiation of infected monocytes. However, the extent to which HCMV regulates the cellular translatome of quiescently infected monocytes and the role that the PI3K/AKT/mTORC1 pathway plays in shaping the global translatome remain unclear.

In this study, we report HCMV rapidly stimulates large-scale mRNA translation in quiescently infected monocytes that were sustained for at least 3 days, a time at which monocytes must make a cell fate decision to undergo cell death or survive and

differentiate into macrophages. The viral entry process was sufficient to stimulate cellular protein synthesis as UV-inactivated HCMV phenocopied "live" virus. Importantly, the majority of protein synthesis induced during HCMV infection was dependent on mTOR signaling, while growth factor-induced protein synthesis was independent, demonstrating the vital role of mTOR in maintaining cellular translation within quiescently infected monocytes. Polyribosomal profiling identified several prosurvival factors to be specifically translated in HCMV-infected, but not myeloid growth factor-treated, monocytes. We confirmed the elevated expression of sirtuin 1 (SIRT1), an NAD+-dependent deacetylase that promotes Akt activity, within infected monocytes. Importantly, SIRT1 was found to promote a feed forward loop to sustain AKT/mTORC1 signaling and the long-term expression of antiapoptotic factors. Our study suggests that HCMV entry triggers profound changes to the translatome dependent on mTORC1 in order to allow for the robust synthesis of prosurvival factors necessary for the differentiation of HCMV-infected monocytes into long-lived replication permissive macrophages.

RESULTS

HCMV stimulates mRNA translation within quiescently infected monocytes

During lytic replication, HCMV stimulates protein synthesis through a multitude of viral gene products, including TRS1, IRS1, and pUL38 (45, 46, 48–51). Accordingly, HCMV-infected fibroblasts pulsed with puromycin, which is incorporated into the elongating peptide chains (SUnSET labeling) (52, 53), exhibited a global increase in protein levels at 48 h post infection (hpi) that was abrogated by the presence of cycloheximide (CHX; a translation inhibitor) (Fig. 1A and B). These results are consistent with several groups demonstrating that HCMV infection stimulates protein synthesis during lytic infection (49, 51, 54–56). Elevated levels of translation were maintained through 72 hpi. UV-inactivated HCMV (UV-HCMV) had no effect on protein expression levels over the 72-h infection period, consistent with other studies showing the involvement of viral gene products in stimulating mRNA translation during a lytic infection (48, 49). We have previously shown that HCMV lytic transcripts are not expressed during the infection of monocytes (24, 57) and now confirm the absence of protein expression (Fig. 2A). To circumvent the lack of lytic prosurvival proteins, HCMV induces the translation of select cellular antiapoptotic

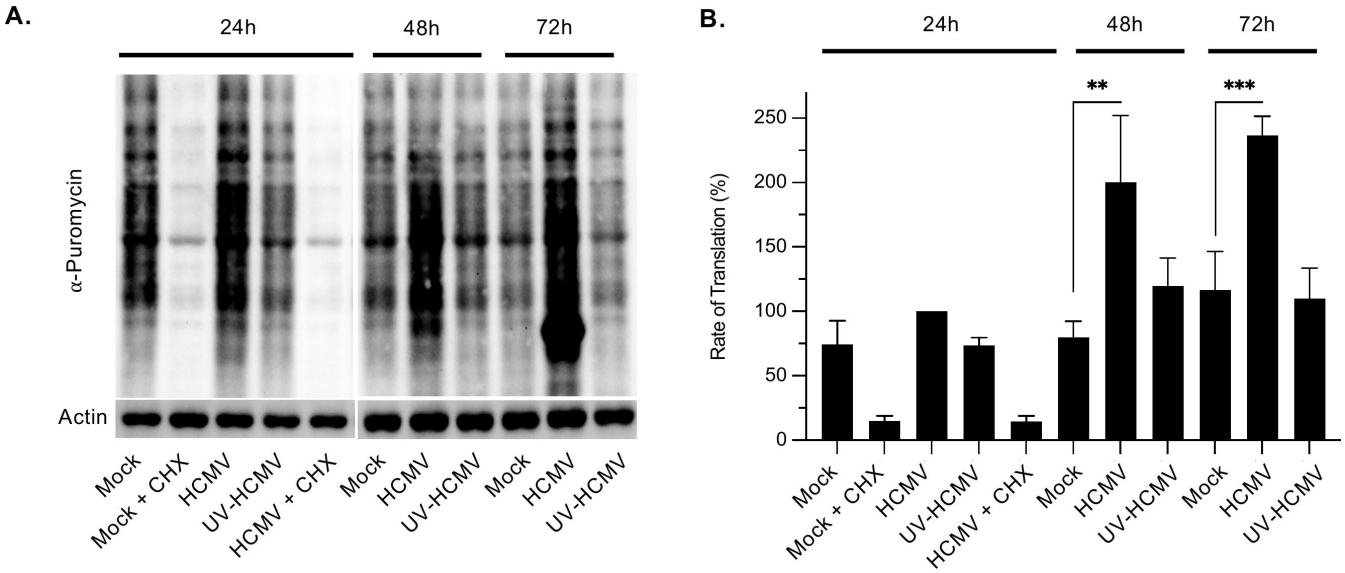

FIG 1 HCMV stimulates mRNA translation in fibroblasts dependent on *de novo* synthesized viral gene products. (A and B) Confluent human embryonic lung 299 fibroblasts were mock, HCMV, or UV-HCMV infected for 24, 48, or 72 h. Cycloheximide (10 µg/mL) was added to the culture media of appropriate treatment groups at 16 hpi prior to SUnSET peptide labeling. Densitometry was performed to determine total protein levels, which were normalized to actin. (B) Quantification was from three to five biological replicates from independent blood donors. **, $P < 0.005$; ***, $P < 0.0005$.

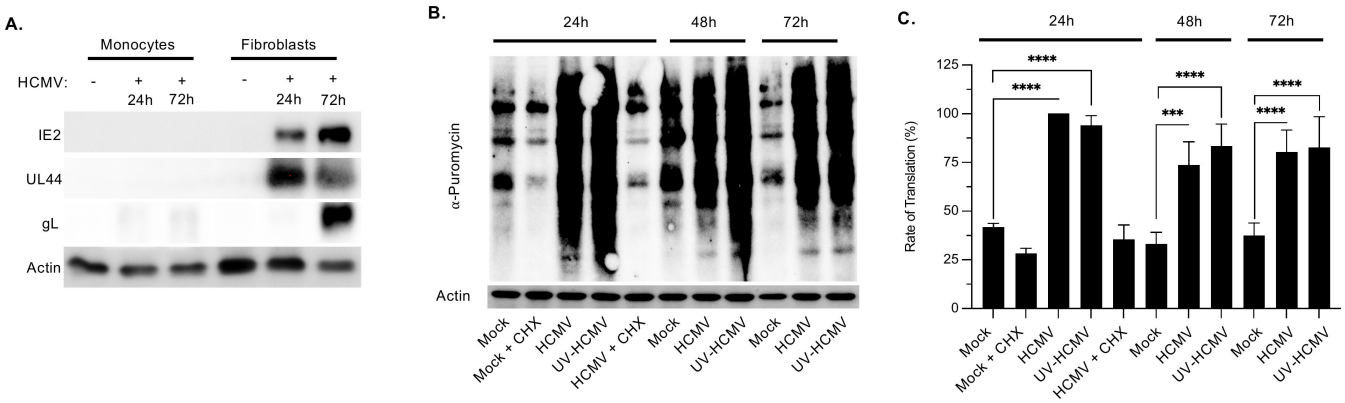

FIG 2 HCMV stimulates mRNA translation in infected monocytes independent of *de novo* synthesized viral gene products. (A) Fibroblasts or peripheral blood monocytes were mock or HCMV infected for 24 or 72 h. Expression lytic viral gene products, including IE (IE2), E (UL44), and L (gL), were determined by immunoblotting. Membranes were probed for actin as a loading control. Data are representative of three biological replicates. Monocyte data are representative of three independent blood donors. (B and C) Peripheral blood monocytes were mock, HCMV, or UV-HCMV infected for 24, 48, or 72 h. Cycloheximide (10 µg/mL) was added to the culture media of appropriate treatment groups at 16 hpi prior to SUnSET peptide labeling. Densitometry was performed to determine total protein levels, which were normalized to actin. (C) Quantification was from three to five biological replicates from independent blood donors. ***, $P < 0.0005$; ****, $P < 0.00005$.

mRNAs within quiescently infected monocytes (24). Yet, little is known about how HCMV governs global protein synthesis during silent infections, given the absence of lytic viral gene products. To begin to address the effects of HCMV on mRNA translation during a quiescent infection, we performed SUnSET labeling on primary peripheral blood monocytes infected over a 72-h time course. Similar to fibroblasts, HCMV promoted large-scale protein synthesis within quiescently infected monocytes (Fig. 2B and C). In contrast to fibroblasts, infection of monocytes with UV-HCMV led to a similar increase in protein synthesis across the 72-h time course as infection with replication competent HCMV, suggesting that mRNA translation is driven by the viral entry process and is maintained independently of *de novo* synthesized viral gene products. Taken together, these data indicate that signaling induced during HCMV entry into monocytes stimulates and maintains increased rates of mRNA translation despite the absence of *de novo* synthesized lytic proteins.

HCMV utilizes mTORC1 to drive protein synthesis

HCMV infection stimulates mTOR to drive the translation of select prosurvival factors to promote the survival of infected monocytes (24, 47). Accordingly, HCMV and myeloid growth factors were able to phosphorylate mTOR at S2448, a marker of mTORC1 activation (58–60) (Fig. 3A). Activated mTORC1 phosphorylates 4E-BP1, which promotes assembly of the eIF4F complex at the 5′ end of mRNA to initiate translation (61, 62). A substantial shift from hypo- to hyper-phosphorylated 4E-BP1 occurred following infection with HCMV (Fig. 3A). Surprisingly, the shift from hypo- to hyper-phosphorylated 4E-BP1 following treatment with GMCSF or MCSF was significantly attenuated relative to HCMV infection despite a similar induction of mTOR phosphorylation, suggesting either mTORC1 signaling to 4E-BP1/eIF4F is interrupted in growth factor-treated monocytes or hyper-phosphorylated 4E-BP1 is rapidly degraded. mTORC1 also promotes translation by phosphorylation of S6K (63, 64). Activated S6K mediates an inhibitory phosphorylation of eEF2K, which results in the dephosphorylation and activation of eEF2, a critical factor for translation elongation (65). We found HCMV-activated mTORC1 phosphorylated eEF2K leading to the subsequent reduction in eEF2 phosphorylation. Consistent with a defect in mTORC1 signaling following growth factor treatment, GMCSF and MCSF had minimal effect on the levels of phosphorylated eEF2K and eEF2. These results argue HCMV infection stimulates protein synthesis by activating mTORC1 to promote translation

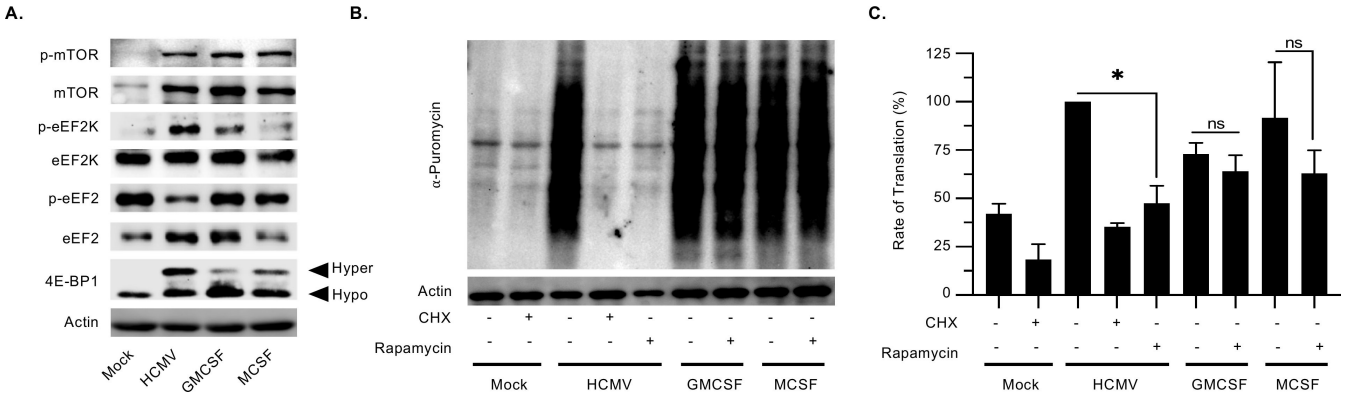

FIG 3 HCMV utilizes mTORC1-mediated signaling to promote protein synthesis. (A, B, C) Peripheral blood monocytes were mock infected, HCMV infected, GMCSF treated, or MCSF treated for 24 h. (A) Expression levels of p-mTOR, mTOR, p-eEF2K, eEF2K, p-eEF2, eEF2, and 4E-BP1 were determined by immunoblotting. Membranes were probed for actin as a loading control. Data are representative of three independent blood donors. (B and C) CHX (10 µg/mL) or rapamycin (10 µM) was added to the culture media at 16 h post treatment. At 24 h post treatment, cells were subjected to SUnSET peptide labeling. (C) Densitometry was performed to determine total protein levels, which were normalized to actin. Quantification was from three independent blood donors. ns, not significant; *, $P < 0.05$.

initiation and elongation. Indeed, the presence of rapamycin (an mTORC1 selective inhibitor) abrogated the ability of HCMV to stimulate protein synthesis (Fig. 3B and C). Importantly, the inhibition of mTORC1 had little effect on growth factor-stimulated mRNA translation, indicating myeloid growth factors drive mRNA translation independent of mTORC1 activation. These data provide evidence that HCMV-activated mTOR rapidly reshapes the translation landscape within infected monocytes.

HCMV infection generates a distinct translatome during the establishment of a quiescent infection

We have previously identified individual prosurvival transcripts tightly associated with polyribosomes during HCMV infection of monocytes (24). Given the extensive mTOR-mediated translational reprogramming following HCMV infection, polyribosomal profiling was performed on monocytes isolated from a single blood donor as an initial screen to globally identify mRNAs with actively bound ribosomes during HCMV infection. Although combining RNA-seq data sets from at least two replicates is typically needed to identify reproducible changes in response to a specific treatment, primary blood monocytes inherently exhibit large donor variability as it relates to the magnitude of change in gene expression following a particular treatment. As such, often only genes that exhibit large changes across different donors are identified as significant, while subtle changes, which could have profound biological impact, are lost. Alternatively, to ensure the reproducibility of potentially biologically relevant changes identified by our initial transcriptomic and/or translatomic screens, we will validate changes in the expression of genes of interest using a multitude of biochemical approaches with ≥3 independent blood donors. Because of the limited starting material from primary blood monocytes, cytoplasmic lysates were fractionated over a non-linear, rather than a conventional continuous linear, sucrose gradient in order to isolate polyribosome-associated mRNAs into a single identifiable peak (Fig. 4A) (66, 67). As expected with the use of a discontinuous gradient, the 40S, 60S, and 80S ribosomes were not individually resolved but instead found near the 5% and 34% sucrose interface. We also found the area under the curve (AUC) in the polyribosome-associated fractions (≥3 ribosomes) was greater in HCMV-infected monocytes versus uninfected monocytes, which is in support of HCMV infection stimulating mRNA translation. Accordingly, the AUC corresponding to less efficiently translated mRNAs (≤2 ribosome) was greater in uninfected cells. Since EDTA disrupts polyribosome association with mRNAs, control treatments with EDTA were performed to validate that the peak corresponds to polyribosomes (Fig. 4B). Next, we

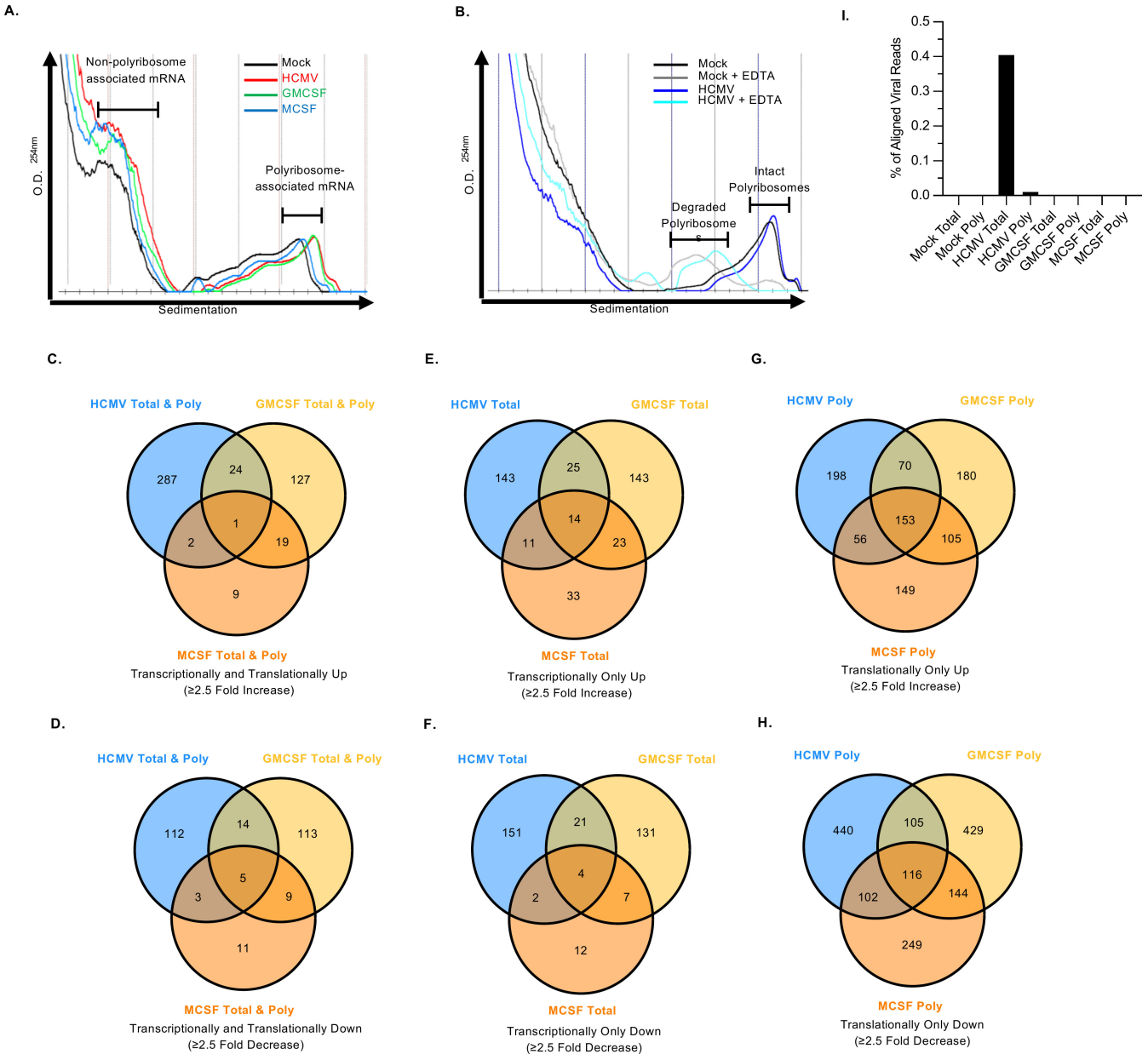

FIG 4 HCMV infection stimulates a unique translatome within quiescently infected monocytes. (A and B) Peripheral blood monocytes were mock infected, HCMV infected, GMCSF treated, or MCSF treated for 24 h. Following treatment, cell lysates were centrifuged through a non-linear sucrose gradient. (B) Samples were treated with or without EDTA prior to centrifugation as a control to disrupt polyribosome association with mRNAs. Non-polyribosome- and polyribosome-associated mRNAs were visualized with continuous monitoring of the UV absorbance at OD254. RNA from whole-cell lysate or from polyribosome-associated fractions was sequenced, and mRNAs were trimmed, aligned, and quantified using Partek genomics suite software. (C and D) Cellular genes upregulated or downregulated in both total RNA samples (total) and polyribosome fractions (poly) unique and/or shared between GMCSF, MCSF, and HCMV were plotted as Venn diagrams. (E and F) Cellular genes upregulated or downregulated only in total RNA samples unique and/or shared between GMCSF, MCSF, and HCMV were plotted as Venn diagrams. (G and H) Cellular genes upregulated or downregulated only in polyribosome fractions unique and/or shared between GMCSF, MCSF, and HCMV were plotted as Venn diagrams. (I) Genes which failed to align to the human genome were then aligned to a reference TB40E strain HCMV genome. The percentage of aligned reads was then plotted for total and polyribosome-associated mRNAs. All figures were generated within GraphPad Prism, Partek flow, or the VennDetail R package.

generated HCMV-regulated total mRNA and polyribosome-associated mRNA gene sets using a ≥2.5-fold cutoff relative mock-infected monocytes (Table S1). The polyribosome-associated mRNA gene sets represent ribosome-bound transcripts irrespective of their

total abundance. We found 287 genes increased and 112 genes decreased ≥2.5-fold in both the total and polyribosome-associated mRNA pools from HCMV-infected monocytes, suggesting that the expression of these genes is regulated, at least in part, at the transcriptional level (Fig. 4C and D). A total of 143 genes were upregulated ≥2.5-fold but not associated with increased levels of ribosomes, suggesting ribosome loading onto these transcripts was attenuated in HCMV-infected monocytes (Fig. 4E). And, 151 genes were downregulated ≥2.5-fold but not associated with decreased amounts of ribosomes, suggesting ribosome loading onto these transcripts may be enhanced to maintain protein expression levels in infected monocytes (Fig. 4F). Also, 198 and 440 genes were uniquely enriched or reduced, respectively, in the HCMV-infected monocyte polyribosome-associated mRNA fraction, indicating HCMV infection regulated the expression of these cellular genes specifically through translation (Fig. 4G and H). Next, the presence of viral transcripts was assessed by aligning non-human mRNAs to the genome of HCMV. As expected, HCMV transcripts were only found in infected monocytes (Fig. 4I). However, <0.5% of the total mRNA reads and <0.001% of the polyribosome-associated mRNA reads aligned to the HCMV genome. The detected HCMV transcripts were a mixture of E and L genes. However, IE transcripts were not detected, highly suggesting that the lytic replication cycle was never initiated in infected monocytes and that present HCMV mRNAs were likely delivered from the tegument during viral entry. Overall, these data demonstrate HCMV stimulates extensive changes to the cellular translational profile of infected monocytes independent of viral gene expression.

SIRT1 contributes to the survival of HCMV-infected monocytes

HCMV stimulates the survival of short-lived monocytes to promote viral dissemination (21, 22, 24, 25, 47, 57). Thus, we generated an unbiased list of negative regulators of cell death mRNAs exhibiting increased translation efficiency as well as positive regulators of cell death mRNAs exhibiting decreased translation efficiency unique to HCMV-infected monocytes (Fig. 5A and B). We identified 11 prosurvival transcripts that were highly associated with increased ribosome loading and 14 prodeath transcripts with decreased ribosome loading. Of particular interest was SIRT1, an NAD+-dependent deacetylase with cytoprotective effects during times of cellular stress (68–72). SIRT1 is a known regulator of Akt, which we have previously shown to be aberrantly activated during HCMV infection to stimulate the survival of infected monocytes (24, 27, 35, 47, 57).

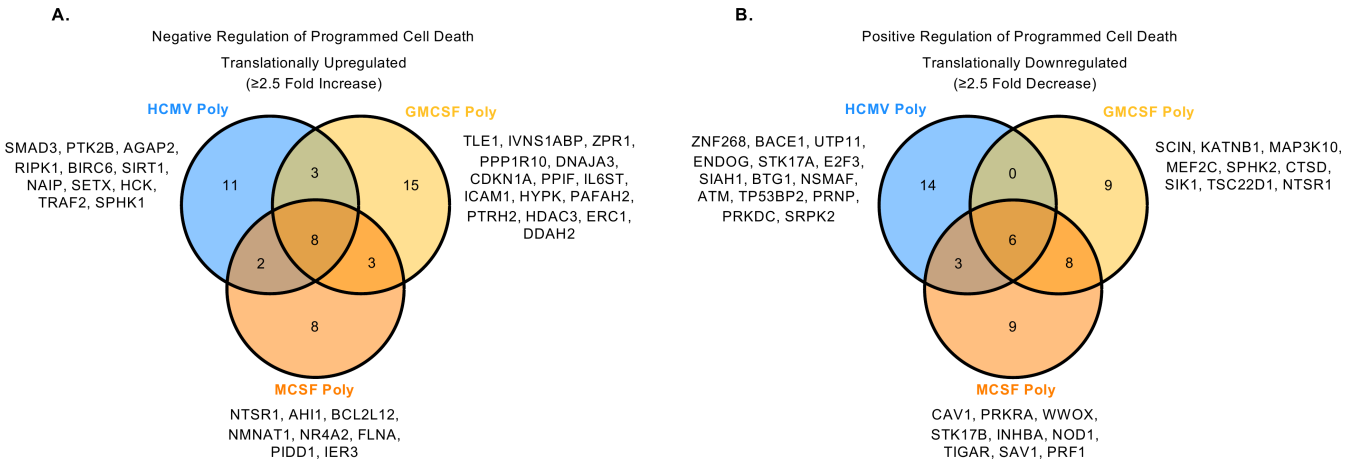

FIG 5 Regulation of program cell death transcripts uniquely translated during HCMV infection. Unbiased lists of human genes involved in the positive or negative regulation of programmed cell death were generated from the AmiGO2 gene ontology repository. These lists were then used to filter the polyribosomal profiling data set from HCMV-infected, GMCSF-treated, or MCSF-treated monocytes. (A) Transcripts exhibiting increased translation involved in the negative regulation of programmed cell death or (B) transcripts exhibiting decreased translation involved in the positive regulation of programmed cell death were ordered into Venn diagrams. All plots were generated in GraphPad Prism or by using the VennDetail R package. Listed are transcripts uniquely regulated by each treatment group.

Our initial screen indicated no increase in total SIRT1 mRNA levels in HCMV-infected monocytes and a threefold induction in polyribosome-associated SIRT1 transcripts when compared to uninfected monocytes (Table S1). In contrast, both GMCSF- and MCSF-treated monocytes had no increase in total SIRT1 mRNA and similar levels of polyribosome-associated SIRT1 mRNA relative to mock-infected cells (Table S1). We next validated total SIRT1 transcripts were not upregulated by qPCR (Fig. 6A) and that protein expression was increased specifically in HCMV-infected monocytes by western blot (Fig. 6B and C). To determine if SIRT1 is necessary for the survival of HCMV-infected monocytes, infected cells were treated with a SIRT1 selective small-molecule inhibitor, EX527 (73). Flow cytometric analysis following annexin V and propidium iodide (PI) staining showed SIRT1 inhibition reduced the viability of HCMV-infected monocytes in a dose-dependent manner at concentrations consistent with other studies (73–78) while having minimal effect on the viability of uninfected and growth factor-treated monocytes (Fig. 6D). To confirm that the effects of EX527 were not due to off-target effects, siRNA knockdown of SIRT1 was performed, which reduced protein expression by ~90% in HCMV-infected monocytes (Fig. 6E). Indeed, SIRT1 knockdown led to significant cell death of HCMV-infected monocytes (~18%) but not uninfected cells (Fig. 6F). While an ~18% reduction in live cells may appear modest, monocytes are highly sensitive to electroporation, and thus the full effect of SIRT1 depletion on cell viability is likely masked by the cell death induced by transfection of siRNAs. Nonetheless, both pharmacological and genetic approaches indicate HCMV selectively promotes the translation of SIRT1 in order to drive the long-term survival of infected monocytes.

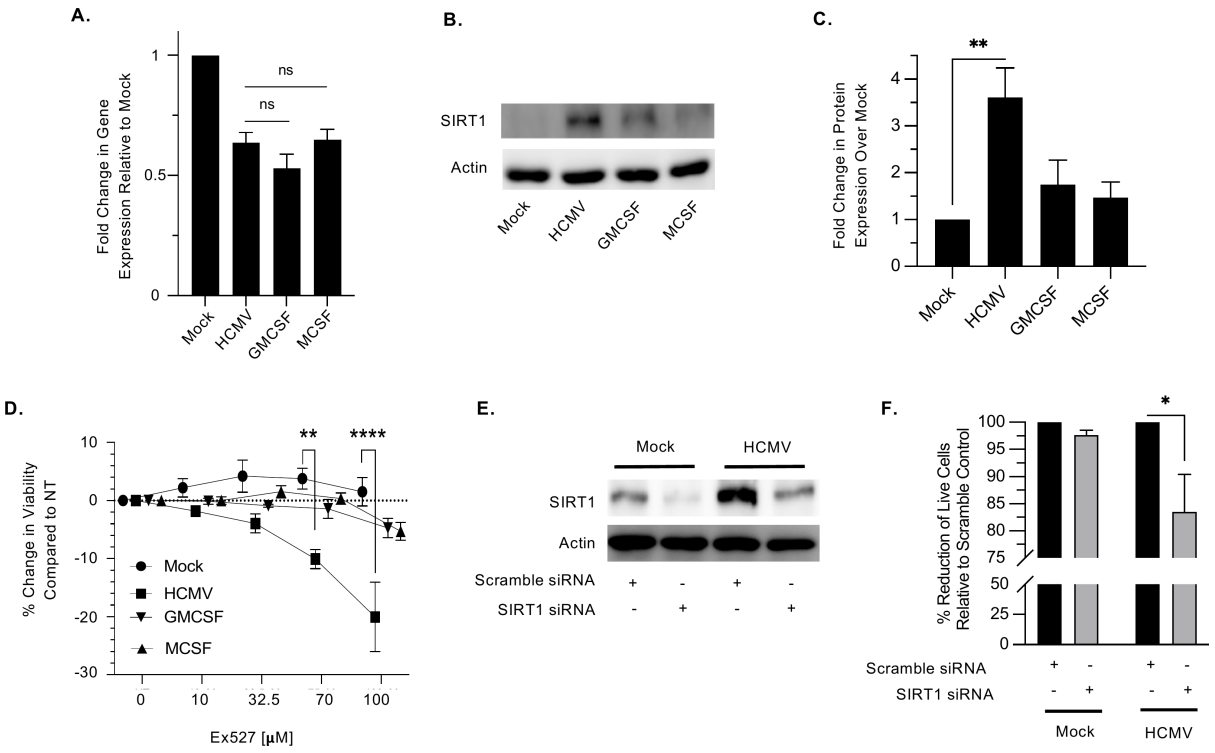

FIG 6 SIRT1 promotes the survival of HCMV-infected monocytes. (A, B, C) Peripheral blood monocytes were mock infected, HCMV infected, GMCSF treated, or MCSF treated for 24 h. (A) SIRT1 mRNA transcript abundance was determined by qRT-PCR. (B and C) Total SIRT1 protein expression was determined by immunoblotting. Membranes were probed for actin as a loading control. (C) Densitometry was performed to quantify SIRT1 levels. (D) Monocytes were pretreated with increasing concentrations of EX527 or vehicle control for 30 min. Cells were then mock infected, HCMV infected, GMCSF treated, or MCSF treated for 24 h. Cell viability was determined by annexin V and PI staining followed by flow cytometric analysis. (E and F) Monocytes were transfected with a SIRT1-specific siRNA (250 nM) or a control siRNA and incubated for 24 h. Cells were then infected with HCMV for an additional 24 h. (E) Total SIRT1 and actin expression levels were determined by immunoblotting. (F) Cells were stained with annexin V and PI to assess cellular viability by flow cytometry. All blots and data are representative of at least three independent blood donors. ns, not significant; *, $P < 0.05$; **, $P < 0.005$; ****, $P < 0.00005$.

SIRT1 promotes AKT/mTORC1 signaling to drive translation of prosurvival factors within HCMV-infected monocytes

SIRT1 directly deacetylates Akt to allow binding to phosphoinositol lipids and the subsequent Akt activating phosphorylation events (79, 80). Since HCMV modifies Akt activity to promote the upregulation of prosurvival factors necessary for the survival

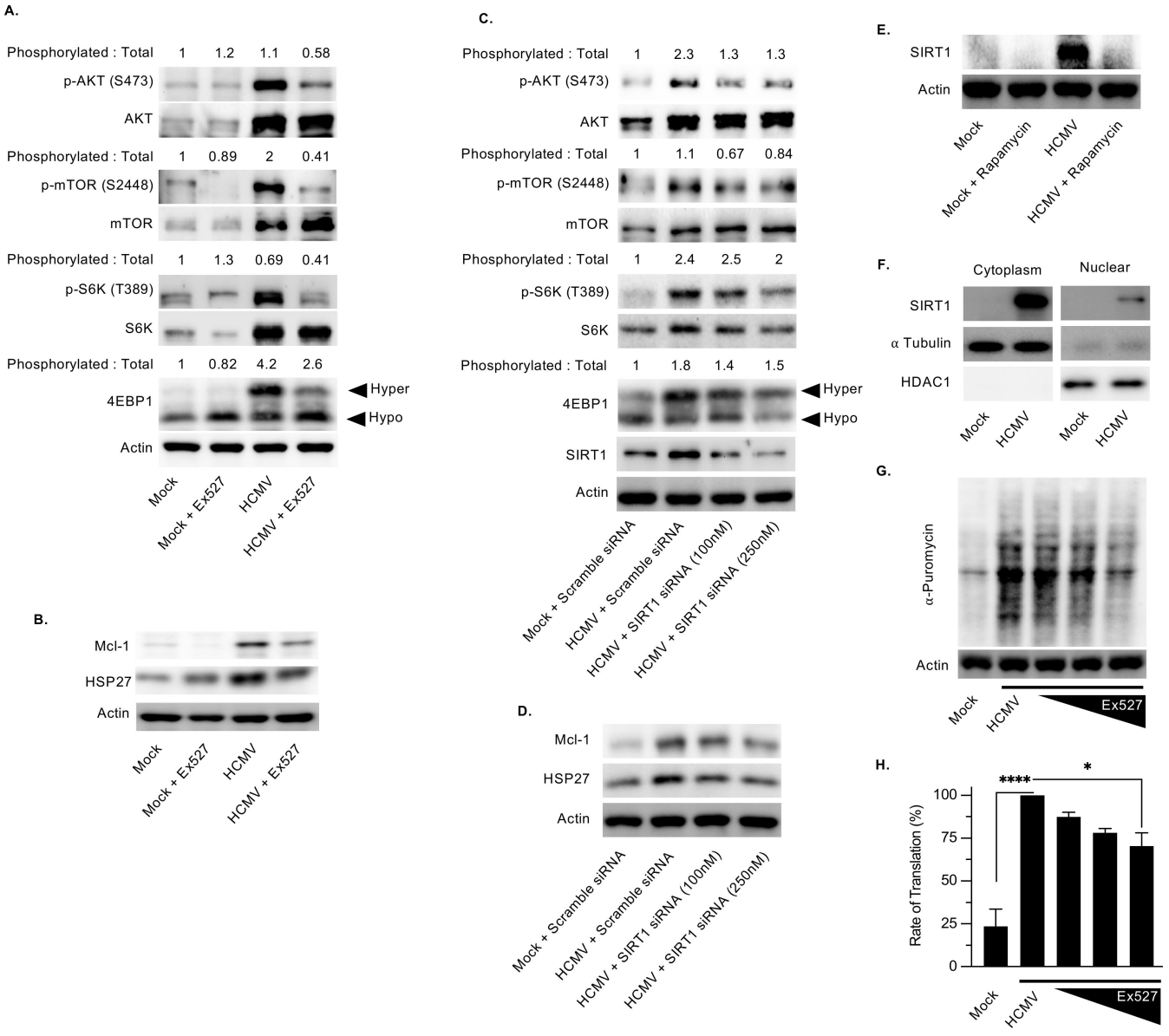

FIG 7 SIRT1 promotes the translation of prosurvival factors within HCMV-infected monocytes through the AKT/mTORC1 signaling pathway. (A and B) Peripheral blood monocytes were mock or HCMV infected in the presence or absence of a sub-cytotoxic concentration of EX527 (10 µM). (C and D) Monocytes were transfected with a SIRT1-specific siRNA or a control siRNA and incubated for 24 h. Cells were then infected with HCMV. At 24 hpi, p-Akt, Akt, p-mTOR, mTOR, p-S6K, S6K, 4EBP1, Mcl-1, HSP27, and SIRT1 levels were assessed by immunoblot. Membranes were probed for actin as a loading control. Densitometry was performed to determine the ratio of phosphorylated to total protein levels for Akt, mTOR, S6K, and 4EBP1. Note the total levels of 4EBP1 were determined by combining hyper- and hypo-phosphorylated levels. (E) Monocytes were mock or HCMV infected in the presence or absence of rapamycin (10 µM). SIRT1 levels were determined by immunoblot. (F) Monocytes were mock or HCMV infected for 24 h, and cytosolic and nuclear extracts collected. SIRT1 levels were determined by immunoblot. GAPDH and HDAC1 expression served cytosolic and nuclear loading controls, respectively. (G and H) Monocytes were pretreated at increasing sub-cytotoxic concentrations of EX527 (10–40 µM) for 1 h prior to HCMV infection for 24 h. Cells were subjected to SUnSET peptide labeling. Densitometry was performed to measure total protein levels. All blots and data are representative of at least three independent blood donors. *, $P < 0.05$; ****, $P < 0.00005$.

of infected monocytes (24–27, 40, 47), we examined the role of SIRT1 in regulating HCMV-induced Akt activity. We found HCMV increased the levels of p-Akt (S473), p-mTOR (S2448), p-S6K (T389), and p-4E-BP1(T37/46) at 24 hpi, which corresponded with increases in total protein levels. Importantly, the presence of a sub-cytotoxic concentration of EX527 (10 µM) reduced the levels of phosphorylated Akt, mTOR, S6K, and 4E-BP1 without affecting total protein expression resulting in reduced phosphorylated to total protein ratios (Fig. 7A), indicating SIRT1 plays a critical role in maintaining HCMV-induced Akt/mTORC1 signaling. Accordingly, SIRT1 inhibition limited the Akt-dependent increase of Mcl-1 and HSP27 in HCMV-infected monocytes (Fig. 7B). SiRNA knockdown of SIRT1 also reduced the ratio of p-AKT (S473), p-mTOR (S2448), and p-S6K (T389) to total protein levels (Fig. 7C) as well as Mcl-1 and HSP27 levels (Fig. 7D) in infected monocytes, validating SIRT1's role in regulating the AKT/mTORC1 signaling axis. Additionally, the expression of SIRT1 was dependent on mTOR as treatment with rapamycin ablated SIRT1 protein expression in HCMV-infected monocytes (Fig. 7E). As a histone deacetylase, SIRT1 is primarily localized to the cell nucleus under homeostatic conditions (81). However, SIRT1 has been documented to be rapidly shuttled to the cytoplasm in cancer models in a PI3K-dependent manner (82). We found SIRT1 was expressed in the cytoplasm of HCMV-infected monocytes and that uninfected monocytes do not express nuclear SIRT1 (Fig. 7F), suggesting that increased protein synthesis during HCMV infection is responsible for the cytoplasmic expression of SIRT1. Thus, SIRT1 cytosolic localization in HCMV-infected monocytes provides a unique regulatory mechanism for the regulation of the Akt/mTORC1 signaling axis. These data further suggest that SIRT1 may play a critical role in further stimulating mRNA translation during HCMV infection. Indeed, SUnSET labeling in the presence of sub-cytotoxic concentrations of EX527 demonstrated a dose-dependent decrease in the rate of protein synthesis in monocytes following HCMV infection (Fig. 7G and H). Based on these data, we propose a positive feedback mechanism whereby HCMV infection stimulates PI3K/Akt/mTORC1 signaling pathway to increase SIRT1 expression, which then further potentiates Akt and mTORC1 activity to enhanced translation of antiapoptotic factors necessary for the viability of HCMV-infected monocytes.

DISCUSSION

Peripheral blood monocytes are essential to HCMV's lifecycle as these blood sentinels are involved in the systemic dissemination of the virus; a prerequisite for the establishment of latency within the bone marrow (16, 83–85). However, monocytes are inherently short-lived cells with a lifespan of ~48 h and are not fully permissive for HCMV replication (14, 17, 86). To overcome these obstacles, HCMV rapidly reshapes the cellular signalsome during viral entry to promote the functional changes necessary for the survival and differentiation of infected monocytes into long-lived replication permissive macrophages (20–22, 24–27, 47, 57, 87). We demonstrate a major outcome of the HCMV-specific signalsome is the large-scale induction of mRNA translation. Polyribosomal profiling of the translatome revealed increases in the translation of mRNAs with potentially proviral effects, including transcripts linked with the negative regulation of cell death. We identified several ribosome-associated transcripts that were elevated during HCMV infection but not during normal myeloid growth factor treatment. Specifically, we determined the increased synthesis of SIRT1 functions as a positive feedback loop to sustain elevated Akt/mTORC1 signaling and the subsequent translation of prosurvival factors required for the survival of infected monocytes (Fig. 8). This study establishes the critical importance of inducing cellular mRNA translation in the absence of early *de novo* synthesized viral gene products to allow for the survival and differentiation of quiescently infected monocytes.

Many viruses have evolved to limit protein synthesis as a means to avoid immune detection by preventing the synthesis of host antiviral factors (54, 55, 88–90). In contrast, HCMV increases protein synthesis during lytic infection to facilitate viral replication through several viral gene products, including TRS1, IRS1, and pUL38 (45, 46, 48–51,

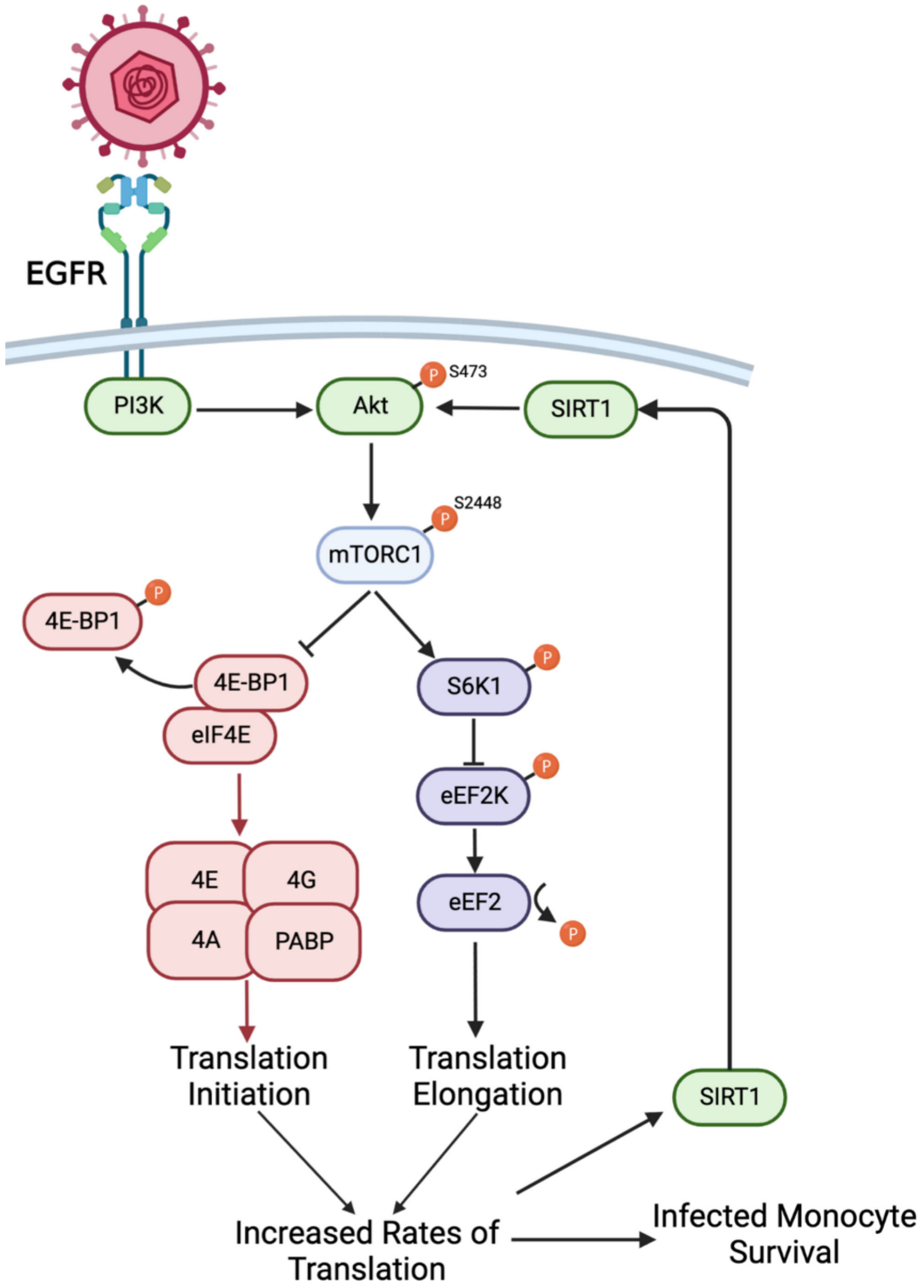

FIG 8 Proposed model for HCMV-induced mTORC1-mediated protein synthesis in quiescently infected monocytes. During HCMV entry into monocytes, glycoproteins gB and gH bind with EGFR and integrin β1, respectively, which leads to the unique activation of Akt. HCMV-activated Akt phosphorylates mTORC1 triggering downstream signaling events that promote translation initiation and elongation of prosurvival factors, including SIRT1. The preferential translation of SIRT transcripts and subsequent cytosolic protein expression in HCMV-infected monocytes sustain AKT signaling following HCMV entry by mediating a positive feedback loop.

54, 55). In this study, we confirm the involvement of viral proteins in stimulating global translation in lytically infected fibroblasts as "live" HCMV, but not UV-inactivated virus, induced protein synthesis (Fig. 1). Furthermore, there was a 24-h delay in the induction of mRNA translation consistent with the time needed for the *de novo* synthesis of viral gene products. In contrast, HCMV stimulated mRNA translation in infected monocytes by 24 hpi, suggesting a mechanism of induction distinct from lytically infected cells. Indeed, UV-HCMV infection of monocytes induced and maintained translation to equivalent levels as HCMV infection, indicating viral binding and entry are sufficient to promote protein synthesis. These data underscore the highly cell-specific mechanisms utilized by HCMV to stimulate translation. In quiescently infected monocytes, the viral entry process likely modulates cellular signaling cascades to generate a cellular state conducive for protein synthesis. Why viral entry into fibroblasts is not sufficient to induce translation is unclear. Fibroblasts and monocytes express a different repertoire of HCMV entry receptors with distinct downstream cellular substrates. Thus, the unique signaling network generated during viral entry into monocytes may be responsible for accelerating mRNA translation in quiescently infected cells.

During HCMV entry into monocytes, viral glycoproteins gB and gH bind to EGFR and integrin β1, respectively, leading to a non-canonical Akt signaling network (20, 22, 27, 87, 91, 92). A functional outcome of HCMV-induced Akt is the activation of mTOR (Fig. 2) (24, 47), which promotes mRNA translation through 4EBP-1 and eEF2K (56) (64, 93). Accordingly, we found HCMV infection to stimulate 4EBP-1 and eEF2K phosphorylation. Surprisingly, despite also inducing mTOR phosphorylation, normal myeloid growth factors were unable to stimulate 4EBP-1 and eEF2K activity, indicating the connection between mTOR and its downstream effectors of translation is severed. A possible explanation in the differential ability of HCMV- versus growth factor-activated mTOR to phosphorylate downstream targets could lie in the presence of other post-translation modification. Several post-translational modifications are known to regulate mTOR activity (94–96), and perhaps, HCMV-activated Akt has distinct effects on these modifications. Alternatively, HCMV infection could differentially activate other kinases required for the full activation of mTOR and/or its downstream targets. Regardless, HCMV infection globally stimulates mRNA translation through the Akt/mTORC1 signaling axis, while growth factors drive protein synthesis through an alternative mechanism (Fig. 3). These data also indicate that HCMV infection generates a highly unique translatome within infected monocytes presumably to support the establishment of a quiescent infection.

Since the survival of short-lived monocytes is essential for the establishment of a quiescent infection, we further explored negative regulators of programmed cell death and identified 11 transcripts that were uniquely translated within HCMV-infected monocytes. SIRT1 was of particular interest as it has long been implicated in promoting cell viability during cellular stress and has been reported to deacetylate Akt to allow binding to phosphatidylinositol phosphate lipids at the plasma membrane and subsequent activation (69, 70, 72, 79, 80). Indeed, HCMV increases the expression of cytoplasmic SIRT1 to promote the Akt-dependent antiapoptotic state within infected monocytes (Fig. 6 and 7). Interestingly, despite SIRT1 primarily being found in the nucleus of other cell types (81), inactivated peripheral blood monocytes do not appear to express basal levels of nuclear SIRT1, suggesting that cytoplasmic expression of SIRT1 is due to the increased synthesis of SIRT1 and not from an increase in shuttling of SIRT1 from the nucleus. These data demonstrate that, after the initial gB/EGFR-mediated burst of Akt activation, HCMV stimulates the translation of SIRT1 to sustain Akt activity through critical viability checkpoints along the myeloid differentiation process.

To the best of our knowledge, this is the first study to define the global reshaping of the cellular translatome during the establishment of a quiescent infection within monocytes. The rapid induction of translation during HCMV entry into monocytes is further enhanced by a feed-forward mechanism between Akt, mTORC1, and SIRT1 to generate a translational landscape that supports the survival and/or differentiation of

quiescently infected monocytes. Current HCMV antivirals are limited to blocking various steps of the lytic replication cycle and thus ineffective against quiescently infected monocytes (97–103). Defining the unique mechanisms utilized by HCMV to ensure the survival of infected monocytes offers insight into new therapeutic targets that could eliminate quiescently infected cells and subsequently limit systemic spread of HCMV within high-risk patients.

MATERIALS AND METHODS

Human peripheral blood monocyte isolation

Isolation of peripheral blood monocytes was performed as previously described (19, 20, 23, 57, 104, 105). Briefly, blood was drawn by venipuncture from random donors, diluted in RPMI 1640 medium (ATCC, Product # 30–2001, Manassas, VA), and centrifuged through Histopaque-1077 (MilliporeSigma, St. Louis, MO) to remove red blood cells and neutrophils. Mononuclear cells were collected and washed with saline to remove platelets and then separated by centrifugation though and Percoll (GE Healthcare, Wilkes-Barre, PA) gradient (40.48% and 47.7%). More than 90% of isolated peripheral blood mononuclear cells were monocytes as determined by CD14-positive staining. Cells were washed with saline and resuspended in RPMI 1640 (ATCC, Manassas, VA). All experiments were performed in a 5% CO_2 incubator, unless otherwise stated. University Institutional Review Board and Health Insurance Portability and Accountability Act guidelines for the use of human subjects were followed for all experimental protocols in our study (IRB #: 262458-19). All small-molecule inhibitors, including cycloheximide, rapamycin, and Ex527, were purchased from Selleckchem (Houston, TX).

Virus preparation and infection

Human embryonic lung (HEL) 299 fibroblasts (CCL-137, ATCC, Manassas, VA) of low passage (P7-15) were subcultured in Dulbecco's Modified Eagle medium (DMEM) (Lonza, Morristown, NJ) with 2.5 µg/mL plasmocin (Invivogen, San Diego, CA) and 10% fetal bovine serum (FBS) (MilliporeSigma). When culture reached 100% confluency, cells were infected with HCMV in DMEM + 4% FBS. Virus was purified from supernatant on a 20% sorbitol cushion to remove cellular contaminants and resuspended in RPMI 1640 medium. A multiplicity of infection (MOI) of 1 was used for experiments with fibroblasts. An MOI of 5 was used for experiment with monocytes (20, 21). Mock infection was performed by adding an equivalent volume of RPMI 1640 medium to monocytes, while GMCSF or MCSF treatment was performed by adding an equivalent volume of RPMI 1640 medium with recombinant human GMCSF or MCSF at 100 ng/mL (R&D Systems, Minneapolis, MI). UV-inactivated virus was prepared by incubating virus under a 30-W germicidal (UVC wavelength of 254 nm) ultraviolet lamp (G30 T8, GE Lighting, East Cleveland, OH) for 20 min on ice and was used in the same manner as "live" virus. The UV-inactivated virus did not replicate or produce any detectable levels of immediate early (IE) gene products.

Preparation of cytosolic and nuclear extracts

Cytoplasmic and nuclear extracts were isolated from 3×10^6 peripheral blood monocytes as previously described with minor modifications (106, 107). Briefly, live blood monocytes were loaded on top of an iso-osmolar discontinuous iodixanol (MilliporeSigma)-based gradient. During centrifugation at 1,000 × *g* for 10 min in a swinging bucket rotor, monocytes travel through a preliminary cell wash layer prior to encountering a mild cell lysis layer (0.5% IPEGAL CA-630) (MilliporeSigma), which disrupts the plasma membrane while leaving nuclei intact. Undamaged nuclei then pass through a subsequent wash layer prior to encountering a hyper-dense float layer. Soluble cytoplasmic fractions were isolated from the cell lysis layer, and crude nuclei were harvested from the interface between the second wash and float layer.

Western blotting analysis

Monocytes were harvested in modified radioimmunoprecipitation assay buffer [50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 100 mM NaCl, 1% Triton X-100, 0.1% SDS, and 10% glycerol] supplemented with protease inhibitor cocktail (MilliporeSigma) and phosphatase inhibitor cocktails 2 and 3 (MilliporeSigma) for 30 min on ice. The lysates were cleared from the cell debris by centrifugation at 4°C (5 min, 21,000 × *g*) and stored at −20°C until further analysis. Protein samples were solubilized in Laemmli SDS sample non-reducing (6×) buffer (Boston Bioproducts, Boston, MA) supplemented with β-mercaptoethanol (Amresco, Solon, OH) by incubation at 95°C for 10 min, unless otherwise stated. Equal amounts of total protein from each sample were loaded in each well, separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA). Blots were blocked in 5% bovine serum albumin (BSA) (Fisher Scientific, Waltham, MA) for 1 h at room temperature (RT) and then incubated with primary antibodies overnight at 4°C. The following antibodies were purchased from the indicated companies: α-puromycin (MilliporeSigma); α-p-mTOR (Santa Cruz Biotechnology, Dallas, TX); α-p-eif2α, α-eIF2α, α-mTOR, α-eEf2K, α-p-eEf2k, α-eEf2, α-p-eEf2, α-SIRT1, α-4EBP-1, α-Akt, α-p-Akt, α-α-tubulin, α-HDAC1 (Cell Signaling Technology, Danvers, MA). Rhodamine α-β actin antibody (Bio-Rad) was used as loading control. The blots were then incubated with horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology) for 30 min at RT, and chemiluminescence was detected using the Clarity Western ECL substrate (Bio-Rad). Images were captured using the Bio-Rad ChemiDoc XRS+ Molecular Imager (Bio-Rad), and densitometry analyses were performed using Image Lab software (Bio-Rad).

Quantitative PCR

Total mRNAs were isolated with an RNeasy Mini Kit (Qiagen, Germantown, MD), as per the manufacturer's recommendation. Contaminating DNA was removed from the samples with a Turbo DNA-free Kit (ThermoFisher, Waltham, MA). For quantitative real-time PCR, iTaq Universal SYBR Green One-Step Kit (Bio-Rad, Hercules, CA) was used to detect the expression of *SIRT1* (sense, 5′- CTGGGGAAGGAGACAATGG-3′; antisense, 5′- GTCGTCGTCTTCGTCGTACA-3′), and 18s rRNA (sense, 5′-GCAATTATTCCCCATGAACG-3′; antisense, 5′-GGGACTTAATCAACGCAAGC-3′) with a CFX Connect Real Time PCR System (Bio-Rad, Hercules, CA).

SUnSET assay peptide labeling

In vitro SUnSET assays were performed as previously described (52, 108). Briefly, 3×10^6 monocytes or 5×10^5 confluent HEL299 fibroblasts were held in culture under various experimental conditions. Following treatment, nascently synthesized peptides were labeled via the addition of low dose puromycin (1 µM; MilliporeSigma) into the culture media for 30 min prior to lysis. Cell lysates were then subject to SDS-PAGE analysis using a puromycin-specific antibody. Total lane density was assessed with Bio-Rad's Image Lab software to measure total protein levels.

Analysis of polysome-associated RNAs

Monocytes (10×10^7 cells) were treated with 0.1 mg/mL CHX (MilliporeSigma) at 37°C for 10 min prior to harvest. Cells were then washed with PBS containing CHX at 4°C and pelleted by centrifugation. Pellets were resuspended in polysome lysis buffer [20 mM Tris-HCl (pH 7.4), 140 mM KCl, 5 mM $MgCl_2$, Triton X-100, 10 mM dithiothreitol, and 0.1 mg/mL CHX] and passed through a 27-gauge needle five times. Residual insoluble debris and mitochondria were pelleted by centrifugation for 10 min at 15,000 × *g* in a microcentrifuge. The clarified lysate was layered onto a non-linear sucrose gradient as previously described (67). Briefly, the non-linear sucrose gradient comprised three

increasingly dense concentrations of sucrose (5%/34%/55%). Because polyribosome-bound RNA cannot enter the hyper-dense 55% sucrose, all polyribosome-associated RNA can be collected into one fraction. Fractions containing ribosomal subunits, monosomes, and polysomes were determined by continuous monitoring of the absorbance at an optical density of 254 nm (OD254) during gradient fractionation using a Brandel gradient fractionator system (Brandel, Gaithersburg, MD) coupled to a type 11 spectrophotometer optical unit (Teledyne ISCO, Lincoln, NE). Total RNA was extracted from an equal volume of each gradient fraction using TRIzol (Thermo Fisher Scientific, Waltham, MA), and contaminating DNA was removed with DNase (Thermo Fisher Scientific).

RNA-Seq analysis

RNAs were isolated out of TRIzol prior to library prep. RNA sequencing library preparation was performed according to the Illumina Dual-Stranded mRNA Preparation Kit. Library quality and quality control metrics were assessed and confirmed to adhere to Illumina's standards using a bioanalyzer (Agilent Technologies, Santa Clara, CA). Completed RNA-Seq libraries were then sequenced using the NextSeq system with a 400-million read sequencing chip (Illumina, San Diego, CA). Sequence bases were then trimmed, aligned, and quantified to the hg38 human reference genome using the Partek Flow Analysis Suite (Partek, Chesterfield, MO). Following quantile normalization, differential gene expression analysis was conducted using Partek's GSA (gene-specific analysis) computational algorithm. All graphs and figures were generated using Partek flow or various R-coding packages. The raw and processed data have been deposited in NCBI GEO (accession number: GSE248927).

Flow cytometry

Monocytes were washed in PBS and incubated in blocking solution consisting of fluorescence-activated cell sorting buffer (145 mM NaCl, 8.45 mM Na_2HPO_4, 1.83 mM NaH_2PO_4, and 0.1% NaN_3), 5% BSA, and human FcR-binding inhibitor (eBioscience, San Diego, CA) for 20 min on ice. After blocking, cells were stained with an allophycocyanin (APC)-anti-CD14 or APC-anti-mouse IgG1 isotype control antibody (BioLegend, San Diego, CA) on ice and then washed and stained with fluorescein isothiocyanate-annexin V and PI (Thermo Fisher Scientific) to detect dead and dying cells. Cells were then analyzed by flow cytometry using an LSRFortessa cell analyzer and BD FlowJo software (BD Biosciences, Franklin Lakes, NJ). Double-negative cells represent live cells, whereas double- and single-positive cells represent dead and/or dying cells.

Transient transfection and RNA silencing

Monocytes (2–3 × 10^6/transfection) were resuspended in 100 µL of RT nucleofection solution (Amaxa P3 primary cell solution; Amaxa Biosystems, Cologne, Germany) containing 250 nM *Silencer* Select SIRT1 targeting siRNA (Thermo Fisher Scientific) or 250 nM *Silencer* Select scramble control siRNA (Thermo Fisher Scientific). Cells were than transfected with a 4D-Nucleofector system using pulse code EI-100. Following transfection, cells were incubated in RPMI 1640 (Cellgro) supplemented with 1% human AB serum in RPMI at 37°C (Lonza) for 24 h. Monocytes were then mock- or HCMV infected for 24 h and subjected to immunoblot or flow cytometry analysis.

Statistical analyses

All experiments were performed with a minimum of three biological replicates using primary monocytes isolated from different blood donors. Data were analyzed with GraphPad Prism (San Diego, CA) software using the Student's *t* test or one-way ANOVA with multiple comparisons when appropriate. *P*-values less than 0.05 were considered statistically significant.

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AUTHOR CONTRIBUTIONS

Michael J. Miller, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing | Dilruba Akter, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review and editing | Jamil Mahmud, Data curation, Formal analysis, Investigation, Methodology, Validation | Gary C. Chan, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review and editing

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Table S1 (JVI01888-23-s0001.xlsx). Cellular genes upregulated or downregulated >2.5-fold following HCMV infection, GMCSF treatment, or MCSF treatment.

REFERENCES

1. Staras SAS, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. 2006. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis 43:1143–1151. https://doi.org/10.1086/508173
2. Lawlor G, Moss AC. 2010. Cytomegalovirus in inflammatory bowel disease: pathogen or innocent bystander? Inflamm Bowel Dis 16:1620–1627. https://doi.org/10.1002/ibd.21275
3. Nerheim PL, Meier JL, Vasef MA, Li WG, Hu L, Rice JB, Gavrila D, Richenbacher WE, Weintraub NL. 2004. Enhanced cytomegalovirus infection in atherosclerotic human blood vessels. Am J Pathol 164:589–600. https://doi.org/10.1016/S0002-9440(10)63148-3
4. Emery VC. 2001. Investigation of CMV disease in immunocompromised patients. J Clin Pathol 54:84–88. https://doi.org/10.1136/jcp.54.2.84

5. Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, Bland KI, Cobbs CS. 2002. Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. Lancet 360:1557–1563. https://doi.org/10.1016/S0140-6736(02)11524-8
6. Cobbs CS, Soroceanu L, Denham S, Zhang W, Kraus MH. 2008. Modulation of oncogenic phenotype in human glioma cells by cytomegalovirus IE1-mediated mitogenicity. Cancer Res 68:724–730. https://doi.org/10.1158/0008-5472.CAN-07-2291
7. Krstanović F, Britt WJ, Jonjić S, Brizić I. 2021. Cytomegalovirus infection and inflammation in developing brain. Viruses 13:1078. https://doi.org/10.3390/v13061078
8. Schleiss MR. 2011. Congenital cytomegalovirus infection: molecular mechanisms mediating viral pathogenesis. Infect Disord Drug Targets 11:449–465. https://doi.org/10.2174/187152611797636721
9. Britt WJ. 2017. Congenital human cytomegalovirus infection and the enigma of maternal immunity. J Virol 91:e02392-16. https://doi.org/10.1128/JVI.02392-16
10. Griffiths P, Baraniak I, Reeves M. 2015. The pathogenesis of human cytomegalovirus. J Pathol 235:288–297. https://doi.org/10.1002/path.4437
11. Fulkerson HL, Nogalski MT, Collins-McMillen D, Yurochko AD. 2021. Overview of human cytomegalovirus pathogenesis. Methods Mol Biol 2244:1–18. https://doi.org/10.1007/978-1-0716-1111-1_1
12. Bissinger AL, Sinzger C, Kaiserling E, Jahn G. 2002. Human cytomegalovirus as a direct pathogen: correlation of multiorgan involvement and cell distribution with clinical and pathological findings in a case of congenital inclusion disease. J Med Virol 67:200–206. https://doi.org/10.1002/jmv.2208
13. Sinzger C, Plachter B, Grefte A, The TH, Jahn G. 1996. Tissue macrophages are infected by human cytomegalovirus *in vivo*. J Infect Dis 173:240–245. https://doi.org/10.1093/infdis/173.1.240
14. Sinzger C, Jahn G. 1996. Human cytomegalovirus cell tropism and pathogenesis. Intervirology 39:302–319. https://doi.org/10.1159/000150502
15. Sinzger C, Digel M, Jahn G. 2008. Cytomegalovirus cell tropism. Curr Top Microbiol Immunol 325:63–83. https://doi.org/10.1007/978-3-540-77349-8_4
16. Taylor-Wiedeman J, Sissons JG, Borysiewicz LK, Sinclair JH. 1991. Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. J Gen Virol 72:2059–2064. https://doi.org/10.1099/0022-1317-72-9-2059
17. Sinclair J, Sissons P. 1996. Latent and persistent infections of monocytes and macrophages. Intervirology 39:293–301. https://doi.org/10.1159/000150501
18. Hoeffel G, Ginhoux F. 2018. Fetal monocytes and the origins of tissue-resident macrophages. Cell Immunol 330:5–15. https://doi.org/10.1016/j.cellimm.2018.01.001
19. Smith MS, Bentz GL, Smith PM, Bivins ER, Yurochko AD. 2004. HCMV activates PI(3)K in monocytes and promotes monocyte motility and transendothelial migration in a PI(3)K-dependent manner. J Leukoc Biol 76:65–76. https://doi.org/10.1189/jlb.1203621
20. Chan G, Nogalski MT, Yurochko AD. 2009. Activation of EGFR on monocytes is required for human cytomegalovirus entry and mediates cellular motility. Proc Natl Acad Sci U S A 106:22369–22374. https://doi.org/10.1073/pnas.0908787106
21. Chan G, Nogalski MT, Yurochko AD. 2012. Human cytomegalovirus stimulates monocyte-to-macrophage differentiation via the temporal regulation of caspase 3. J Virol 86:10714–10723. https://doi.org/10.1128/JVI.07129-11
22. Chan G, Nogalski MT, Stevenson EV, Yurochko AD. 2012. Human cytomegalovirus induction of a unique signalsome during viral entry into monocytes mediates distinct functional changes: a strategy for viral dissemination. J Leukoc Biol 92:743–752. https://doi.org/10.1189/jlb.0112040
23. Chan G, Bivins-Smith ER, Smith MS, Yurochko AD. 2009. NF-kappaB and phosphatidylinositol 3-kinase activity mediates the HCMV-induced atypical M1/M2 polarization of monocytes. Virus Res 144:329–333. https://doi.org/10.1016/j.virusres.2009.04.026
24. Peppenelli MA, Arend KC, Cojohari O, Moorman NJ, Chan GC, Frueh K. 2016. Human cytomegalovirus stimulates the synthesis of select Akt-dependent antiapoptotic proteins during viral entry to promote survival of infected monocytes. J Virol 90:3138–3147. https://doi.org/10.1128/JVI.02879-15
25. Cojohari O, Peppenelli MA, Chan GC, Frueh K. 2016. Human cytomegalovirus induces an atypical activation of Akt to stimulate the survival of short-lived monocytes. J Virol 90:6443–6452. https://doi.org/10.1128/JVI.00214-16
26. Cojohari O, Mahmud J, Altman AM, Peppenelli MA, Miller MJ, Chan GC. 2020. Human cytomegalovirus mediates unique monocyte-to-macrophage differentiation through the PI3K/SHIP1/Akt signaling network. Viruses 12:652. https://doi.org/10.3390/v12060652
27. Mahmud J, Miller MJ, Altman AM, Chan GC, Goodrum F. 2020. Human cytomegalovirus glycoprotein-initiated signaling mediates the aberrant activation of Akt. J Virol 94:e00167-20. https://doi.org/10.1128/JVI.00167-20
28. Yurochko AD, Hwang ES, Rasmussen L, Keay S, Pereira L, Huang ES. 1997. The human cytomegalovirus UL55 (gB) and UL75 (gH) glycoprotein ligands initiate the rapid activation of Sp1 and NF-kappaB during infection. J Virol 71:5051–5059. https://doi.org/10.1128/JVI.71.7.5051-5059.1997
29. Compton T, Nowlin DM, Cooper NR. 1993. Initiation of human cytomegalovirus infection requires initial interaction with cell surface heparan sulfate. Virology 193:834–841. https://doi.org/10.1006/viro.1993.1192
30. Compton T. 2004. Receptors and immune sensors: the complex entry path of human cytomegalovirus. Trends Cell Biol 14:5–8. https://doi.org/10.1016/j.tcb.2003.10.009
31. Zhang H, Domma AJ, Goodrum FD, Moorman NJ, Kamil JP, Shenk T. 2022. The Akt forkhead box O transcription factor axis regulates human cytomegalovirus replication. mBio 13:e0104222. https://doi.org/10.1128/mbio.01042-22
32. Yung HW, Charnock-Jones DS, Burton GJ. 2011. Regulation of Akt phosphorylation at Ser473 and Thr308 by endoplasmic reticulum stress modulates substrate specificity in a severity dependent manner. PLoS One 6:e17894. https://doi.org/10.1371/journal.pone.0017894
33. Manning BD, Cantley LC. 2007. AKT/PKB signaling: navigating downstream. Cell 129:1261–1274. https://doi.org/10.1016/j.cell.2007.06.009
34. Balasuriya N, Davey NE, Johnson JL, Liu H, Biggar KK, Cantley LC, Li SS-C, O'Donoghue P. 2020. Phosphorylation-dependent substrate selectivity of protein kinase B (AKT1). J Biol Chem 295:8120–8134. https://doi.org/10.1074/jbc.RA119.012425
35. Altman AM, Mahmud J, Nikolovska-Coleska Z, Chan G. 2019. HCMV modulation of cellular PI3K/AKT/mTOR signaling: new opportunities for therapeutic intervention? Antiviral Res 163:82–90. https://doi.org/10.1016/j.antiviral.2019.01.009
36. Yang H, Jiang X, Li B, Yang HJ, Miller M, Yang A, Dhar A, Pavletich NP. 2017. Mechanisms of mTORC1 activation by RHEB and inhibition by PRAS40. Nature 552:368–373. https://doi.org/10.1038/nature25023
37. Thoreen CC, Chantranupong L, Keys HR, Wang T, Gray NS, Sabatini DM. 2012. A unifying model for mTORC1-mediated regulation of mRNA translation. Nature 485:109–113. https://doi.org/10.1038/nature11083
38. Szwed A, Kim E, Jacinto E. 2021. Regulation and metabolic functions of mTORC1 and mTORC2. Physiol Rev 101:1371–1426. https://doi.org/10.1152/physrev.00026.2020
39. Inoki K, Zhu T, Guan KL. 2003. TSC2 mediates cellular energy response to control cell growth and survival. Cell 115:577–590. https://doi.org/10.1016/s0092-8674(03)00929-2
40. Zhao Y, Hu X, Liu Y, Dong S, Wen Z, He W, Zhang S, Huang Q, Shi M. 2017. ROS signaling under metabolic stress: cross-talk between AMPK and AKT pathway. Mol Cancer 16:79. https://doi.org/10.1186/s12943-017-0648-1
41. Herzig S, Shaw RJ. 2018. AMPK: guardian of metabolism and mitochondrial homeostasis. Nat Rev Mol Cell Biol 19:121–135. https://doi.org/10.1038/nrm.2017.95
42. Gordon BS, Kelleher AR, Kimball SR. 2013. Regulation of muscle protein synthesis and the effects of catabolic states. Int J Biochem Cell Biol 45:2147–2157. https://doi.org/10.1016/j.biocel.2013.05.039
43. Kudchodkar SB, Del Prete GQ, Maguire TG, Alwine JC. 2007. AMPK-mediated inhibition of mTOR kinase is circumvented during immediate-early times of human cytomegalovirus infection. J Virol 81:3649–3651. https://doi.org/10.1128/JVI.02079-06

44. McArdle J, Moorman NJ, Munger J. 2012. HCMV targets the metabolic stress response through activation of AMPK whose activity is important for viral replication. PLoS Pathog 8:e1002502. https://doi.org/10.1371/journal.ppat.1002502
45. Rodríguez-Sánchez I, Schafer XL, Monaghan M, Munger J, Dittmer DP. 2019. The human cytomegalovirus UL38 protein drives mTOR-independent metabolic flux reprogramming by inhibiting TSC2. PLoS Pathog 15:e1007569. https://doi.org/10.1371/journal.ppat.1007569
46. Bai Y, Xuan B, Liu H, Zhong J, Yu D, Qian Z. 2015. Tuberous sclerosis complex protein 2-independent activation of mTORC1 by human cytomegalovirus pUL38. J Virol 89:7625–7635. https://doi.org/10.1128/JVI.01027-15
47. Peppenelli MA, Miller MJ, Altman AM, Cojohari O, Chan GC. 2018. Aberrant regulation of the Akt signaling network by human cytomegalovirus allows for targeting of infected monocytes. Antiviral Res 158:13–24. https://doi.org/10.1016/j.antiviral.2018.07.015
48. Ziehr B, Vincent HA, Moorman NJ. 2016. Human cytomegalovirus pTRS1 and pIRS1 antagonize protein kinase R to facilitate virus replication. J Virol 90:3839–3848. https://doi.org/10.1128/JVI.02714-15
49. Vincent HA, Ziehr B, Lenarcic EM, Moorman NJ. 2019. Human cytomegalovirus pTRS1 stimulates cap-independent translation. Virology 537:246–253. https://doi.org/10.1016/j.virol.2019.08.026
50. Marshall EE, Bierle CJ, Brune W, Geballe AP. 2009. Essential role for either TRS1 or IRS1 in human cytomegalovirus replication. J Virol 83:4112–4120. https://doi.org/10.1128/JVI.02489-08
51. Braggin JE, Child SJ, Geballe AP. 2016. Essential role of protein kinase R antagonism by TRS1 in human cytomegalovirus replication. Virology 489:75–85. https://doi.org/10.1016/j.virol.2015.11.032
52. Schmidt EK, Clavarino G, Ceppi M, Pierre P. 2009. SUnSET, a nonradioactive method to monitor protein synthesis. Nat Methods 6:275–277. https://doi.org/10.1038/nmeth.1314
53. Ravi V, Jain A, Mishra S, Sundaresan NR. 2020. Measuring protein synthesis in cultured cells and mouse tissues using the non-radioactive SUnSET assay. Curr Protoc Mol Biol 133:e127. https://doi.org/10.1002/cpmb.127
54. Walsh D, Perez C, Notary J, Mohr I. 2005. Regulation of the translation initiation factor eIF4F by multiple mechanisms in human cytomegalovirus-infected cells. J Virol 79:8057–8064. https://doi.org/10.1128/JVI.79.13.8057-8064.2005
55. McKinney C, Perez C, Mohr I. 2012. Poly(A) binding protein abundance regulates eukaryotic translation initiation factor 4F assembly in human cytomegalovirus-infected cells. Proc Natl Acad Sci U S A 109:5627–5632. https://doi.org/10.1073/pnas.1202829109
56. Li BB, Qian C, Gameiro PA, Liu CC, Jiang T, Roberts TM, Struhl K, Zhao JJ. 2018. Targeted profiling of RNA translation reveals mTOR-4EBP1/2-independent translation regulation of mRNAs encoding ribosomal proteins. Proc Natl Acad Sci U S A 115:E9325–E9332. https://doi.org/10.1073/pnas.1805782115
57. Chan G, Nogalski MT, Bentz GL, Smith MS, Parmater A, Yurochko AD. 2010. PI3K-dependent upregulation of Mcl-1 by human cytomegalovirus is mediated by epidermal growth factor receptor and inhibits apoptosis in short-lived monocytes. J Immunol 184:3213–3222. https://doi.org/10.4049/jimmunol.0903025
58. Bostner J, Karlsson E, Pandiyan MJ, Westman H, Skoog L, Fornander T, Nordenskjöld B, Stål O. 2013. Activation of Akt, mTOR, and the estrogen receptor as a signature to predict tamoxifen treatment benefit. Breast Cancer Res Treat 137:397–406. https://doi.org/10.1007/s10549-012-2376-y
59. Figueiredo VC, Markworth JF, Cameron-Smith D. 2017. Considerations on mTOR regulation at serine 2448: implications for muscle metabolism studies. Cell Mol Life Sci 74:2537–2545. https://doi.org/10.1007/s00018-017-2481-5
60. Chiang GG, Abraham RT. 2005. Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. J Biol Chem 280:25485–25490. https://doi.org/10.1074/jbc.M501707200
61. Thoreen CC. 2013. Many roads from mTOR to eIF4F. Biochem Soc Trans 41:913–916. https://doi.org/10.1042/BST20130082
62. Pópulo H, Lopes JM, Soares P. 2012. The mTOR signalling pathway in human cancer. Int J Mol Sci 13:1886–1918. https://doi.org/10.3390/ijms13021886
63. Aoki M, Fujishita T. 2017. Oncogenic roles of the PI3K/AKT/mTOR axis. Curr Top Microbiol Immunol 407:153–189. https://doi.org/10.1007/82_2017_6
64. Magnuson B, Ekim B, Fingar DC. 2012. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. Biochem J 441:1–21. https://doi.org/10.1042/BJ20110892
65. Kaul G, Pattan G, Rafeequi T. 2011. Eukaryotic elongation factor-2 (eEF2): its regulation and peptide chain elongation. Cell Biochem Funct 29:227–234. https://doi.org/10.1002/cbf.1740
66. Panda AC, Martindale JL, Gorospe M. 2017. Polysome fractionation to analyze mRNA distribution profiles. Bio Protoc 7:e2126. https://doi.org/10.21769/BioProtoc.2126
67. Liang S, Bellato HM, Lorent J, Lupinacci FCS, Oertlin C, van Hoef V, Andrade VP, Roffé M, Masvidal L, Hajj GNM, Larsson O. 2018. Polysome-profiling in small tissue samples. Nucleic Acids Res 46:e3–e3. https://doi.org/10.1093/nar/gkx940
68. Ma C, Zhang D, Ma Q, Liu Y, Yang Y. 2021. Arbutin inhibits inflammation and apoptosis by enhancing autophagy via SIRT1. Adv Clin Exp Med 30:535–544. https://doi.org/10.17219/acem/133493
69. Lee SH, Lee JH, Lee HY, Min KJ. 2019. Sirtuin signaling in cellular senescence and aging. BMB Rep 52:24–34. https://doi.org/10.5483/BMBRep.2019.52.1.290
70. Kim JK, Silwal P, Jo EK. 2022. Sirtuin 1 in host defense during infection. Cells 11:2921. https://doi.org/10.3390/cells11182921
71. Garten A, Grohmann T, Kluckova K, Lavery GG, Kiess W, Penke M. 2019. Sorafenib-induced apoptosis in hepatocellular carcinoma is reversed by SIRT1. Int J Mol Sci 20:4048. https://doi.org/10.3390/ijms20164048
72. Luo G, Jian Z, Zhu Y, Zhu Y, Chen B, Ma R, Tang F, Xiao Y. 2019. SIRT1 promotes autophagy and inhibits apoptosis to protect cardiomyocytes from hypoxic stress. Int J Mol Med 43:2033–2043. https://doi.org/10.3892/ijmm.2019.4125
73. Broussy S, Laaroussi H, Vidal M. 2020. Biochemical mechanism and biological effects of the inhibition of silent information regulator 1 (SIRT1) by EX-527 (SEN0014196 or selisistat). J Enzyme Inhib Med Chem 35:1124–1136. https://doi.org/10.1080/14756366.2020.1758691
74. Gertz M, Fischer F, Nguyen GTT, Lakshminarasimhan M, Schutkowski M, Weyand M, Steegborn C. 2013. Ex-527 inhibits sirtuins by exploiting their unique NAD+-dependent deacetylation mechanism. Proc Natl Acad Sci U S A 110:E2772–81. https://doi.org/10.1073/pnas.1303628110
75. Kim BS, Lee CH, Chang GE, Cheong E, Shin I. 2016. A potent and selective small molecule inhibitor of sirtuin 1 promotes differentiation of pluripotent P19 cells into functional neurons. Sci Rep 6:34324. https://doi.org/10.1038/srep34324
76. Andersen JL, Thompson JW, Lindblom KR, Johnson ES, Yang CS, Lilley LR, Freel CD, Moseley MA, Kornbluth S. 2011. A biotin switch-based proteomics approach identifies 14-3-3ζ as a target of SIRT1 in the metabolic regulation of caspase-2. Mol Cell 43:834–842. https://doi.org/10.1016/j.molcel.2011.07.028
77. Kim HW, Kim SA, Ahn SG. 2016. Sirtuin inhibitors, EX527 and AGK2, suppress cell migration by inhibiting HSF1 protein stability. Oncol Rep 35:235–242. https://doi.org/10.3892/or.2015.4381
78. Mourits VP, Helder LS, Matzaraki V, Koeken V, Groh L, de Bree LCJ, Moorlag S, van der Heijden C, Keating ST, van Puffelen JH, Jaeger M, Joosten LAB, Netea MG. 2021. The role of sirtuin 1 on the induction of trained immunity. Cell Immunol 366:104393. https://doi.org/10.1016/j.cellimm.2021.104393
79. Sundaresan NR, Pillai VB, Wolfgeher D, Samant S, Vasudevan P, Parekh V, Raghuraman H, Cunningham JM, Gupta M, Gupta MP. 2011. The deacetylase SIRT1 promotes membrane localization and activation of Akt and PDK1 during tumorigenesis and cardiac hypertrophy. Sci Signal 4:ra46. https://doi.org/10.1126/scisignal.2001465
80. Pillai VB, Sundaresan NR, Gupta MP. 2014. Regulation of Akt signaling by sirtuins: its implication in cardiac hypertrophy and aging. Circ Res 114:368–378. https://doi.org/10.1161/CIRCRESAHA.113.300536
81. Aguilar-Arnal L, Ranjit S, Stringari C, Orozco-Solis R, Gratton E, Sassone-Corsi P. 2016. Spatial dynamics of SIRT1 and the subnuclear distribution of NADH species. Proc Natl Acad Sci U S A 113:12715–12720. https://doi.org/10.1073/pnas.1609227113
82. Byles V, Chmilewski LK, Wang J, Zhu L, Forman LW, Faller DV, Dai Y. 2010. Aberrant cytoplasm localization and protein stability of SIRT1 is

regulated by PI3K/IGF-1R signaling in human cancer cells. Int J Biol Sci 6:599–612. https://doi.org/10.7150/ijbs.6.599
83. Gnann JW, Ahlmén J, Svalander C, Olding L, Oldstone MB, Nelson JA. 1988. Inflammatory cells in transplanted kidneys are infected by human cytomegalovirus. Am J Pathol 132:239–248.
84. Crawford LB, Hancock MH, Struthers HM, Streblow DN, Yurochko AD, Caposio P, Goodrum FD, Nelson JA, Longnecker RM. 2021. CD34$^+$ hematopoietic progenitor cell subsets exhibit differential ability to maintain human cytomegalovirus latency and persistence. J Virol 95:e02105-20. https://doi.org/10.1128/JVI.02105-20
85. Taylor-Wiedeman J, Sissons P, Sinclair J. 1994. Induction of endogenous human cytomegalovirus gene expression after differentiation of monocytes from healthy carriers. J Virol 68:1597–1604. https://doi.org/10.1128/JVI.68.3.1597-1604.1994
86. Whitelaw DM. 1972. Observations on human monocyte kinetics after pulse labeling. Cell Tissue Kinet 5:311–317. https://doi.org/10.1111/j.1365-2184.1972.tb00369.x
87. Nogalski MT, Chan GCT, Stevenson EV, Collins-McMillen DK, Yurochko AD, Nelson JA. 2013. The HCMV gH/gL/UL128-131 complex triggers the specific cellular activation required for efficient viral internalization into target monocytes. PLoS Pathog 9:e1003463. https://doi.org/10.1371/journal.ppat.1003463
88. Stern-Ginossar N, Thompson SR, Mathews MB, Mohr I. 2019. Translational control in virus-infected cells. Cold Spring Harb Perspect Biol 11:a033001. https://doi.org/10.1101/cshperspect.a033001
89. Au HHT, Jan E. 2014. Novel viral translation strategies. Wiley Interdiscip Rev RNA 5:779–801. https://doi.org/10.1002/wrna.1246
90. Stern-Ginossar N, Weisburd B, Michalski A, Le VTK, Hein MY, Huang S-X, Ma M, Shen B, Qian S-B, Hengel H, Mann M, Ingolia NT, Weissman JS. 2012. Decoding human cytomegalovirus. Science 338:1088–1093. https://doi.org/10.1126/science.1227919
91. Fulkerson HL, Chesnokova LS, Kim JH, Mahmud J, Frazier LE, Chan GC, Yurochko AD. 2020. HCMV-induced signaling through gB-EGFR engagement is required for viral trafficking and nuclear translocation in primary human monocytes. Proc Natl Acad Sci U S A 117:19507–19516. https://doi.org/10.1073/pnas.2003549117
92. Mahmud J, Chan GC. 2021. Analysis of cytomegalovirus glycoprotein and cellular receptor interactions. Methods Mol Biol 2244:199–211. https://doi.org/10.1007/978-1-0716-1111-1_10
93. Tavares MR, Pavan ICB, Amaral CL, Meneguello L, Luchessi AD, Simabuco FM. 2015. The S6K protein family in health and disease. Life Sci 131:1–10. https://doi.org/10.1016/j.lfs.2015.03.001
94. Acosta-Jaquez HA, Keller JA, Foster KG, Ekim B, Soliman GA, Feener EP, Ballif BA, Fingar DC. 2009. Site-specific mTOR phosphorylation promotes mTORC1-mediated signaling and cell growth. Mol Cell Biol 29:4308–4324. https://doi.org/10.1128/MCB.01665-08
95. Yin S, Liu L, Gan W. 2021. The roles of post-translational modifications on mTOR signaling. Int J Mol Sci 22:1784. https://doi.org/10.3390/ijms22041784
96. Dan HC, Ebbs A, Pasparakis M, Van Dyke T, Basseres DS, Baldwin AS. 2014. Akt-dependent activation of mTORC1 complex involves phosphorylation of mTOR (mammalian target of rapamycin) by IκB kinase α (IKKα). J Biol Chem 289:25227–25240. https://doi.org/10.1074/jbc.M114.554881
97. Santos Bravo M, Plault N, Sánchez Palomino S, Mosquera Gutierrez MM, Fernández Avilés F, Suarez Lledo M, Sabé Fernández N, Rovira M, Alain S, Marcos Maeso MÁ. 2021. Phenotype and genotype study of novel C480F maribavir-ganciclovir cross-resistance mutation detected in hematopoietic stem cell and solid organ transplant recipients. J Infect Dis 224:1024–1028. https://doi.org/10.1093/infdis/jiab029
98. Paolucci S, Campanini G, Cassaniti I, Tebaldi A, Novazzi F, Fratini A, Meini A, Girelli F, Palumbo L, Plebani A, Baldanti F. 2021. Emergence of Letermovir-resistant HCMV UL56 mutant during rescue treatment in a liver transplant recipient with ganciclovir-resistant infection HCMV: a case report. BMC Infect Dis 21:994. https://doi.org/10.1186/s12879-021-06694-4
99. Maertens J, Cordonnier C, Jaksch P, Poiré X, Uknis M, Wu J, Wijatyk A, Saliba F, Witzke O, Villano S. 2019. Maribavir for preemptive treatment of cytomegalovirus reactivation. N Engl J Med 381:1136–1147. https://doi.org/10.1056/NEJMoa1714656
100. Lurain NS, Chou S. 2010. Antiviral drug resistance of human cytomegalovirus. Clin Microbiol Rev 23:689–712. https://doi.org/10.1128/CMR.00009-10
101. Kim ES. 2018. Letermovir: first global approval. Drugs 78:147–152. https://doi.org/10.1007/s40265-017-0860-8
102. Kendle JB, Fan-Havard P. 1998. Cidofovir in the treatment of cytomegaloviral disease. Ann Pharmacother 32:1181–1192. https://doi.org/10.1345/aph.17312
103. Erice A, Jordan MC, Chace BA, Fletcher C, Chinnock BJ, Balfour HH. 1987. Ganciclovir treatment of cytomegalovirus disease in transplant recipients and other immunocompromised hosts. JAMA 257:3082–3087. https://doi.org/10.1001/jama.1987.03390220080025
104. Yurochko AD, Huang ES. 1999. Human cytomegalovirus binding to human monocytes induces immunoregulatory gene expression. J Immunol 162:4806–4816. https://doi.org/10.4049/jimmunol.162.8.4806
105. Smith MS, Bentz GL, Alexander JS, Yurochko AD. 2004. Human cytomegalovirus induces monocyte differentiation and migration as a strategy for dissemination and persistence. J Virol 78:4444–4453. https://doi.org/10.1128/jvi.78.9.4444-4453.2004
106. Katholnig K, Poglitsch M, Hengstschläger M, Weichhart T. 2015. Lysis gradient centrifugation: a flexible method for the isolation of nuclei from primary cells. Methods Mol Biol 1228:15–23. https://doi.org/10.1007/978-1-4939-1680-1_2
107. Poglitsch M, Katholnig K, Säemann MD, Weichhart T. 2011. Rapid isolation of nuclei from living immune cells by a single centrifugation through a multifunctional lysis gradient. J Immunol Methods 373:167–173. https://doi.org/10.1016/j.jim.2011.08.012
108. Goodman CA, Hornberger TA. 2013. Measuring protein synthesis with SUnSET: a valid alternative to traditional techniques? Exerc Sport Sci Rev 41:107–115. https://doi.org/10.1097/JES.0b013e3182798a95