

**Human cytomegalovirus degrades DMXL1
to inhibit autophagy, lysosomal acidification
and viral assembly**

Hanqi Li^{1,2,8}, Alice Fletcher-Etherington^{1,2,8}, Leah M. Hunter^{1,2}, Swati Keshri^{1,3}, Ceri A. Fielding⁴, Katie Nightingale^{1,2}, Benjamin Ravenhill^{1,2}, Luis Nobre^{1,2}, Martin Potts^{1,2}, Robin Antrobus^{1,2}, Colin M. Crump⁵, David C. Rubinsztein^{1,3}, Richard J. Stanton^{4,6}, Michael P. Weekes^{1,6,7}

Affiliations:

¹ Cambridge Institute for Medical Research, University of Cambridge, Hills Road,
Cambridge CB2 0XY, UK

² Department of Medicine, University of Cambridge, Hills Road, Cambridge, CB2 2QQ, UK

³ UK Dementia Institute, The Keith Peters Building, University of Cambridge, Hills Road,
Cambridge CB2 0XY

⁴ Cardiff University School of Medicine, Division of Infection and Immunity, Henry
Wellcome Building, Heath Park, Cardiff CF14 4XN, UK

⁵ Division of Virology, Department of Pathology, University of Cambridge, Cambridge, UK

⁶ Senior author

⁷ Lead contact

⁸ These authors contributed equally

Correspondence: mpw1001@cam.ac.uk

SUMMARY

Human cytomegalovirus (HCMV) is an important human pathogen that regulates host immunity and hijacks host compartments including lysosomes to assemble virions. We combined a quantitative proteomic analysis of HCMV infection with a database of proteins involved in vacuolar acidification, revealing Dmx-like protein-1 (DMXL1) as the only protein that acidifies vacuoles yet is degraded by HCMV. Systematic comparison of viral deletion mutants revealed the uncharacterised 7kDa US33A protein as necessary and sufficient for DMXL1 degradation, which occurred via recruitment of the E3 ubiquitin ligase Kip1 ubiquitination-promoting complex (KPC). US33A-mediated DMXL1 degradation inhibited lysosome acidification and autophagic cargo degradation. Formation of the virion assembly compartment, which requires lysosomes, occurred significantly later with US33A-expressing virus infection, with reduced viral replication. These data thus identify a viral strategy for cellular remodelling, with the potential to employ US33A in therapies for viral infection or rheumatic conditions, in which inhibition of lysosome acidification can attenuate disease.

Keywords: proteomics; systems virology; human cytomegalovirus; lysosome; pH; viral replication; viral assembly; autophagy

INTRODUCTION

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that persistently infects more than 60% of all people worldwide¹. Reactivation of HCMV from latency in immunocompromised people including organ transplant recipients can cause significant morbidity and mortality^{2,3}. HCMV is also a leading cause of congenital disease, affecting 0.5-4% of pregnancies⁴. Here, it can cause a spectrum of disease including sensorineural deafness, intellectual disability and microcephaly⁴. However, only four anti-HCMV drugs have been approved, all with significant toxicity and problematic drug resistance⁵.

As a virus that persists lifelong, and that has co-evolved with its human host over millions of years, HCMV is regarded as a master cellular regulator, systemically modulating multiple processes critical to infection. We previously found that >900 host proteins are downregulated >3 fold over the course of infection, with at least 163 proteins degraded in the proteasome or lysosome⁶⁻⁸. These studies highlighted previously unrecognised facets of innate immunity, including that helicase-like transcription factor (HLTF) and Schlafen family member 11 (SLFN11) are novel antiviral restriction factors targeted by the viral UL145 and RL1 proteins respectively, and that mixed lineage kinase domain-like protein (MLKL) is degraded by HCMV UL36 to inhibit necroptotic cell death⁷⁻⁹. Small molecules that inhibit these and other HCMV-host interactions may offer novel therapeutic potential¹⁰.

During productive infection *in vitro*, expression of HCMV genes is conventionally divided into immediate-early, early and late phases during a ~72-96 h lytic replication cycle¹¹. We previously characterised five temporal classes of viral protein expression, offering finer definition of protein expression profiles⁷. Late during infection, HCMV hijacks host intracellular compartments (vacuoles) to facilitate viral replication, especially virion assembly and egress. For example, in fibroblasts, HCMV reorganises the cytoskeleton and endocytic pathway to form virion assembly compartments (vAC), which contain virions and early endosomes in the core, and late endosomes/lysosomes at the periphery^{12,13}. HCMV forms similar structures in endothelial cells, containing lysosomes and autophagosomes, but not early endosomes¹⁴. These studies suggest that lysosomes are necessary to assemble the HCMV virion. Lysosomes are also required during viral replication to degrade a variety of molecules important in innate and adaptive immunity¹⁵⁻²⁰. In comparison to the cytoplasm and other intracellular vesicles with a neutral pH, lysosomes are acidic (pH 4.5 - 5), requiring the host V-ATPase to pump protons into the lumen²¹. Although the impact of lysosomal pH on viral replication is poorly defined, vacuolar acidification conducted by the ATP6V0C subunit of the V-ATPase may be necessary for vAC formation²².

74 To determine how HCMV may globally modulate lysosome function, we combined our
75 proteomic analyses with a database of proteins involved in vacuolar acidification. Dmx-like
76 protein 1 (DMXL1) was the only protein that acidifies vacuoles (via interaction with the v-
77 ATPase²³) and is also degraded by HCMV. We characterise the function of each of seven viral
78 genes from a genetic block we identify as necessary to target DMXL1, determining that the
79 previously uncharacterised HCMV US33A protein degrades DMXL1 by recruiting the Kip1
80 complex (KPC) E3 ligase. HCMV-induced DMXL1 degradation was sufficient to inhibit
81 lysosomal acidification, autophagic cargo degradation and vAC formation, all previously
82 unidentified functions for viral proteins.

RESULTS

DMXL1 stimulates vacuolar acidification but is targeted for degradation by HCMV US33A

To identify host factors with roles in lysosomal acidification that are antagonized by HCMV, we overlapped data from our previous publications in which we identified proteins degraded early⁷ and/or late⁹ during HCMV infection with a database of proteins that regulate acidification of the vacuole²⁴. Only Dmx-like 1 (DMXL1) fulfilled both criteria (**Figures 1a-c**), suggesting that this protein may be of substantial importance both to the cell and to virus. DMXL1 has only been characterised in three prior publications, which identified that the 338 kDa protein is highly conserved with multiple WD40 repeats that fold to form β -propellers^{23,25,26}. DMXL1 and DMXL2 interact with WD repeat-containing protein 7 (WDR7) to form the complex Rabconnectin-3, which interacts with the V-ATPase in mouse kidney lysates^{23,27,28}. The DMXL proteins are partially homologous to regulator of V-ATPase in vacuolar membrane protein 1 (RAV1), a component of the yeast regulator of H⁺-ATPase of vacuolar and endosomal membranes (RAVE) complex which is involved in the reassembly of dissociated V-ATPase V1 and V0 sectors²⁹. In addition to a potential role in the control of V-ATPase trafficking, the DMXL proteins may play analogous roles to RAV1 in the assembly of V-ATPase subunits via WD40 domain interactions, and are thought to promote intracellular vesicle acidification^{23,26,27}.

To study whether HCMV regulates lysosomal acidification via DMXL1, we first needed to identify which of the 171 HCMV genes is involved. We previously conducted a proteomic screen using a panel of recombinant viruses deleted for one or other of a series of blocks of genes non-essential for viral replication *in vitro*⁹. This identified that DMXL1 is targeted by the uncharacterised viral *US29-US34A* block (**Figure 1d**). To determine which gene(s) in this block is required, we generated a library of HCMV single gene deletion mutants in *US29-US34A*, and infected immortalised primary human fetal foreskin fibroblasts (HFFF-TERTs) with these or the wild-type (WT) strain (**Figure S1a**). Multiplexed proteomic analysis of cellular lysates using TMT peptide labels and MS3 mass spectrometry quantified 7687 human and 158 viral proteins, revealing a diversity of functions for this gene block. These included regulation of innate immunity via inhibition of multiple interferon-stimulated genes by US30 and US34A; regulation of proteins involved in mitotic cell division by US31 and regulation of protein involved in secretion by US34 (**Figure S1b, Tables S1-2**). Data from proteomic

experiments in this study are shown in **Table S3**. Here, the worksheet “Plots” is interactive, enabling generation of graphs of protein expression of any of the human and viral proteins quantified.

DMXL1 expression was only rescued by deletion of US33A, a small 7 kDa protein with a single predicted α -helix (**Figures 1e-f, S1, S2a**). DMXL1 and the related WDR7 protein were the only proteins significantly upregulated after 72 h infection with Δ US33A HCMV in comparison to WT virus (**Figures 1g, S1b, S3a**). Overlap of this data with a complementary proteomic screen comparing stably overexpressed US33A to control suggested that this protein is both necessary and sufficient for DMXL1 downregulation. Furthermore, the combination of both screens suggested that DMXL1 and to a lesser extent WDR7 are the only bona fide targets of US33A (**Figures 1h, S3b, Table S1**). However, addition of either an N- or C-terminal tag ablated US33A-mediated DMXL1 degradation (**Figure S3c, d**), leading us to use untagged US33A for subsequent functional experiments. We did not detect any US33A peptides either in this study or previously using mass spectrometry, likely reflecting its small size and a paucity of peptides suitable for proteomic analysis. However, *US33A* transcript was detected within the first 24 h of HCMV infection, consistent with the kinetics of DMXL1 degradation (**Figures 1i-j**).

DMXL degradation by US33A disrupts lysosomal acidification

To determine whether degradation of DMXL1 by US33A inhibits lysosomal acidification, HFFF-TERTs stably expressing US33A or control were stained with LysoTracker DND 99. US33A expression reduced LysoTracker staining, indicative of a less acidic lysosome (**Figure 2a**). These findings were confirmed using the pH-sensitive probe LysoSensor Yellow/Blue (**Figure 2b**). Whereas LysoTracker quantitation is dependent on the total cellular LysoTracker signal, ratiometric quantification of LysoSensor staining at two different wavelengths minimises the potential for error due to differences in dye uptake by each stained cell³⁰. US33A-mediated impairment of lysosomal acidification was confirmed in HFFF-TERTs stably expressing US33A or control, infected with adenoviruses expressing US33A (RAd-US33A) or control and infected with WT or Δ US33A HCMV (**Figure 2c, S4a**). Cells infected with RAd-US33A expressed a higher level of US33A mRNA, consistent with the increased magnitude of effect observed (**Figure S3e**).

A previous publication suggested that in mouse cells, knockdown of DMXL1 does not impact intravesicular acidification under baseline conditions, however re-acidification of intracellular vesicles after Bafilomycin A1 (BafA1) treatment is suppressed³¹. This indicated that cellular functions requiring significant and sustained upregulation of V-ATPase activity may be dependent on DMXL1. We designed experiments to re-capitulate this phenotype with regards to US33A (**Figure 2d**). Lysosomes in HFFF-TERTs expressing US33A lost their ability to recover an acidic pH after 1 h BafA1 treatment. Infection of HFFF-TERTs with Δ US33A HCMV enhanced lysosomal pH recovery after 1 h BafA1 treatment compared to WT HCMV-infected cells (**Figure 2d**). Small interfering RNA (siRNA) knockdown of DMXL1 phenocopied US33A expression and inhibited lysosomal pH recovery in HFFF-TERTs (**Figure S4b, c**). These results suggest that DMXL1 degradation by US33A causes functional lysosomal deficiency, which may be due to impaired V-ATPase regulation.

US33A inhibits autophagic cargo degradation

A functional V-ATPase and acidic lysosome are required for the terminal stages of autophagy: autophagosome-lysosome fusion and autophagosome degradation (**Figure 3a**)³². To determine whether the lysosomal deficiency caused by US33A-mediated DMXL1 degradation impacts autophagy, we quantified LC3B-II. Accumulation of this classical marker of autophagosome abundance either indicates enhanced autophagosome formation or impaired autophagosome degradation³³⁻³⁵. To distinguish between these two possibilities, we also applied the V-ATPase inhibitor BafA1 to block autophagosome/LC3B-II degradation completely, meaning that measurement of LC3B-II would indicate autophagosome synthesis^{36,37}. US33A expression in HFFF-TERTs via stable lentiviral transduction or adenoviral infection significantly enhanced LC3B-II accumulation in the absence, but not in the presence of BafA1 (**Figures 3b-c, Figure S5a-b**). These findings suggest that US33A leads to inhibition of autophagosome degradation. To recapitulate the experiment during HCMV infection, we used a high MOI=25 to ensure infection of the greatest number of cells. Pre-incubation of HFFF-TERTs with serum-free medium can enable high-percentage infection at a lower MOI^{8,20,38}, however nutrient starvation can rapidly upregulate autophagic flux and autophagosome degradation³⁹ which would confound data. In comparison to infection with WT HCMV, infection with Δ US33A HCMV reduced LC3B-II accumulation in DMSO-treated cells but had no significant effect in BafA1-treated cells, which is consistent with US33A-mediated inhibition of autophagosome degradation (**Figure 3d, Figure S5c**). siRNA knockdown of DMXL1 phenocopied US33A

expression and inhibited LC3B-II degradation, suggesting that inhibition of autophagosome degradation by US33A is mediated by DMXL1 degradation (**Figure S5d**).

A reduction in autophagosome degradation might be caused by a decrease in autophagic cargo degradation due to reduced lysosome activity, impaired lysosomal-autophagosome fusion, or both³⁰. To identify which applied here, co-localisation between LC3B (autophagosome marker) and LAMP1/CD63 (lysosome/late endosome markers) was measured. Stable US33A expression enhanced co-localisation of endogenous LC3B with LAMP1 and increased expression of both markers, suggesting that autophagosome-lysosome fusion was not impaired upon DMXL1 degradation and that LC3B-II degradation was likely to have been retarded, as we have previously observed in similar circumstances^{30,40} (**Figures 3e, f, S5e, Video S1**). Infection with Δ US33A HCMV led to reduced LC3B-LAMP1 co-localisation and expression in comparison to WT HCMV infection (**Figures 3g, h, S5f**). These results suggest that US33A-mediated DMXL1 deficiency inhibits autophagosome degradation in lysosomes, rather than inhibiting the fusion between lysosomes and autophagosomes.

US33A degrades DMXL1 via recruitment of the KPC E3 ligase complex

We previously conducted a systematic interactome analysis of 169 canonical HCMV proteins, and a subset of non-canonical HCMV proteins, in infected cells³⁸. Although this study purified complexes using C-terminal V5 tags, which ablate US33A-mediated DMXL1 degradation (**Figure S3c**), it was nevertheless possible that US33A retained the ability to recruit the relevant DMXL1 degradation machinery. Interactome analysis revealed that US33A-V5 interacts with RNF123 and UBAC1, the catalytic and non-catalytic subunits of the KPC E3 ligase (**Figure 4a**). We confirmed the interaction between US33A and RNF123/UBAC1 by co-IP (**Figures 4b, S6a**), and, using a series of US33A deletion or truncation mutants, determined that the US33A N-terminus is necessary to recruit KPC (**Figures 4c, S6b**).

To determine whether KPC is required for US33A to degrade DMXL1, CRISPR/Cas9 was used to knock out RNF123, UBAC1 or both in HFFF-TERTs, followed by infection with WT or Δ US33A HCMV. In all three knockout cell types, DMXL1 was rescued during WT HCMV infection to a level similar to during Δ US33A infection (**Figures 4d, S6c, Table S4**), indicating that US33A degrades DMXL1 via recruitment of KPC.

US33A delays formation of the virion assembly compartment and negatively regulates replication of HCMV and HSV-1

To determine whether the effects of US33A on lysosomal pH and autophagy may modulate viral assembly compartment (vAC) formation, we performed a temporal analysis during infection with WT and Δ US33A HCMV. Mature vACs were identified as a juxtanuclear structure formed by a ring of giantin, a marker for the Golgi body, outside a core of the HCMV envelope glycoprotein B (gB)⁴¹⁻⁴⁶ (**Figure 4e, Figure S7a**). Early/immature vACs were identified as infected cells expressing gB but without ring-shaped giantin^{43,44}. vACs formed significantly earlier in cells infected with Δ US33A HCMV compared to wild-type (**Figure 4e, Figure S7-S9**), suggesting that vAC formation may depend either on vacuolar pH, autophagy or both, and that this is delayed in the absence of DMXL1. To determine whether US33A delays vAC formation by modulating vacuolar pH, we infected HFFF-TERTs with WT or Δ US33A HCMV in the presence of BafA1. BafA1 significantly delayed the formation of vACs at 48 hpi in Δ US33A HCMV infected cells with a similar but non-significant trend in WT-infected cells, and completely inhibited vAC formation with both WT and Δ US33A HCMV infection at 72 h (**Figure S9**). This provides a mechanistic link between US33A-mediated inhibition of lysosomal acidification / autophagosome degradation and delayed vAC formation.

To determine whether US33A-mediated effects on vAC formation are reflected in viral replication, we quantified the number and size of plaques formed in DMXL1 shRNA knockdown cells compared to control (**Figure 4f, g**). As Merlin-strain viruses are recognised to plaque inefficiently⁴⁷, for this experiment, strain AD169-GFP was employed¹⁵. Knockdown of DMXL1 inhibited both the number and size of plaques. To determine whether DMXL1 degradation by US33A also inhibits viral replication, HFFF-TERTs were infected with the same WT or Δ US33A Merlin-strain HCMV as used in all of our other experiments at MOI=0.1 for six days, to facilitate approximately two viral replication cycles. Infection with Δ US33A HCMV significantly increased both cell-associated and released viral titres compared to wild-type infection (**Figure 4h**), indicating that US33A negatively regulates HCMV replication. Furthermore, infection with Δ US33A HCMV did not significantly increase viral release compared to WT in cells knocked down for DMXL1 using siRNA, highlighting the relevance of DMXL1 as US33A's target (**Figure S10c**). The greater effect of DMXL1 KD on viral release compared to infection with WT (US33A-expressing) virus likely reflects (a) more complete abrogation of DMXL1 expression by siRNA compared to infection with a US33A-expressing

249 virus, and (b) knockdown of DMXL1 from the beginning of the viral replication cycle via
250 siRNA compared to US33A expression-dependent DMXL1 degradation. To determine
251 whether US33A has a more general impact on other herpesviruses, we infected HFFF-TERTs
252 expressing US33A or control with HSV-1, which shares a similar vAC-based viral replication
253 strategy yet does not express a US33A homologue⁴⁸. HSV-1 replication was also significantly
254 inhibited in cells expressing US33A compared to control (**Figure 4h**).

DISCUSSION

Herpesviruses achieve lifelong persistence in infected individuals by utilizing a wide range of strategies to modulate cellular processes of the host. These include the deployment of proteins to target host factors for degradation. Degradation of DMXL1 by US33A and the KPC E3 ligase complex increases lysosomal pH, inhibits autophagy, and delays assembly of the vAC, reducing viral replication. US33A therefore functions to globally regulate cellular remodeling during infection. Functions were also ascribed to each of the other members of the previously uncharacterised HCMV US29-34A block of viral genes.

Only a few studies have hitherto examined manipulation of host lysosomes by HCMV. These have chiefly focused on subversion of lysosome function to degrade certain key molecules important in host innate or adaptive immunity, for example MHC class I polypeptide-related sequences (MIC) A/B, Toll-like receptor (TLR) 3/4, NK-cell regulator B7-H6, HLA-DR and multidrug resistance-associated protein 1 (MRP1)¹⁵⁻²⁰. We previously systematically defined host proteins targeted for lysosomal degradation throughout the course of HCMV infection via application of the lysosomal protease inhibitor leupeptin^{7,20}. However, there was no enrichment at any time point in the average abundance of these degraded proteins in cells infected with Δ US33A virus compared to wild-type. Use of published data relating change in lysosomal pH to change in LysoSensor emission suggested that the pH difference between lysosomes from cells infected with WT HCMV or Δ US33A HCMV was ~ 0.3 ^{49,50}. This relatively modest change may be insufficient to modulate lysosomal degradation, yet sufficient to modify autophagy and vAC formation.

Although no other HCMV proteins are known to modulate lysosome function, a systematic microRNA screen demonstrated that HCMV miR-US25-1 targets the ATP6V0C transcript, which encodes an essential component of the lysosomal v-ATPase. siRNA knockdown of ATP6V0C reduced replication of the highly-passaged laboratory HCMV strain AD169^{22,51}. It remains to be determined whether virally-expressed miR-US25-1 reproduces this phenotype, reduces lysosomal acidification and inhibits autophagy, as might be expected from our present study. If so, this would suggest that HCMV encodes two complementary strategies to achieve the same aim, and that the virus is willing to sacrifice replication efficiency to facilitate its overall lifecycle. Assuming DMXL1 is also downregulated in other cell types infected with HCMV *in vitro*, possible explanations might include (a) immune evasion (for example, in

dendritic cells), since endosomal acidification is required for efficient MHC class II presentation and efficient signalling by toll-like receptors^{52,53}; (b) promotion of latent infection by inhibition of viral assembly in monocytes or other latent reservoirs⁵⁴ or (c) prevention of excessive viral replication, tissue destruction and inflammation whose overall effects might affect the balance between immune control and viral clearance, or change the duration of transmission from host-to-host. Prior studies in which HCMV was systematically mutagenised identified other temperance factors that also reduce viral replication including RL13, UL9 and US30^{55,56}, however none have hitherto been mechanistically characterised. US33A was not included in these studies, having only been identified in 2011⁵⁷.

A systems-based analysis of HCMV-induced organelle remodelling revealed that during late infection, lysosomes are distributed into two distinct populations⁵⁸. The first, denser fraction, was found in discrete punctate structures and was defined by expression of molecules involved in carbohydrate catabolism such as lysosomal alpha-glucosides. The second, less-dense LAMP1-positive fraction localised to the vAC⁵⁸. Our data suggests that this latter population may play important roles with regards to US33A function, given the co-localisation we observed between LAMP1 and LC3B in Δ US33A HCMV infected cells.

The impact of HCMV infection on autophagy remains incompletely defined and varies according to the stage of viral replication⁵⁹. Autophagy is promoted early during infection via increased lipidation of LC3-I into LC3-II and increased generation of autophagosomes^{60,61}. By 24 hours, expression of HCMV TRS1 and IRS1 block the first stages of autophagy by targeting the Beclin 1 complex, a key regulator of phagophore and autophagosome formation^{60,62}. US33A may thus complement IRS1/TRS1 function by blocking the last stages of autophagy, namely autophagic cargo degradation. The impact of autophagy on HCMV replication also remains incompletely defined, and conflicting phenotypes have been described. A proviral role has been suggested by recent studies that have either employed knock-down of autophagy components or chemical modulators^{46,60}. Our data support these observations, relating inhibited autophagy during US33A expression to reduced viral replication.

The overall effect of US33A is somewhat paradoxically to delay vAC formation and diminish viral replication. Reduced autophagic cargo degradation might have been expected to increase availability of membrane for forming HCMV particles, and a more alkaline lysosome might

have been expected to degrade new HCMV particles to a lesser degree. However, the key factor that determines viral replication efficiency might be pH in the endosome/lysosome/vAC, with a more acidic vacuole promoting vAC formation. As discussed above, earlier vAC formation in the absence of US33A might present an overall disadvantage to the viral lifecycle, for example by facilitating viral antigen presentation at a stage of replication in which adaptive immune evasion is critical.

The power of our prior HCMV interactome analysis to reveal viral mechanism is exemplified by our identification that the KPC complex is required for DMXL1 degradation. Given that this study identified interactions between 51 HCMV proteins and E3 ligases³⁸, additional insights are likely to result for other proteins degraded by HCMV that so far remain mechanistically uncharacterised. The lack of an observed direct interaction between US33A and DMXL1 is most likely to reflect the effect of the V5 tag, which in the present study completely ablated DMXL1 degradation when applied either at US33A's N- or C-terminus. Given our demonstration of the interaction between US33A's N-terminus and the KPC complex, it is plausible that the US33A C-terminus may interact with DMXL1. Further analyses will require generation of an antibody specific for unmodified US33A. Functionally, the 57 amino acid US33A is most likely to act in a manner akin to a viral proteolysis-targeting chimera (PROTAC), linking KPC together with DMXL1 to facilitate its degradation⁶³.

US33A is conserved across 264 strains of HCMV with several minor mutations and in chimpanzee cytomegalovirus (CCMV), which is the closest relative of HCMV (**Figure S11a, Data S1**). However, no homologues are found in other primate CMVs, or other betaherpesviruses such as human herpesvirus-6 and -7 (HHV-6 and HHV-7). The divergence of HCMV from HHV-6 and HHV-7 occurred prior to the branching of CMVs into species-specific lineages (**Figure S11b**), suggesting that the acquisition of US33A by HCMV and CCMV occurred during transmission between great apes in the past few million years⁶⁴⁻⁶⁷.

As US33A is not conserved in any other human pathogen and is a small protein, an intriguing possibility is its potential use as a therapeutic agent. In addition to herpesviruses, multiple viruses require an acidic endosome at one or other point during their replication cycle. For example, cellular entry of RNA viruses including SARS-CoV-2, Influenza A, and human rhinoviruses is highly dependent on endosomal pH⁶⁸⁻⁷¹. Replication of *Alphaviruses* such as Sindbis virus also requires acidic endosomes⁷². Although it is unclear at present whether the

relatively modest change in lysosomal pH offered by US33A-mediated DMXL1 degradation will be sufficient to inhibit these processes, smaller changes in endosomal pH may result in fewer toxic effects, than for strong protein flux inhibitors such as BafA1⁷³. Although HCMV entry into human fibroblasts is pH-independent⁷⁴⁻⁷⁷, via fusion at plasma membrane, and we did not observe an effect of US33A expression on viral entry into retinal epithelial cells, in which entry is pH-dependent ⁷⁵(**Figure S10d**), it is nevertheless possible that US33A may impair HCMV entry into other cell types in which entry is pH dependent, such as other epithelial cells or myeloid lineage cells. Chloroquine and hydroxychloroquine are lysosomotropic agents that raise intracellular pH, impair autophagic protein degradation and are widely prescribed to patients with rheumatic diseases such as lupus and rheumatoid arthritis^{78,79}. However, these drugs can exhibit off-target effects, for example via interaction with TLR ligands and mitochondria, and toxicity remains a major limitation⁸⁰⁻⁸⁴. A more selective inhibitor of lysosomal acidification such as US33A might therefore be a worthy therapeutic candidate. Furthermore, over-acidification of lysosomes due to genetic deficiencies can lead to metabolic disorders, for example mucopolysaccharidosis plus (MPS+) and Mucopolysaccharidosis IV (MLIV)⁸⁵⁻⁸⁷. Here, the over-acidified environment within lysosomes may result in the malfunction of various lysosomal hydrolases, leading to a substrate accumulation, which might be ameliorated via US33A-based therapy. Finally, the scope of targeted PROTAC-based protein degradation will increase commensurate with an increased diversity of E3 ligases that can be hijacked. Modification of US33A to the minimal essential sequence that enables recruitment of KPC and chosen target(s) of interest may therefore provide a tool to this expanding armoury⁶³.

ACKNOWLEDGEMENTS

We are grateful to Prof. Steve Gygi for providing access to the “MassPike” software pipeline for quantitative proteomics. We are also grateful to the Cambridge Institute for Medical Research core facilities in flow cytometry and microscopy. This work was supported by a Medical Research Council Project Grant (MR/X000516/1) to MPW, a MRC grant (MR/S00971X/1) to RJS, a Royal Society University Research Fellowship (UF090010) and BBSRC grant (BB/M021424/1) to CMC. Funding was also received from the UK Dementia Research Institute (funded by the MRC, Alzheimer’s Research UK and the Alzheimer’s Society), The Cambridge Commonwealth, European & International Trust (SK), the Nehru Trust for Cambridge University and the Trinity-Henry Barlow Scholarship (SK) and the NIHR

Cambridge Biomedical Research Centre (NIHR203312). The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social care.

AUTHOR CONTRIBUTIONS

Conceptualization: M.P.W. Investigation: H.L., A.F.E., L.M.H., S.K., C.A.F, K.N., B.R., L.N., R.A., C.M.C. Data analysis: H.L., A.F.E., K.N., M.P.W. Funding acquisition: M.P.W. Supervision: D.C.R., R.J.S., M.P.W. Writing: H.L., A.F.E., M.P.W. All authors edited the manuscript.

DECLARATION OF INTERESTS

DCR is a consultant for Aladdin Healthcare Technologies Ltd., Mindrank AI, Nido Biosciences, Drishti Discoveries, Retro Biosciences and PAQ Therapeutics. None of the other authors have any interests to declare.

MAIN FIGURE TITLES AND LEGENDS

Figure 1. DMXL1 is targeted for degradation in the proteasome by HCMV US33A.

(a) Venn diagram showing the overlap between 163 proteins we previously identified to be degraded during early and/or late HCMV infection with a database of 1232 proteins that have roles in vacuolar acidification, from AmiGo (accession: GO:0007035)²⁴. The following three figures (b) – (d) are based on data from prior publications^{7,8}, and, along with data shown later in **Figure 4A** are the only previously published data in this manuscript. (b) Three orthogonal protein degradation screens showing that DMXL1 is degraded with high confidence during early HCMV infection, from Nightingale *et al.*⁷. Left panel: DMXL1 was rescued from degradation by the proteasome inhibitor MG132 in HCMV-infected cells, but not during mock infection, infection with UV-irradiated HCMV (HCMV^{UV}), or inhibition with the lysosomal protease inhibitor Leupeptin (see also **Figure 1** from Nightingale *et al.*⁷). Middle panel: Increased rate of DMXL1 degradation during HCMV compared to mock infection. Cells were pre-labelled with medium SILAC amino acids prior to infection, then switched to heavy amino acids at the point of infection. Quantification of medium-labelled proteins over time using TMT-based multiplexing facilitated quantification of protein degradation rates (see also **Figure 2** from Nightingale *et al.*⁷). Right panel: relative abundance of DMXL1 transcript over 72 h infection. Although DMXL1 protein was not identified in this screen to enable simultaneous quantitation, DMXL1 was downregulated in our other experiments over a comparable timecourse suggesting that it is degraded (left panels and (c); see also **Figure 3** from Nightingale *et al.*⁷). (c) DMXL1 is degraded during late HCMV infection (data from Fletcher-Etherington *et al.*⁸). The y-axis scale is the same as panel (b). (d) A proteomic screen of viral block deletion mutants determined that the US29-34A gene block is required to downregulate DMXL1. Bars of same colour represent biological replicates, and all viruses were examined in duplicate or triplicate across three independent experiments (data from Nightingale *et al.*⁹). Fold change was calculated in comparison to strain Merlin wild-type 1 (WT1) control. A second control was included, comprising WT1 that lacked UL16 and UL18 genes⁷. Strain AD169 HCMV was used as the $\Delta U_L/b'$ strain in this study, and we confirmed that AD169 as well as Merlin-strain HCMV downregulates DMXL1 (**Figure S2b**). (e) A proteomic screen of viral single gene-deletion mutants determined that US33A is necessary for downregulation of DMXL1 (each virus was used at MOI=7.5, n=2, 60 h infection). Fold change (FC) was calculated from the average signal:noise for both $\Delta US33$ biological replicates divided by the

average signal:noise for both control replicates. The Z-score was calculated as described in **Figure S1**. See also **Table S3**. (f) An AlphaFold model of US33A suggested that the protein is chiefly unstructured, with a central alpha helix (see **Figure S2a** for all models generated). (g) Scatter plot of human proteins from (e) with $Z > 0$ and $FC > 1$ identified that DMXL1 and WDR7 significantly increase in abundance upon infection with Δ US33A compared to WT HCMV. (h) Overlap of data from (g) with a complementary proteomic screen comparing protein abundance in cells stably overexpressing US33A or a control vector, which quantified 7687 proteins (see also **Figure S3a-d**). (i) Proteomic quantitation of the relative abundance of DMXL1 over 72 h of infection as indicated (MOI=5). 7994 human and 143 viral proteins were quantified. See also **Table S3**. (j) Quantification of US33A transcript over 24 h of infection at MOI = 10 by RT-qPCR. Error bars show standard error of the mean (SEM) for three replicates.

Figure 2. US33A reduces lysosomal acidification and impairs lysosomal pH maintenance.

(a) LysoTracker DND-99 staining of lysosomes in HFFF-TERTs expressing US33A or a control vector. BafA1 treatment was at 400 nM for 4 h and LysoTracker at 1 μ M for 30 min. Left: Representative images. Scale bar: 20 μ m. Right: Representative flow cytometry results. (b) LysoSensor Yellow/Blue DND-160 staining of lysosomes in HFFF-TERTs expressing US33A or a control vector. Scale bar: 10 μ m. To measure fluorescence of LysoSensor in both confirmations using live-cell imaging, paired excitation/emission wavelengths 405/435 and 458/493nm were employed as per previously published protocols^{30,88}. Ten images with >10 cells per image were quantified. The box and whisker plot shows mean and range of the 435/493 emission ratio. The p-value for a difference between control and US33A-expressing cells was estimated using an unpaired two-tailed t-test. * $p < 0.05$. (c) Quantification of LysoSensor staining using a microplate reader with excitation at 340 or 380 nm and emission at 527 nm as per previous protocols^{88,89}. Staining with LysoSensor was at 5 μ M for 10 min. Left panel: HFFF-TERTs expressing US33A or a control vector. Middle panel: HFFF-TERTs expressing the coxsackie virus and adenovirus receptor (HFFF-CARs) infected with adenovirus expressing US33A or GFP (MOI 5 for 72 h). Right panel: HFFF-TERTs infected with WT or Δ US33A HCMV (MOI 5 for 24 h). Values shown are mean $\pm$ SEM (n=3 independent experiments; each value was calculated from the mean of five technical replicates). (d) US33A inhibits lysosomal re-acidification. Left panels: schematics of workflow. Right panels: quantification of lysosomal acidity via LysoTracker staining in HFFF-TERTs expressing US33A or a control vector (top; 400 nM BafA1 for 1 h), or infected with parental

or Δ US33A HCMV (bottom; 100 nM BafA1 for 1 h). Values shown are mean $\pm$ SEM (n=3 independent experiments). p-values were estimated using an unpaired two-tailed t-test. *p<0.05.

Figure 3. Autophagic cargo degradation is inhibited by US33A.

(a) Schematic of the autophagy pathway after the formation of autophagosomes. (b-d) LC3B-II expression in (b) HFFF-TERTs stably expressing US33A or control; (c) HFFF-CARs infected with adenovirus expressing US33A or GFP at MOI 5 for 72 h, or (d) HFFF-TERTs infected with WT or Δ US33A HCMV at MOI 25 for 48 h. Cells were treated with vehicle (DMSO) or BafA1 at 400 nM for 4 h as indicated. Left panels: immunoblots of LC3B. Right panels: quantification of ratios of LC3B-II:GAPDH. Error bars show SEM for three independent experiments. P-values were estimated using two-way ANOVA. *p<0.05, ***p<0.001, ns: not significant, p>0.05. For (d), for each experiment, LC3B-II/GAPDH (middle panel) or IE1/GAPDH ratios (right panel) were first normalised to the ratio of DMSO-treated WT HCMV prior to calculating mean values. (e) Co-localisation of LC3B and LAMP1 in HFFF-TERTs stably expressing US33A or a control vector, by immunofluorescence. Scale bar: 20 μ m. (f) Quantification of (e), using >10 cells from each of a total of 36 images from three independent experiments. (g) Co-localisation of LC3B and LAMP1 in HFFF-TERTs infected with WT or Δ US33A HCMV at MOI 0.5 for 24 h. GFP fluorescence was used to identify infected cells. Scale bar: 10 μ m. (h) Quantification of (g), 93 WT HCMV and 97 Δ US33A infected cells from over 30 images from three independent experiments. Pearson's correlation was used for (f) and (h), with p-values estimated using unpaired two-tailed t-tests: ****p<0.0001. Full data quantifying Pearson's correlation is shown in **Table S5**.

Figure 4. HCMV US33A recruits the KPC E3 ligase to degrade DMXL1 and inhibit vAC formation.

(a) High-confidence interacting proteins for US33A from our previous systematic interactome analysis of all canonical HCMV genes in infected cells (data from Nobre *et al*³⁸). The NWD score is calculated from the fraction of runs in which an interacting protein is observed, the number of peptides from this protein and the standard deviation of this number across all IPs, and the reproducibility of detection across US33A replicates. (b) Co-IP validating that US33A interacts with RNF123 and UBAC1. (c) Co-IP showing that the US33A N-terminus is necessary to recruit RNF123 and UBAC1. Expression of the N-terminus, alpha-helix and C-

terminal regions of US33A-V5 in isolation was validated by qPCR as the proteins were of insufficient size to detect by immunoblot (**Figure S6b**). (**d**) HCMV US33A downregulates DMXL1 in a KPC-dependent manner. Polyclonal bulk populations of HFFF-TERTs edited using CRISPR/Cas9 as indicated were infected with WT or Δ US33A HCMV at MOI 5 for 72 h. The left panel shows a representative immunoblot from two independent experiments. Blotting for HCMV IE1/2 confirmed similar infection in each cell type. Immunoblots for RNF123 and UBAC1 are shown in **Figure S6c**. The right panel shows the quantification of DMXL1 for three independent experiments (error bars: mean $\pm$ SEM). P-values were estimated using two-way ANOVA, * $p < 0.05$, ** $p < 0.01$. (**e**) vAC formation timecourse, see **Figure S7** for full data. Cells were infected with WT or Δ US33A HCMV at MOI 1. At 24 h post-infection (hpi), both viruses infected a similar proportion of cells (**Figure S7b**). Left panel: representative images at 48 hpi. Pink arrows: examples of mature vACs. Orange arrows: examples of early/immature vACs. These were counted separately for the purpose of quantification (**Figures S7c, S9c**). Scale bar: 20 μ m. Right panel: percentage of infected cells with mature vACs at each time point. Similar results were observed in a second independent experiment (**Figure S8**). The number of images with infected cells assessed at each time point were: 24 h (n=21 images with 112 infected cells for WT; n=22, 115 for Δ US33A); 36 h (n=31, 122 for WT; n=21, 146 for Δ US33A); 48 h (n=21, 240 for WT, n=27, 241 for Δ US33A); 72 h (n=22, 205 for WT, n=21, 188 for Δ US33A); 96 h (n=25, 389 for WT, n=27, 321 for Δ US33A). P-values were estimated using a two-tailed unpaired t-test. *** $p < 0.001$, **** $p < 0.0001$. (**f**) Top panel: number of plaques (defined as a group of >5 cells) formed by AD169-GFP in HFFF-TERTs treated with DMXL1 or control shRNA. AD169 degrades DMXL1 similarly to WT Merlin strain, as shown in **Figures 1d and S2b**. Error bars show SEM of three independent experiments. ** $p < 0.01$ (unpaired two-tailed t-test). Bottom panel: immunoblot showing DMXL1 knockdown efficiency by shRNA. We confirmed that US33A expression was not affected by DMXL1 shRNAs (**Figure S10a, b**). (**g**) Left panel: quantification of the plaque size from the experiment described in (f). Error bars show SEM for >60 plaques. **** $p < 0.0001$ (unpaired two-tailed t-test). Right panel: representative images of plaque morphology. Scale bar: 1 mm. (**h**) Left panel: Relative titres of released and cell-associated WT or Δ US33A HCMV after six days of infection at MOI 0.1. Because absolute titres varied from experiment to experiment, relative titres were calculated for each experiment and then averaged between experiments (see Methods). Right panel: Titre of released and cell-associated herpes simplex virus-1 (HSV-1) in HFFF-TERTs expressing US33A or control after 24 h of infection at MOI

534 0.1. Error bars show SEM for three independent experiments. P-values were estimated using
535 unpaired two-tailed t-tests. * $p < 0.05$, ** $p < 0.01$.
536
537
538

STAR METHODS

Resource Availability

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Michael Weekes (mpw1001@cam.ac.uk).

Materials Availability

Materials generated in this study are available on request to the corresponding author.

Data and Code Availability

Unprocessed peptide data files for Figure 1 is available at: <https://data.mendeley.com/datasets/rvjz3wbz9m/draft?a=318040f4-52fd-49b5-bd21-cdb0a3343173>. This file includes details of peptide sequence, redundancy, protein assignment raw unprocessed TMT reporter intensities and isolation specificity. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE⁹⁰ partner repository with the dataset identifier PXD044273. No new code was generated in this study. All raw data and any information required to reanalyze the data in this manuscript are available on request.

Experimental Model and Subject Details

Cells and cell culture

Human fetal foreskin fibroblast cells immortalised with human telomerase (HFFF-TERTs, male), HEK293T cells (female), and ARPE-19 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with foetal bovine serum (FBS: 10% v/v, SIGMA) at 37°C in 5% v/v CO₂. HFFF-TERTs have been tested at regular intervals since isolation to confirm that human leukocyte antigen (HLA) and MHC Class I Polypeptide-Related Sequence A (MICA) genotypes, cell morphology and antibiotic resistance are unchanged. In addition, HCMV strain Merlin grows only in human fibroblast cells (dermal or foreskin in origin), further reducing the possibility that they have been contaminated with another cell type. HEK293T cells were obtained as a gift from Professor Paul Lehner and had been authenticated by Short Tandem Repeat profiling⁹¹. All cells were confirmed to be mycoplasma-negative (Lonza MycoAlert).

Viruses

The genome sequence of HCMV strain Merlin (GenBank accession AY446894) is designated the reference HCMV sequence (RefSeq accession NC_006273.2) by the National Center for Biotechnology Information, and was originally sequenced after three passages in human fibroblast cells^{92,93}. A recombinant version (RCMV1111) of this strain was derived by transfection of a sequenced BAC clone⁹³. RCMV1111 contains point mutations in two genes (RL13 and UL128) that enhance replication in fibroblasts⁹³. HCMV expressing rGFP from a P2A self-cleaving peptide at the 3'-end of the UL36 coding region (RCMV2582) was generated by recombineering the strain Merlin BAC as described previously⁹³. The single gene deletion mutants: Δ US29 (RCMV3082), Δ US30 (RCMV3066), Δ US31 (RCMV3083), Δ US32 (RCMV3045), Δ US33A (RCMV3037), Δ US34 (RCMV2936), Δ US34A (RCMV2935) were generated on a background of RCMV2582 (**Table S6**). RCMV288 was used for plaque assays, and is based on HCMV strain AD169 with one copy of the EGFP (enhanced green fluorescent protein) gene inserted in one copy of the HCMV long repeat under the control of the HCMV RNA2.7 early promoter⁹⁴. Viral stocks were prepared in HFFF-TERTs as described previously⁹⁵. Viruses were titrated by plating 1.5×10^5 cells in 12-well plates. After 24 h, cells were infected with viral stocks diluted at 1:16, 1:64, 1:256, 1:1024 in DMEM for 2 h prior to replacement with DMEM+10% FBS. Cells were harvested for flow cytometry at 24 h infection, and the viral titre estimated from the percentage of GFP positive cells. Whole-genome consensus sequences of passage 1 of all recombinant viruses were determined using the Illumina platform as described previously.

A replication deficient recombinant adenovirus vector (RAd) type 5 expressing US33A was made as described previously⁹⁶. In brief, the entire US33A ORF was amplified from HCMV strain Merlin DNA using primers recognising the 3' or 5' end of US33A (**Table S7a**), and inserted into pAdZ5 by recombineering. After verifying the insert by Sanger sequencing, vector DNA was purified using Qiagen Plasmid Maxi kit and transfected into 293TREx cells. Virus was amplified in 293TREx cells, extracted using tetrachloroethylene, and titrated by plaque assay on HEK293T monolayers.

A fluorescent HSV-1 strain encoding UL47 with an in-frame C-terminal fusion to EYFP (A206K) was constructed using bacterial artificial chromosome (BAC)-cloned KOS strain of HSV-1⁹⁷ and the two-step Red recombination technique⁹⁸ using the primers COL444 and

COL445 (**Key Resource Table**). Viral stocks were prepared in Vero cells and titrated using a plaque assay on HFFF-TERT monolayers.

Viral infections

For HCMV and HSV-1 infection, the required volume of viral stock to achieve the multiplicity of infection (MOI) described in the results section was diluted in the serum-free DMEM, mixed gently and applied to HFFF-TERTs. Mock infections were performed identically but with DMEM instead of viral stock. Time zero was considered the time at which cells first came into contact with virus. Cells were incubated with virus for 2 h at 37°C on a rocking platform, and the medium was replaced with DMEM+FBS or DMEM+FBS supplemented with 10 nM BafA1 or DMSO.

For RAd infections, the required volume of viral stock to achieve MOI 5 was diluted in serum-free DMEM, mixed gently, and applied to HFFF-CARs. Expression of the CAR receptor boosts the efficiency of adenovirus infection. Cells were incubated with virus for 2 h at 37°C on a rocking platform, then medium was replaced with DMEM+FBS. Cells were infected with RAd for 72 h at 37°C before further analysis.

Plasmid construction

US33A was amplified from an adenoviral template, expressing the gene under the control of the HCMV major immediate early promoter (MIEP). Primers were designed to recognize the 3' end of the MIEP (forward) and the 3' end of the gene if untagged (reverse). Both primers had flanking Gateway attB sequences for the purpose of cloning, and where required V5 and additional linker sequences (**Key Resources Table, Table S7b, c**)³⁸. PCR employed PfuUltra II Fusion HS DNA polymerase (Agilent). Constructs were subsequently cloned into lentiviral destination vector pHAGE-pSFFV using Gateway system (ThermoScientific)⁷. The untagged version of US33A, US33A with an N-terminal V5 tag separated by a serine-alanine linker (V5-SA-US33A), US33A with an N-terminal V5 tag separated by a glycine-glycine-glycine-serine linker (V5-GGGS-US33A) and US33A with a C-terminal V5 tag separated by a glycine-glycine-glycine-serine linker (US33A-GGGS-V5) were cloned from the pHAGE-pSFFV-US33A-V5 vector (**Key Resources Table, Table S7c**).

Truncations of US33A-V5 were synthesized as double-stranded DNA fragments (gBlocks, Integrated DNA Technologies, detailed in **Key Resource Table, Table S7b**). Gene fragments

were cloned into a linearised (MluI-HF and NheI-HF (NEB, R3198S, R3131S)), gel-purified doxycycline-inducible lentiviral vector pCW57-MCS1-2A-MCS2 (plasmid #71782, Addgene) via a Gibson Assembly reaction (NEB, E2621S) at a 5:1 ratio.

To generate shRNA constructs, two partially complementary oligonucleotides were annealed, with all sequences shown in **Table S7d**. The resulting product was ligated as a BamHI-EcoRI fragment into the pHR-SIREN vector (a gift from Prof. Paul Lehner, University of Cambridge) using T4 ligase (Thermo Scientific). All constructed plasmids were transformed into 5-alpha Competent E. coli (NEB) and selected on antibiotic-containing luria broth (LB) agar plates. All plasmid inserts were sequenced fully to check for mutations. Two different non-targeting control shRNA sequences are shown in **Table S7d**, which also lists the sequences of all primers and oligonucleotides.

Transient transfection of HEK293T cells with US33A truncation mutants

3x10⁶ HEK293T cells were seeded into 10 cm dishes the day preceding transfection. 2.5 µg of plasmid DNA was transfected into HEK293T cells with LipofectamineTM 3000 transfection reagent (Invitrogen). After 4 h, 2 µg/ml doxycycline was added to induce gene expression for 24 h prior to co-immunoprecipitation.

Stable cell line production

Lentiviral particles were generated through transfection of HEK293T cells with the lentiviral transfer vector plus four helper plasmids (VSVG, TAT1B, MGPM2, CMV-Rev1B), using TransIT-293 transfection reagent (Mirus) according to the manufacturer's recommendations. Viral supernatant was typically harvested 48 h after transfection, cell debris was removed with a 0.22 µm filter, and target cells were transduced for 48 h then subjected to antibiotic selection for two weeks.

CRISPR/Cas9-mediated gene disruption

A pool of three sgRNAs targeting RNF123 or UBAC1 (Synthego) were delivered to low passage HFFF-TERTs by nucleofection using a 4D-X Core unit (Lonza). Immunoblot was used to confirm that expression of the relevant protein had reduced in polyclonal populations of nucleofected cells, which were used for all experiments in this study.

siRNA knockdown

24 h prior to transfection, HFFF-TERTs were plated in 6- or 12-well plates. Cells were transfected with a pool of DMXL1 siRNAs (L-012091-01-0005, Dharmacon) or control (D-001810-10, Dharmacon) at a final concentration of 50 nM for 24 h, followed by 50 nM DMXL1 or control siRNAs for another 24 h. After 48 h, cells were harvested for immunoblot or lysosomal pH measurement. For viral release assays, where required, HFFF-TERTs were transfected with 100 nM DMXL1 or control siRNA for 24 h, followed by 50 nM siRNAs for another 24 h prior to infection.

Proteomic analyses

Whole cell lysate protein digestion

Cells were washed twice with phosphate buffered saline (PBS), and 250 µl lysis buffer added (6M Guanidine/50 mM HEPES pH 8.5). Cell lifters (Corning) were used to scrape cells in lysis buffer, which was removed to an eppendorf tube, vortexed extensively then sonicated. Cell debris was removed by centrifuging at 21,000 g for 10 min twice. Dithiothreitol (DTT) was added to half of the sample to a final concentration of 5mM and incubated at room temperature for 20 mins. Cysteines were alkylated with 15 mM iodoacetamide and incubated for 20 min at room temperature in the dark. Excess iodoacetamide was quenched with DTT for 15 mins. Samples were diluted with 200 mM HEPES pH 8.5 to 1.5 M Guanidine followed by digestion at room temperature for 3 h with LysC protease at a 1:100 protease-to-protein ratio. Samples were further diluted with 200mM HEPES pH8.5 to 0.5 M Guanidine. Trypsin was then added at a 1:100 protease-to-protein ratio followed by overnight incubation at 37°C. The reaction was quenched with 5% formic acid and centrifuged at 21,000 g for 10 min to remove undigested protein. Peptides were subjected to C18 solid-phase extraction (SPE, Sep-Pak, Waters) and vacuum-centrifuged to near-dryness.

Peptide labelling with tandem mass tags (TMT)

In preparation for TMT labelling, desalted peptides were dissolved in 200mM HEPES pH8.5. Peptide concentration was measured by microBCA (Pierce), and 25 µg of peptide labelled with TMT reagent. TMT reagents (0.8 mg) were dissolved in 43 µl anhydrous acetonitrile and 3 µl added to peptide at a final acetonitrile concentration of 30% (v/v). Sample labelling was as indicated in **Table S7e**. Following incubation at room temperature for 1 h, the reaction was quenched with hydroxylamine to a final concentration of 0.3% (v/v). TMT-labelled samples were combined at a 1:1:1:1:1:1:1:1:1:1:1:1:1:1:1:1 ratio. The sample was vacuum-centrifuged to near dryness and subjected to C18 SPE (Sep-Pak, Waters). An unfractionated

single-shot was analysed initially to ensure similar peptide loading across each TMT channel, thus avoiding the need for excessive electronic normalization. As all normalisation factors were >0.5 and <2, data for each single-shot experiment was analysed with data for the corresponding fractions to increase the overall number of peptides quantified. Normalisation is discussed in 'Data Analysis' and high pH reversed-phase (HpRP) fractionation is discussed below.

Offline HpRP Fractionation

TMT-labelled tryptic peptides were subjected to HpRP fractionation using an Ultimate 3000 RSLC UHPLC system (Thermo Fisher Scientific) equipped with a 2.1 mm internal diameter (ID) x 25 cm long, 1.7 µm particle Kinetix Evo C18 column (Phenomenex). Mobile phase consisted of A: 3% acetonitrile (MeCN), B: MeCN and C: 200 mM ammonium formate pH 10. Isocratic conditions were 90% A/10% C, and C was maintained at 10% throughout the gradient elution. Separations were conducted at 45 °C. Samples were loaded at 200 µl/minute for 5 minutes. The flow rate was then increased to 400 µl/minute over 5 minutes, after which the gradient elution proceeded as follows: 0-19% B over 10 minutes, 19-34% B over 14.25 minutes, 34-50% B over 8.75 minutes, followed by a 10 minutes wash at 90% B. UV absorbance was monitored at 280 nm and 15 s fractions were collected into 96 well microplates using the integrated fraction collector. Fractions were recombined orthogonally in a checkerboard fashion, combining alternate wells from each column of the plate into a single fraction, and commencing combination of adjacent fractions in alternating rows. Wells were excluded prior to the start or after the cessation of elution of peptide-rich fractions, as identified from the UV trace. This yielded two sets of 12 combined fractions, A and B, which were dried in a vacuum centrifuge and resuspended in 10 µl MS solvent (4% MeCN/5% formic acid) prior to LC-MS3. 12 set 'A' fractions were used for MS analysis.

LC-MS3

MS data were generated using an Orbitrap Fusion Lumos (Thermo). An Ultimate 3000 RSLC UHPLC machine equipped with a 300 µm internal diameter × 5 mm Acclaim PepMap µ-Precolumn (Thermo) and a 75 µm internal diameter × 50 cm 2.1 µm particle Acclaim PepMap RSLC analytical column were used. The loading solvent was 0.1% FA. The analytical solvent consisted of 0.1% FA (A) and 80% AcN + 0.1% FA (B). All separations were carried out at 40 °C. Samples were loaded at 5 µl/min for 5 min in loading solvent. The analytical gradient consisted of 3-7% B over 3 min, 7-37% B over 173 min, followed by a 4 min wash at 95% B

and equilibration at 3% B for 15 min. Each analysis used a MultiNotch MS3-based TMT method^{99,100}. The following settings were used: MS1: 380-1500 Th, 120,000 resolution, 2×10^5 automatic gain control (AGC) target, 50 ms maximum injection time. MS2: Quadrupole isolation at an isolation width of mass-to-charge ratio (m/z) 0.7, collision-induced dissociation fragmentation (normalised collision energy (NCE) 34) with ion trap scanning in turbo mode from m/z 120, 1.5×10^4 AGC target, 120 ms maximum injection time. MS3: In Synchronous Precursor Selection mode, the top 10 MS2 ions were selected for higher energy collision dissociation (HCD) fragmentation (NCE 45) and scanned in the Orbitrap at 60,000 resolution with an AGC target of 1×10^5 and a maximum accumulation time of 150 ms. Ions were not accumulated for all parallelisable time. The entire MS/MS/MS cycle had a target time of 3 s. Dynamic exclusion was set to ± 10 ppm for 70 s. MS2 fragmentation was triggered on precursors 5×10^3 counts and above.

Immunoblotting

HFFF-TERTs were used for all experiments. For most immunoblots, cells were washed with ice-cold PBS and lysed for 30 min on ice with a Nonidet P-40 (NP-40) based lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM EDTA, 10% (v/v) glycerol, 1% (v/v) Triton X-100 and 0.05% (v/v) NP-40) containing Complete Protease Inhibitor Cocktail (Roche)¹⁰¹. Lysates were reduced with 6X Protein Loading Dye (Tris 375 mM pH 6.8, 12% SDS, 30% glycerol, 0.6 M DTT, 0.06% bromophenol blue) for 5 min at 100°C or 15 min at 75 °C. Protein samples were then separated by polyacrylamide gel electrophoresis (PAGE) using 4-15% TGX Precast Protein Gels (Bio-rad), NuPAGE 4–12% Bis-Tris gels (Invitrogen), NuPAGE 3-8% Tris-Acetate gels (Invitrogen), or 10-20% Tricine gels (Invitrogen), then transferred to 0.2 μ m nitrocellulose membranes using Trans-Blot Systems (Bio-rad). The following primary antibodies were used: anti-LC3B (1:2500, #ab48394, Abcam), anti-RNF123 (1:200, #sc-101122, Santa-Cruz), anti-UBAC1 (1:3000, #67385-1-Ig, Proteintech), anti-DMXL1 (1:100, #A304-685A, Bethyl laboratories), anti-HCMV IE1/2 (1:1000, #MAB810R, Merk), anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:10,000, #MAB5718, R&D Systems), anti-Calnexin (1:1000, #LS-B6881, LSBio), anti-V5 (1:1000, #D3H8Q, CST). Secondary antibodies were: IRDye 680RD goat anti-mouse (925-68070, LI-COR), IRDye 800CW goat anti-rabbit (925-32211, LI-COR), HRP-linked goat-anti mouse (1:10,000, #115-035-146, Jackson), HRP-linked goat anti-rabbit (1:1000, #7074, CST). Fluorescent signals were detected

using a LI-COR Odyssey and HRP signals were detected using Bio-Rad ChemiDoc Imaging Systems, and images were processed using Image Studio Lite (LI-COR) or ImageLab.

Co-immunoprecipitation

Cells were harvested in lysis buffer (50 mM Tris pH 7.5, 300 mM NaCl, 0.5% (v/v) NP40, 1 mM DTT and Roche protease inhibitor cocktail), tumbled for 30 min at 4 °C and then centrifuged at 15,000 g for 14 min at 4 °C. The supernatants were incubated for 3 h with immobilised mouse monoclonal anti-V5 agarose resin. Samples were washed multiple times with lysis buffer, followed by incubation in Protein Loading Dye (components see immunoblotting section) for 10 min at 100 °C, centrifugation at 1000 g for 1 min, then analysis by immunoblotting.

Flow cytometry

Cells were washed in PBS before fixation with 4% PFA fixation buffer (420801 Biolegend) for 15 min at room temperature. 10,000 or 20,000 events were acquired with a Fortessa flow cytometer (Becton Dickinson) and analysed using FlowJo V9 software (Tree Star Inc).

Immunofluorescence microscopy

For LAMP1-LC3B co-localisation analysis, HFFF-TERTs stably expressing US33A or control were seeded onto coverslips and incubated overnight. For LAMP1-LC3B co-localisation analysis in the context of HCMV infection, HFFF-TERTs were infected on coverslips with WT or Δ US33A HCMV at MOI 0.1 for 24 h. For the HCMV vAC formation analysis, HFFF-TERTs were infected on coverslips with WT or Δ US33A at MOI 1 for 24, 36, 48, 72, or 96 h. Cells were then cross-linked with 4% fixation buffer (Biolegend), permeabilised with ice-cold methanol or 0.5% Triton-X100 (Sigma), and blocked with Human TruStain FcX (Biolegend) or HCMV-serotype negative human serum. Primary antibodies were used as indicated and included: rabbit anti-LC3B (1:1000, #ab43894, Abcam), mouse anti-LAMP1 (1:100, #328602, Biolegend), rabbit anti-Giantin (1:1000, #Ab80864, Abcam) and mouse anti-glycoprotein B (1:1000, #Ab6499, Abcam). Secondary antibodies were anti-mouse Alexa Fluor 647 (1:1000, #A31573, Thermo) and anti-rabbit Alexa Fluor 568 (1:1000, #A-11011, Thermo). Cell nuclei were stained with DAPI (Cell Signaling). Fluorescence was observed using a confocal microscope (Zeiss LSM 880).

Super-resolution live cell imaging

HFFF-TERTs expressing US33A or control were transfected with mCherry-LC3 and CD63-GFP plasmids (kind gift of Prof Paul Luzio, Cambridge Institute for Medical Research) by nucleotransfection using a 4D-X core unit (Lonza). After 72 h, cells were starved with serum-free DMEM for 30 min and imaged every 2 s for 3 min using the AiryScan function of a Zeiss LSM880 confocal microscope, with a 63x objective.

RT-qPCR

Total RNA from mock-, HCMV- or Adenovirus-infected HFFF-TERTs was extracted using RNeasy Mini Kit (Qiagen). cDNA was synthesized using GoScript Reverse Transcriptase (Promega). To detect US33A or truncated versions of US33A-V5, qPCR was performed using Fast SYBR Green Master Mix (Applied Biosystems). Primers targeting HCMV US33A, US33A-V5 or GAPDH and 18S (as an internal control) are shown in the **Table S7f**. The PCR program started with activation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 30 s. To detect DMXL1, qPCR was performed using TaqMan primers (Invitrogen, Assay ID: Hs00194128_m1 for DMXL1, Assay ID: Hs02786624_g1 for GAPDH) and TaqMan Universal PCR Master Mix (Invitrogen). The PCR program started with activation at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. Melting curve analyses were performed to verify the amplification specificity. All mock-infected samples exhibited non-singular melting curves, indicating non-specific amplification; values for these samples were set to zero.

Lysosomal pH measurements

For LysoTracker analysis, cells were stained with 100 nM LysoTracker Red DND-99 (L7528 Invitrogen) at 37 °C for 30 min, washed with PBS, fixed with 4% PFA fixation buffer (420801 Biolegend) and imaged using flow cytometry or microscopy.

For LysoSensor analysis, cells were stained with 5 µM LysoSensor Yellow/Blue DND-160 (L7545 Invitrogen) at room temperature for 10 min. LysoSensor was removed, cells were washed three times with PBS then recovered in phenol-red free DMEM (Gibco 31053028) for 15 min. Fluorescence intensity was quantified using a plate reader (CLARIOSTAR) at two excitation wavelengths, 340 nm (F_{340 nm}) and 380 nm (F_{380 nm}), with the emission wavelength set at 527 nm⁸⁸. Five independent wells were analysed for each condition to calculate an

average $F_{340\text{ nm}}/F_{380\text{ nm}}$ for each independent experiment. Images of LysoSensor staining were captured at two emission wavelengths, $430 \pm 25\text{ nm}$ and $465 \pm 30\text{ nm}$.

Plaque assay

1.3×10^5 HFFF-TERTs stably expressing shRNA constructs targeted against DMXL1 or control were seeded in 12-well plates in triplicate 24 h prior to infection with AD169-GFP at MOI 0.005. After 2 h, media was replaced with a 1:1 (v/v) mixture of 2 x DMEM and Avicel (2% w/v in water, FMC BioPolymer). After two weeks, the DMEM/Avicel mixture was removed and cells were washed three times with PBS before fixation in 4% (w/v) paraformaldehyde. The number of GFP-fluorescent plaques per well was counted and an average calculated across three independent wells for each condition.

Plaque size analysis

Images of 12 plaques per well were taken using a Zeiss AxioObserver inverted fluorescence microscope at 5 x magnification. The first 12 plaques encountered that were suitable for imaging were used. Suitable plaques were those that fitted entirely in the field of view, were not located against the edge of the well, and were the only plaque within the field of view. Images were converted into greyscale and plaque area was calculated using Image J Fiji.

Viral release assay

HFFF-TERTs were infected with WT or Δ US33A HCMV, or HSV-1 at MOI 0.1. After 6 days (for HCMV infection) or 24 h (for HSV-1 infection), cell culture medium was harvested, containing released virus. Infected cells were detached using trypsin, followed by sonication to release cell-associated virus, using a UP2000St Ultrasonic lab homogenizer (Hielscher). The programme was: 210 W (100% power), 100% pulse, 100% amplitude, 30 sec, 3 cycles. Cell debris were removed by centrifugation at 500 g for 10 min. Released or cell-associated virus was titred on a fresh batch of HFFF-TERTs using the method described in the 'Viruses' section. 10,000 events were acquired with a Fortessa flow cytometer (Becton Dickinson) flow cytometer and analysed with FlowJo V9 software, to assess %GFP positive cells. In order to combine data from three independent replicate experiments, and to account for variation in absolute titre, relative titres were calculated for each experiment and then averaged. Four conditions were examined: (a) WT, released virus; (b) Δ US33A, released virus; (c) WT, cell associated virus; (d) Δ US33A, cell associated virus. For (a), the relative titre was calculated as:

(a) / $\sum((a) + (b) + (c) + (d))$). Similar calculations were performed for (b), (c) and (d). Mean relative titres for (a), (b), (c) and (d) were then calculated.

Quantification and Statistical Analysis

Data analysis

Mass spectra were processed using a Sequest-based software pipeline for quantitative proteomics, “MassPike”, through a collaborative arrangement with Professor Steven Gygi’s laboratory at Harvard Medical School. MS spectra were converted to mzXML using an extractor built upon Thermo Fisher’s RAW File Reader library (version 4.0.26). In this extractor, the standard mzxml format has been augmented with additional custom fields that are specific to ion trap and Orbitrap mass spectrometry and essential for TMT quantitation. These additional fields include ion injection times for each scan, Fourier Transform-derived baseline and noise values calculated for every Orbitrap scan, isolation widths for each scan type, scan event numbers, and elapsed scan times. This software is a component of the MassPike software platform and is licensed by Harvard Medical School.

A combined database was constructed from (a) the human Uniprot database (26th January, 2017), (b) the HCMV strain Merlin Uniprot database, (c) all additional non-canonical human cytomegalovirus ORFs described by Stern-Ginossar *et al.*¹⁰², (d) a six-frame translation of HCMV strain Merlin filtered to include all potential ORFs of ≥ 8 amino acids (delimited by stop-stop rather than requiring ATG-stop) and (e) common contaminants such as porcine trypsin and endoproteinase LysC. ORFs from the six-frame translation (6FT-ORFs) were named as follows: 6FT_Frame_ORFnumber_length, where Frame is numbered 1-6, and length is the length in amino acids. The combined database was concatenated with a reverse database composed of all protein sequences in reversed order. Searches were performed using a 20 ppm precursor ion tolerance. Fragment ion tolerance was set to 1.0 Th. TMT tags on lysine residues and peptide N termini (Experiment 1: 229.162932 Da; Experiments 2-3: 304.2071 Da) and carbamidomethylation of cysteine residues (57.02146 Da) were set as static modifications, while oxidation of methionine residues (15.99492 Da) was set as a variable modification.

To control the fraction of erroneous protein identifications, a target-decoy strategy was employed¹⁰³. Peptide spectral matches (PSMs) were filtered to an initial peptide-level false

discovery rate (FDR) of 1% with subsequent filtering to attain a final protein-level FDR of 1%. PSM filtering was performed using a linear discriminant analysis, as described previously¹⁰³. This distinguishes correct from incorrect peptide IDs in a manner analogous to the widely used Percolator algorithm (<https://noble.gs.washington.edu/proj/percolator/>), though employing a distinct machine-learning algorithm. The following parameters were considered: XCorr, ΔC_n , missed cleavages, peptide length, charge state, and precursor mass accuracy.

Protein assembly was guided by principles of parsimony to produce the smallest set of proteins necessary to account for all observed peptides (algorithm described in ¹⁰³). Where all PSMs from a given HCMV protein could be explained either by a canonical gene or non-canonical ORF, the canonical gene was picked in preference. In a small number of cases, PSMs assigned to a non-canonical or 6FT-ORF were a mixture of peptides from the canonical protein and the ORF. This most commonly occurred where the ORF was a 5'-terminal extension of the canonical protein (thus meaning that the smallest set of proteins necessary to account for all observed peptides included the ORFs alone). In these cases, the peptides corresponding to the canonical protein were separated from those unique to the ORF, generating two separate entries. In a single case, PSM were assigned to the 6FT-ORF 6FT_6_ORF1202_676aa, which is a 5'-terminal extension of the non-canonical ORF ORFL147C. The principles described above were used to separate these two ORFs.

Proteins were quantified by summing TMT reporter ion counts across all matching peptide-spectral matches using "MassPike", as described previously⁹⁹. Briefly, a 0.003 Th window around the theoretical m/z of each reporter ion (127n, 128n, 130c) was scanned for ions and the maximum intensity nearest to the theoretical m/z was used. The primary determinant of quantitation quality is the number of TMT reporter ions detected in each MS3 spectrum, which is directly proportional to the signal-to-noise (S:N) ratio observed for each ion. An isolation specificity filter with a cut-off of 50% was additionally employed to minimise peptide co-isolation⁹⁹. Peptide-spectral matches with poor quality MS3 spectra (a combined S:N ratio of less than 250 across all TMT reporter ions) or no MS3 spectra at all were excluded from quantitation. Peptides meeting the stated criteria for reliable quantitation were then summed by parent protein, in effect weighting the contributions of individual peptides to the total protein signal based on their individual TMT reporter ion yields. Protein quantitation values were exported for further analysis in Excel.

For protein quantitation, reverse and contaminant proteins were removed, then each reporter ion channel was summed across all quantified proteins and normalised assuming equal protein loading across all channels. For further analysis and display in Figures, fractional TMT signals were used (i.e. reporting the fraction of maximal signal observed for each protein in each TMT channel, rather than the absolute normalized signal intensity). This effectively corrected for differences in the numbers of peptides observed per protein. For the TMT-based experiments, normalised S:N values are presented in **Table S1**. As it was not possible confidently to assign peptides to only two HLA-A, -B or -C alleles, signal:noise values were further summed for each of these alleles to give a single combined result for each of HLA-A, -B or -C.

For each protein in the single viral gene-deletion and overexpression screen, a mean (μ) and standard deviation (σ) for all paired, summed S:N values was calculated (i.e. sum of both Δ US29 replicates, sum of both Δ US30 replicates, sum of both Δ US31 replicates...). In each case, the maximum (x) value was omitted. For example, for DMXL1 in Figure 1e, μ and σ were calculated for WT, Δ US29, Δ US30, Δ US31, Δ US32, Δ US34, Δ US34A but not the maximum Δ US33A. The formula $Z = (x - \mu) / \sigma$ was then applied to calculate the z-score. Fold change compared to WT infection or control vector expression was calculated from normalised S:N values using $FC = \Delta x / WT$ or $x / control$. For Figure S3a, p-values were estimated using the method of Significance A¹⁰⁴.

Pathway analysis

The Database for Annotation, Visualisation and Integrated Discovery (DAVID) was used to determine functional enrichment of Gene Ontology biological process terms and UniProt Keywords¹⁰⁵. A given cluster was always searched against a background of all proteins quantified within the relevant experiment. For **Table S2**, DAVID analysis examined proteins rescued by each gene deletion compared to control by >1.5-fold with a z-score >3. The AimGO vacuolar acidification database (accession: GO:0007035) was used to overlap with our prior databases of protein degraded early and late during HCMV infection^{7,8}. For the former, 133 proteins degraded with medium confidence were considered, and for the latter, 30 additional proteins degraded as defined in Fletcher-Etherington *et al* were considered⁸.

Immunofluorescence microscopy analysis

To quantify the LAMP1-LC3B co-localisation, at least 10 images with over five cells were analysed for each sample of each experiment (details see RESULT). Pearson's coefficients of defined region of interest (ROI) were examined with Volocity software as quantification of co-localisation, with a threshold automatically assigned based on a study by Costes *et al.*, 2004¹⁰⁶ (Table S5). ROI of HFFF-TERTs expressing US33A or control was defined as an entire image, and that of HCMV infected cells was defined by drawing outline of regions with GFP signals.

Abundance of LAMP1 and LC3B in HFFF-TERTs expressing US33A or control, or infected cells was quantified by measurement of mean intensity of ROI using ImageJ. ROI was defined by HCS CellMask™ Blue Stain (Invitrogen) for HFFF-TERTs expressing US33A or control, and GFP signal for HCMV infection using auto-threshold with Huang method.

AlphaFold2 prediction

Potential structures and per-residue local distance difference test (pLDDT) confidence scores for the US33A protein (Uniprot accession: F7V999) were predicted using AlphaFold2 (AF2)¹⁰⁷. Structures were visualised and generated using PyMol Molecular Graphics System (<http://www.pymol.org>).

SUPPLEMENTAL INFORMATION

Table S1. Enrichment of functional pathways for host proteins downregulated by individual genes from the US29-34A block, related to Figure 1.

Table S2. Known characteristics of proteins encoded by the US29-34A block, related to Figure 1.

Table S3. Interactive spreadsheet of all proteomic data in the manuscript, related to Figure 1 and Figure S1-3. The "Plotter" worksheet generates graphs for all human and viral proteins quantified, and enables easy visualization of statistics. The "Data" worksheet shows minimally annotated protein data, for which the only modifications are formatting, normalization, reassignment of 11 non-canonical HCMV ORFs, and combination of peptide data for HLA-B and HLA-C alleles into single combined results, as described in STAR Methods.

Table S4. Quantification of DMXL1 degradation by all proteomic and immunoblot assays in the manuscript, related to Figures 1-4.

Table S5. Full data of Pearson's correlation of LAMP1-LC3 colocalisation assay. (a) Pearson's correlation of HFFF-TERTs expressing US33A and control. **(b)** Pearson's correlation of HFFF-TERTs infected with WT or Δ US33A HCMV.

Table S6. Details of viral gene deletion mutants, related to Figure 1 and STAR Methods.

Table S7. Further details of materials and techniques used in this manuscript, related to STAR Methods. (a) Oligonucleotides used to clone US33A into adenoviruses. **(b)** US33A and UL26 gene sequences of US33A overexpressed in HFFF-TERTs/HEK293Ts. **(c)** Oligonucleotides used to generate stable cell lines. **(d)** Oligonucleotides used for shRNA knockdown of DMXL1. **(e)** Details of TMT labelling. **(f)** Oligonucleotides used for RT-qPCR.

Video S1. Super-resolution microscopy to visualise autophagosome-lysosome fusion and autophagosome degradation in HFFF-TERTs expressing US33A or control, related to Figure 3. HFFF-TERTs expressing US33A or control were transfected with LC3-mCherry (red, marker of autophagosomes) and CD63-GFP (green, marker of lysosomes) for 48 h. After 30 min serum starvation to induce autophagy, imaging was conducted every 2 s for 3 min using the Zeiss LSM880 microscope's AiryScan function. **Video (a)** shows cells expressing a control vector. Here, autophagosomes transiently fuse with lysosomes in a 'kiss-and-run' fashion, and there is a clear progressive decrease in the intensity of red LC3 staining indicative of normal degradation of autophagic cargo¹⁰⁸. For the first 4 s of the video, the left hand arrow shows the initial location of a red autophagosome. At 5-6 s, this fuses with a lysosome, acquires GFP signal then rapidly detaches. Two further fusion-detachment cycles occur at 7-8 s and 11-16 s. The right hand arrow is another example showing autophagosome-lysosome fusion/detachment. **Video (b)** shows cells expressing US33A. Here, most autophagosomes had already fused to lysosomes and the green and red colours had combined to become yellow. Fused autophagosome/lysosome pairs move together excluding the possibility that one was superimposed upon the other in different layers of the cell. As LC3 is not degraded, there was no change in the yellow colour throughout the video. The arrow shows an already-fused autophagosome/lysosome pair that further fuses with another lysosome (11-12 s, 12-13 s). These results are consistent with the LC3-LAMP1 colocalisation assay shown in Figures 3e-f, and demonstrate US33A-mediated inhibition of autophagic cargo degradation.

1037 **Data S1. Conservation of US33A across 264 strains of HCMV, related to Figure S11 and**
1038 **DISCUSSION.**
1039

1040 REFERENCES

- 1041 1 Zuhair, M. *et al.* Estimation of the worldwide seroprevalence of cytomegalovirus: A systematic review
1042 and meta-analysis. *Rev Med Virol* **29**, e2034 (2019). <https://doi.org/10.1002/rmv.2034>
- 1043 2 Reinke, P., Prosch, S., Kern, F. & Volk, H. D. Mechanisms of human cytomegalovirus (HCMV)
1044 (re)activation and its impact on organ transplant patients. *Transpl Infect Dis* **1**, 157-164 (1999).
1045 <https://doi.org/10.1034/j.1399-3062.1999.010304.x>
- 1046 3 Nichols, W. G., Corey, L., Gooley, T., Davis, C. & Boeckh, M. High risk of death due to bacterial and
1047 fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from
1048 seropositive donors: evidence for indirect effects of primary CMV infection. *J Infect Dis* **185**, 273-282
1049 (2002). <https://doi.org/10.1086/338624>
- 1050 4 Buxmann, H., Hamprecht, K., Meyer-Wittkopf, M. & Friese, K. Primary Human Cytomegalovirus
1051 (HCMV) Infection in Pregnancy. *Dtsch Arztebl Int* **114**, 45-52 (2017).
1052 <https://doi.org/10.3238/arztebl.2017.0045>
- 1053 5 Tan, B. H. Cytomegalovirus Treatment. *Curr Treat Options Infect Dis* **6**, 256-270 (2014).
1054 <https://doi.org/10.1007/s40506-014-0021-5>
- 1055 6 Weekes, M. P. *et al.* Quantitative temporal viromics: an approach to investigate host-pathogen
1056 interaction. *Cell* **157**, 1460-1472 (2014). <https://doi.org/10.1016/j.cell.2014.04.028>
- 1057 7 Nightingale, K. *et al.* High-Definition Analysis of Host Protein Stability during Human Cytomegalovirus
1058 Infection Reveals Antiviral Factors and Viral Evasion Mechanisms. *Cell Host Microbe* **24**, 447-460 e411
1059 (2018). <https://doi.org/10.1016/j.chom.2018.07.011>
- 1060 8 Fletcher-Etherington, A. *et al.* Human cytomegalovirus protein pUL36: A dual cell death pathway
1061 inhibitor. *Proc Natl Acad Sci U S A* **117**, 18771-18779 (2020). <https://doi.org/10.1073/pnas.2001887117>
- 1062 9 Nightingale, K. *et al.* Human cytomegalovirus protein RL1 degrades the antiviral factor SLFN11 via
1063 recruitment of the CRL4 E3 ubiquitin ligase complex. *Proc Natl Acad Sci U S A* **119** (2022).
1064 <https://doi.org/10.1073/pnas.2108173119>
- 1065 10 Nathans, R. *et al.* Small-molecule inhibition of HIV-1 Vif. *Nat Biotechnol* **26**, 1187-1192 (2008).
1066 <https://doi.org/10.1038/nbt.1496>
- 1067 11 Mocarski ES, S. T., Pass RF. *Cytomegaloviruses.*, 2701-2772 (Lippincott Williams & Wilkins, 2007).
- 1068 12 Cepeda, V., Esteban, M. & Fraile-Ramos, A. Human cytomegalovirus final envelopment on membranes
1069 containing both trans-Golgi network and endosomal markers. *Cell Microbiol* **12**, 386-404 (2010).
1070 <https://doi.org/10.1111/j.1462-5822.2009.01405.x>
- 1071 13 Krzyzaniak, M. A., Mach, M. & Britt, W. J. HCMV-encoded glycoprotein M (UL100) interacts with
1072 Rab11 effector protein FIP4. *Traffic* **10**, 1439-1457 (2009). <https://doi.org/10.1111/j.1600-0854.2009.00967.x>
- 1073 14 Momtaz, S., Molina, B., Mlera, L., Goodrum, F. & Wilson, J. M. Cell type-specific biogenesis of novel
1074 vesicles containing viral products in human cytomegalovirus infection. *J Virol* **95** (2021).
1075 <https://doi.org/10.1128/JVI.02358-20>
- 1076 15 Fielding, C. A. *et al.* Two novel human cytomegalovirus NK cell evasion functions target MICA for
1077 lysosomal degradation. *PLoS Pathog* **10**, e1004058 (2014). <https://doi.org/10.1371/journal.ppat.1004058>
- 1078 16 Park, A. *et al.* HCMV-encoded US7 and US8 act as antagonists of innate immunity by distinctively
1079 targeting TLR-signaling pathways. *Nat Commun* **10**, 4670 (2019). <https://doi.org/10.1038/s41467-019-12641-4>
- 1080 17 Weekes, M. P. *et al.* Latency-associated degradation of the MRP1 drug transporter during latent human
1081 cytomegalovirus infection. *Science* **340**, 199-202 (2013). <https://doi.org/10.1126/science.1235047>
- 1082 18 Charpak-Amikam, Y. *et al.* Human cytomegalovirus escapes immune recognition by NK cells through
1083 the downregulation of B7-H6 by the viral genes US18 and US20. *Sci Rep* **7**, 8661 (2017).
1084 <https://doi.org/10.1038/s41598-017-08866-2>
- 1085 19 Odeberg, J., Plachter, B., Branden, L. & Soderberg-Naucler, C. Human cytomegalovirus protein pp65
1086 mediates accumulation of HLA-DR in lysosomes and destruction of the HLA-DR alpha-chain. *Blood*
1087 **101**, 4870-4877 (2003). <https://doi.org/10.1182/blood-2002-05-1504>
- 1088 20 Fielding, C. A. *et al.* Control of immune ligands by members of a cytomegalovirus gene expansion
1089 suppresses natural killer cell activation. *Elife* **6** (2017). <https://doi.org/10.7554/eLife.22206>
- 1090 21 Mindell, J. A. Lysosomal acidification mechanisms. *Annu Rev Physiol* **74**, 69-86 (2012).
1091 <https://doi.org/10.1146/annurev-physiol-012110-142317>
- 1092 22 Pavelin, J. *et al.* Cellular v-ATPase is required for virion assembly compartment formation in human
1093 cytomegalovirus infection. *Open Biol* **7** (2017). <https://doi.org/10.1098/rsob.160298>
- 1094 23 Merkulova, M. *et al.* Mapping the H(+) (V)-ATPase interactome: identification of proteins involved in
1095 trafficking, folding, assembly and phosphorylation. *Sci Rep* **5**, 14827 (2015).
1096 <https://doi.org/10.1038/srep14827>
- 1097
- 1098

1099 24 Carbon, S. *et al.* AmiGO: online access to ontology and annotation data. *Bioinformatics* **25**, 288-289
1100 (2009). <https://doi.org/10.1093/bioinformatics/btn615>
1101 25 Breyer, F. *et al.* TPL-2 kinase induces phagosome acidification to promote macrophage killing of
1102 bacteria. *EMBO J* **40**, e106188 (2021). <https://doi.org/10.15252/embj.2020106188>
1103 26 Kraemer, C., Enklaar, T., Zabel, B. & Schmidt, E. R. Mapping and structure of DMXL1, a human
1104 homologue of the DmX gene from *Drosophila melanogaster* coding for a WD repeat protein. *Genomics*
1105 **64**, 97-101 (2000). <https://doi.org/10.1006/geno.1999.6050>
1106 27 Jaskolka, M. C., Winkley, S. R. & Kane, P. M. RAVE and Rabconnectin-3 Complexes as Signal
1107 Dependent Regulators of Organelle Acidification. *Front Cell Dev Biol* **9**, 698190 (2021).
1108 <https://doi.org/10.3389/fcell.2021.698190>
1109 28 Kawabe, H. *et al.* A novel rabconnectin-3-binding protein that directly binds a GDP/GTP exchange
1110 protein for Rab3A small G protein implicated in Ca(2+)-dependent exocytosis of neurotransmitter. *Genes*
1111 *Cells* **8**, 537-546 (2003). <https://doi.org/10.1046/j.1365-2443.2003.00655.x>
1112 29 Kane, P. M. Targeting reversible disassembly as a mechanism of controlling V-ATPase activity. *Curr*
1113 *Protein Pept Sci* **13**, 117-123 (2012). <https://doi.org/10.2174/138920312800493142>
1114 30 Pavel, M. *et al.* CCT complex restricts neuropathogenic protein aggregation via autophagy. *Nat Commun*
1115 **7**, 13821 (2016). <https://doi.org/10.1038/ncomms13821>
1116 31 Brown, B., Siddique, N. & Greene, J. Herpes Simplex Virus Type 1 and Varicella-Zoster Virus
1117 Coinfection in an Immunocompromised Male Patient. *Infect Dis Clin Prac* **30** (2022).
1118 <https://doi.org/10.1097/Ipc.0000000000001155>
1119 32 Yim, W. W. & Mizushima, N. Lysosome biology in autophagy. *Cell Discov* **6**, 6 (2020).
1120 <https://doi.org/10.1038/s41421-020-0141-7>
1121 33 Kabeya, Y. *et al.* LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome
1122 membranes after processing. *EMBO J* **19**, 5720-5728 (2000). <https://doi.org/10.1093/emboj/19.21.5720>
1123 34 Tanida, I., Ueno, T. & Kominami, E. LC3 conjugation system in mammalian autophagy. *Int J Biochem*
1124 *Cell Biol* **36**, 2503-2518 (2004). <https://doi.org/10.1016/j.biocel.2004.05.009>
1125 35 Klionsky, D. J. *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy.
1126 *Autophagy* **8**, 445-544 (2012). <https://doi.org/10.4161/auto.19496>
1127 36 Sarkar, S., Davies, J. E., Huang, Z., Tunnacliffe, A. & Rubinsztein, D. C. Trehalose, a novel mTOR-
1128 independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. *J*
1129 *Biol Chem* **282**, 5641-5652 (2007). <https://doi.org/10.1074/jbc.M609532200>
1130 37 Klionsky, D. J., Elazar, Z., Seglen, P. O. & Rubinsztein, D. C. Does bafilomycin A1 block the fusion of
1131 autophagosomes with lysosomes? *Autophagy* **4**, 849-850 (2008). <https://doi.org/10.4161/auto.6845>
1132 38 Nobre, L. V. *et al.* Human cytomegalovirus interactome analysis identifies degradation hubs, domain
1133 associations and viral protein functions. *Elife* **8** (2019). <https://doi.org/10.7554/eLife.49894>
1134 39 Boya, P., Reggiori, F. & Codogno, P. Emerging regulation and functions of autophagy. *Nat Cell Biol* **15**,
1135 713-720 (2013). <https://doi.org/10.1038/ncb2788>
1136 40 Yadavalli, N. & Ferguson, S. M. LRRK2 suppresses lysosome degradative activity in macrophages and
1137 microglia through MiT-TFE transcription factor inhibition. *Proc Natl Acad Sci U S A* **120**, e2303789120
1138 (2023). <https://doi.org/10.1073/pnas.2303789120>
1139 41 Tandon, R. & Mocarski, E. S. Viral and host control of cytomegalovirus maturation. *Trends Microbiol*
1140 **20**, 392-401 (2012). <https://doi.org/10.1016/j.tim.2012.04.008>
1141 42 Close, W. L., Anderson, A. N. & Pellett, P. E. Betaherpesvirus Virion Assembly and Egress. *Adv Exp*
1142 *Med Biol* **1045**, 167-207 (2018). https://doi.org/10.1007/978-981-10-7230-7_9
1143 43 Lucin, P. *et al.* Cytomegalovirus Generates Assembly Compartment in the Early Phase of Infection by
1144 Perturbation of Host-Cell Factors Recruitment at the Early Endosome/Endosomal Recycling
1145 Compartment/Trans-Golgi Interface. *Front Cell Dev Biol* **8**, 563607 (2020).
1146 <https://doi.org/10.3389/fcell.2020.563607>
1147 44 Cepeda, V., Esteban, M. & Fraile-Ramos, A. Human cytomegalovirus final envelopment on membranes
1148 containing both trans-Golgi network and endosomal markers. *Cell Microbiol* **12**, 386-404 (2010).
1149 <https://doi.org/10.1111/j.1462-5822.2009.01405.x>
1150 45 Das, S., Vasanthi, A. & Pellett, P. E. Three-dimensional structure of the human cytomegalovirus
1151 cytoplasmic virion assembly complex includes a reoriented secretory apparatus. *J Virol* **81**, 11861-11869
1152 (2007). <https://doi.org/10.1128/JVI.01077-07>
1153 46 Taisne, C. *et al.* Human cytomegalovirus hijacks the autophagic machinery and LC3 homologs in order
1154 to optimize cytoplasmic envelopment of mature infectious particles. *Sci Rep* **9**, 4560 (2019).
1155 <https://doi.org/10.1038/s41598-019-41029-z>
1156 47 Stanton, R. J. *et al.* Reconstruction of the complete human cytomegalovirus genome in a BAC reveals
1157 RL13 to be a potent inhibitor of replication. *J Clin Invest* **120**, 3191-3208 (2010).
1158 <https://doi.org/10.1172/JCI42955>

1159 48 Crump, C. Virus Assembly and Egress of HSV. *Adv Exp Med Biol* **1045**, 23-44 (2018).
1160 https://doi.org/10.1007/978-981-10-7230-7_2

1161 49 Ma, L., Ouyang, Q., Werthmann, G. C., Thompson, H. M. & Morrow, E. M. Live-cell Microscopy and
1162 Fluorescence-based Measurement of Luminal pH in Intracellular Organelles. *Front Cell Dev Biol* **5**, 71
1163 (2017). <https://doi.org/10.3389/fcell.2017.00071>

1164 50 Halerow, P. *et al.* Importance of measuring endolysosome, cytosolic, and extracellular pH in
1165 understanding the pathogenesis of and possible treatments for glioblastoma multiforme. *Cancer Rep* **2**
1166 (2019). <https://doi.org/10.1002/cnr2.1193>

1167 51 Pavelin, J. *et al.* Systematic microRNA analysis identifies ATP6V0C as an essential host factor for
1168 human cytomegalovirus replication. *PLoS Pathog* **9**, e1003820 (2013).
1169 <https://doi.org/10.1371/journal.ppat.1003820>

1170 52 Benaroch, P. *et al.* How MHC class II molecules reach the endocytic pathway. *EMBO J* **14**, 37-49 (1995).
1171 <https://doi.org/10.1002/j.1460-2075.1995.tb06973.x>

1172 53 Macfarlane, D. E. & Manzel, L. Antagonism of immunostimulatory CpG-oligodeoxynucleotides by
1173 quinacrine, chloroquine, and structurally related compounds. *J Immunol* **160**, 1122-1131 (1998).

1174 54 Schwartz, M. *et al.* Molecular characterization of human cytomegalovirus infection with single-cell
1175 transcriptomics. *Nat Microbiol* **8**, 455-468 (2023). <https://doi.org/10.1038/s41564-023-01325-x>

1176 55 Dunn, W. *et al.* Functional profiling of a human cytomegalovirus genome. *Proc Natl Acad Sci U S A*
1177 **100**, 14223-14228 (2003). <https://doi.org/10.1073/pnas.2334032100>

1178 56 Wilkinson, G. W. *et al.* Human cytomegalovirus: taking the strain. *Med Microbiol Immunol* **204**, 273-
1179 284 (2015). <https://doi.org/10.1007/s00430-015-0411-4>

1180 57 Gatherer, D. *et al.* High-resolution human cytomegalovirus transcriptome. *Proc Natl Acad Sci U S A* **108**,
1181 19755-19760 (2011). <https://doi.org/10.1073/pnas.1115861108>

1182 58 Jean Beltran, P. M., Mathias, R. A. & Cristea, I. M. A portrait of the human organelle proteome in space
1183 and time during Cytomegalovirus infection. *Cell Syst* **3**, 361-373 e366 (2016).
1184 <https://doi.org/10.1016/j.cels.2016.08.012>

1185 59 Lussignol, M. & Esclatine, A. Herpesvirus and Autophagy: "All Right, Everybody Be Cool, This Is a
1186 Robbery!". *Viruses* **9** (2017). <https://doi.org/10.3390/v9120372>

1187 60 Chaumorcet, M. *et al.* The human cytomegalovirus protein TRS1 inhibits autophagy via its interaction
1188 with Beclin 1. *J Virol* **86**, 2571-2584 (2012). <https://doi.org/10.1128/JVI.05746-11>

1189 61 McFarlane, S. *et al.* Early Induction of Autophagy in Human Fibroblasts after Infection with Human
1190 Cytomegalovirus or Herpes Simplex Virus 1. *Journal of Virology* **85**, 4212-4221 (2011).
1191 <https://doi.org/10.1128/Jvi.02435-10>

1192 62 Mouna, L. *et al.* Analysis of the role of autophagy inhibition by two complementary human
1193 cytomegalovirus BECN1/Beclin 1-binding proteins. *Autophagy* **12**, 327-342 (2016).
1194 <https://doi.org/10.1080/15548627.2015.1125071>

1195 63 Ishida, T. & Ciulli, A. E3 Ligase Ligands for PROTACs: How They Were Found and How to Discover
1196 New Ones. *SLAS Discov* **26**, 484-502 (2021). <https://doi.org/10.1177/2472555220965528>

1197 64 McGeoch, D. J., Rixon, F. J. & Davison, A. J. Topics in herpesvirus genomics and evolution. *Virus Res*
1198 **117**, 90-104 (2006). <https://doi.org/10.1016/j.virusres.2006.01.002>

1199 65 Cagliani, R., Forni, D., Mozzì, A. & Sironi, M. Evolution and Genetic Diversity of Primate
1200 Cytomegaloviruses. *Microorganisms* **8** (2020). <https://doi.org/10.3390/microorganisms8050624>

1201 66 Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J*
1202 *Mol Biol* **215**, 403-410 (1990). [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)

1203 67 Murthy, S. *et al.* Cytomegalovirus distribution and evolution in hominines. *Virus Evol* **5**, vez015 (2019).
1204 <https://doi.org/10.1093/ve/vez015>

1205 68 Perreira, J. M. *et al.* RNASEK Is a V-ATPase-Associated Factor Required for Endocytosis and the
1206 Replication of Rhinovirus, Influenza A Virus, and Dengue Virus. *Cell Rep* **12**, 850-863 (2015).
1207 <https://doi.org/10.1016/j.celrep.2015.06.076>

1208 69 Guinea, R. & Carrasco, L. Requirement for vacuolar proton-ATPase activity during entry of influenza
1209 virus into cells. *J Virol* **69**, 2306-2312 (1995). <https://doi.org/10.1128/JVI.69.4.2306-2312.1995>

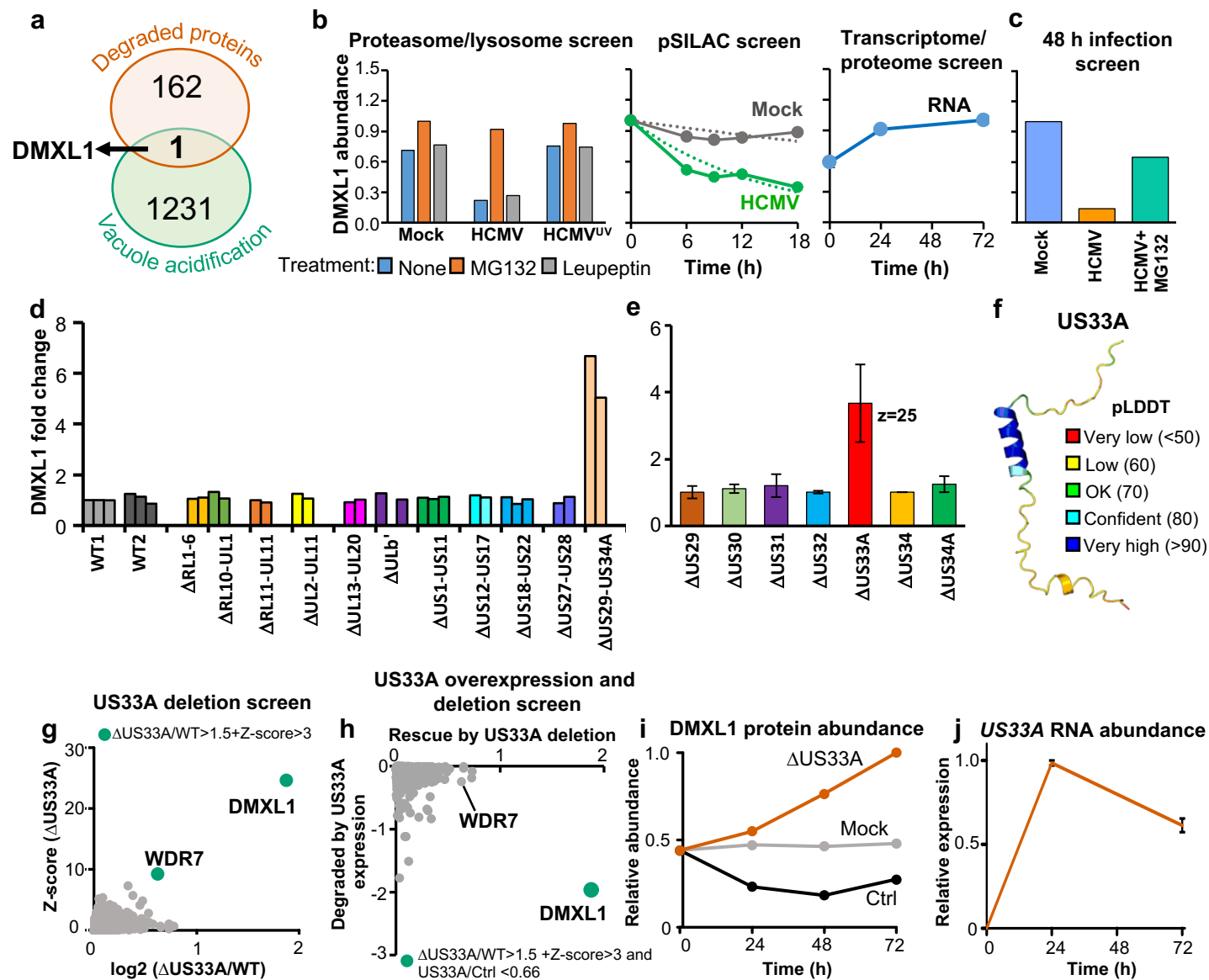
1210 70 Ochiai, H., Sakai, S., Hirabayashi, T., Shimizu, Y. & Terasawa, K. Inhibitory effect of bafilomycin A1,
1211 a specific inhibitor of vacuolar-type proton pump, on the growth of influenza A and B viruses in MDCK
1212 cells. *Antiviral Res* **27**, 425-430 (1995). [https://doi.org/10.1016/0166-3542\(95\)00040-s](https://doi.org/10.1016/0166-3542(95)00040-s)

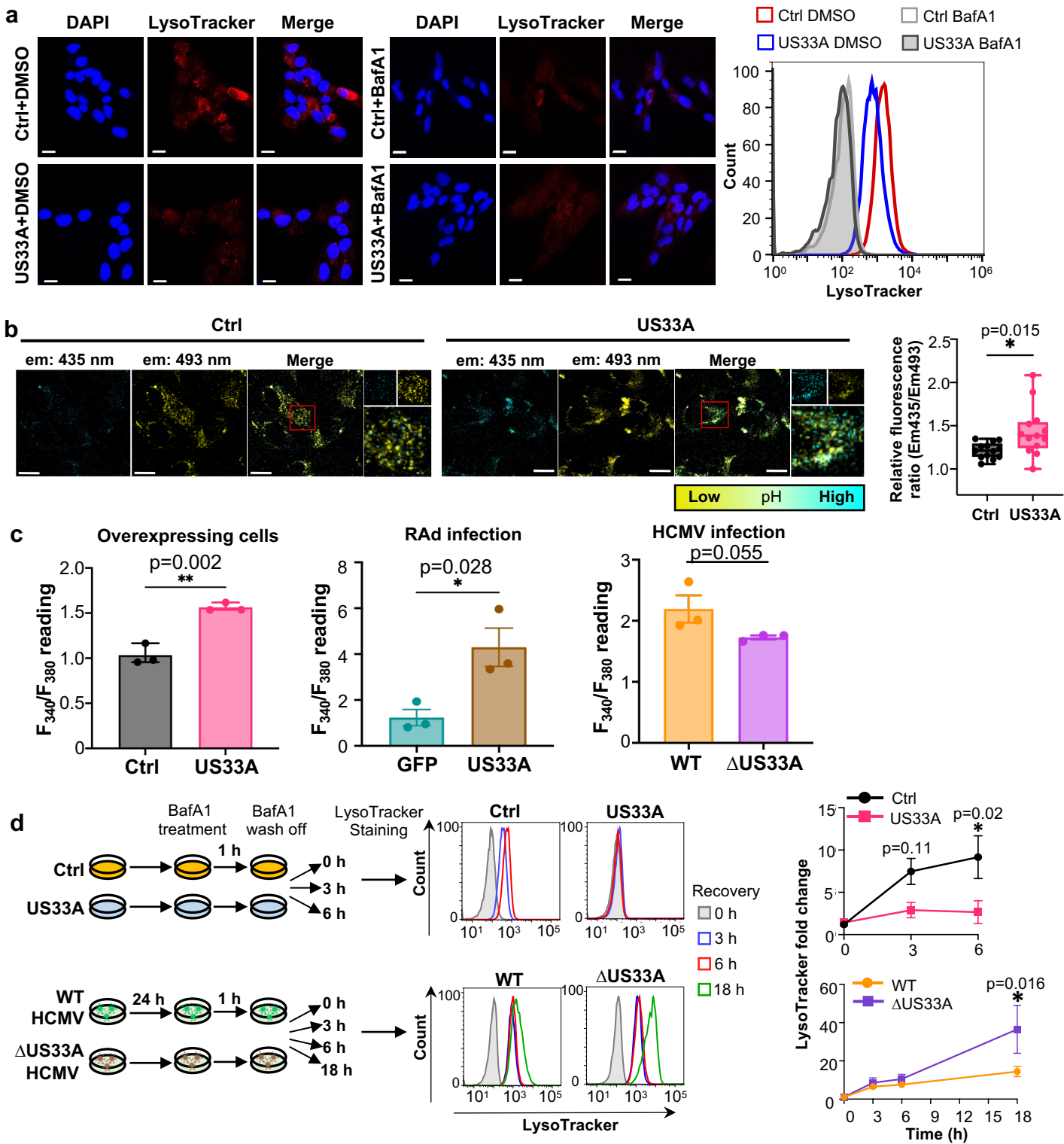
1213 71 Icho, S. *et al.* Dual Inhibition of Vacuolar-ATPase and TMPRSS2 Is Required for Complete Blockade
1214 of SARS-CoV-2 Entry into Cells. *Antimicrob Agents Chemother* **66**, e0043922 (2022).
1215 <https://doi.org/10.1128/aac.00439-22>

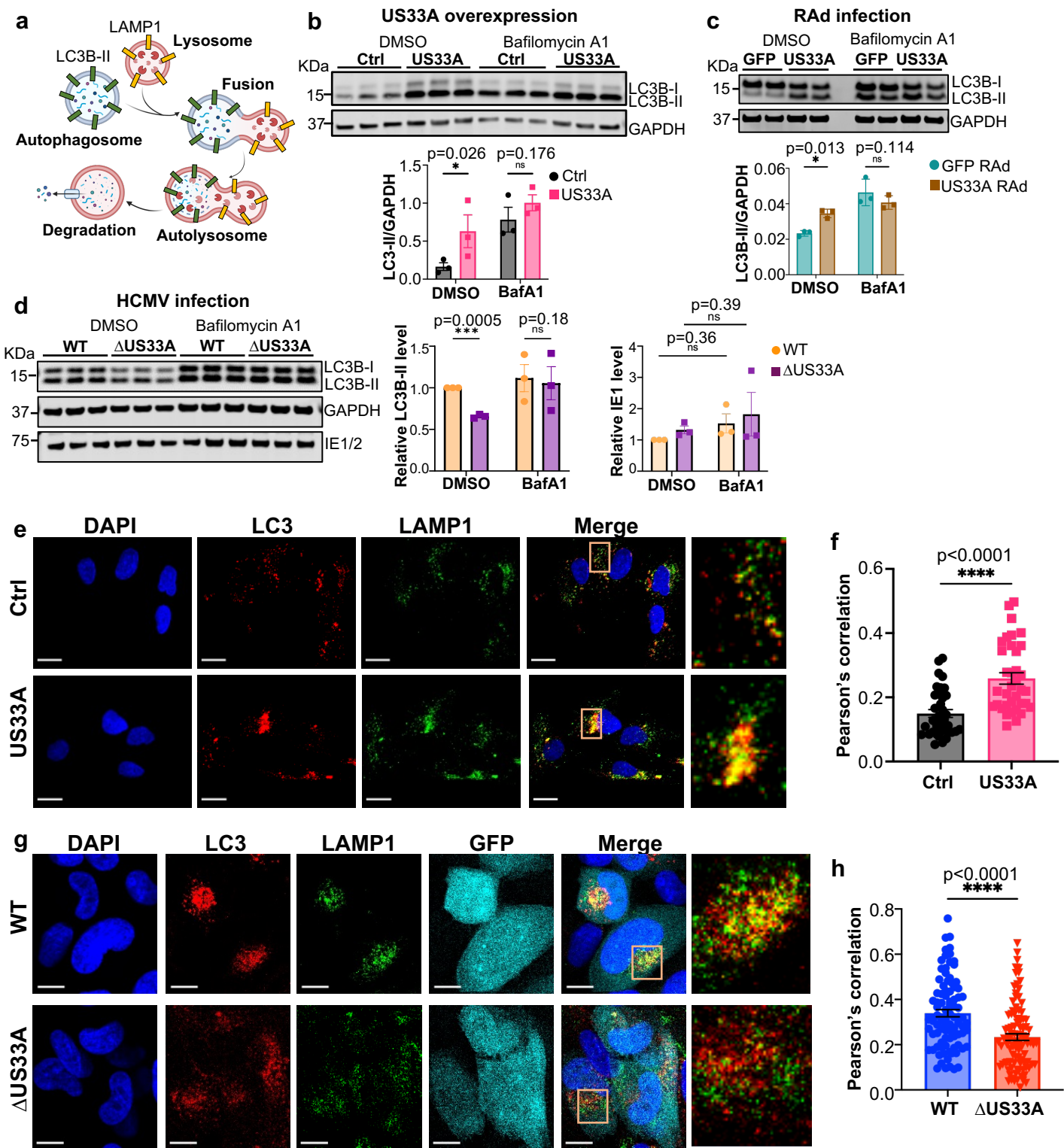
1216 72 Schuchman, R. M. *et al.* Role of the vacuolar ATPase in the Alphavirus replication cycle. *Heliyon* **4**,
1217 e00701 (2018). <https://doi.org/10.1016/j.heliyon.2018.e00701>

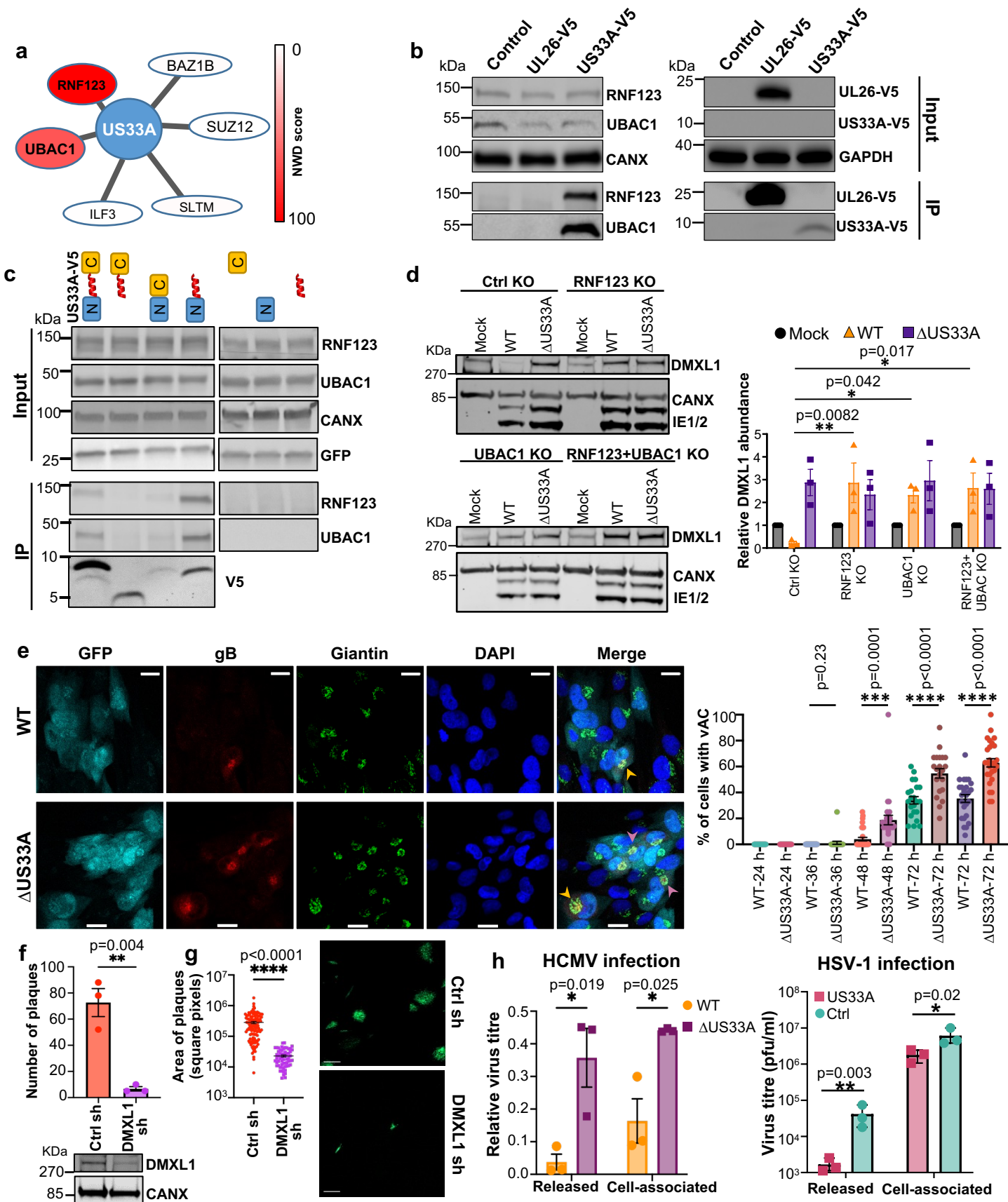
- 73 Yan, Y. *et al.* Bafilomycin A1 induces caspase-independent cell death in hepatocellular carcinoma cells via targeting of autophagy and MAPK pathways. *Sci Rep* **6**, 37052 (2016). <https://doi.org/10.1038/srep37052>
- 74 Vanarsdall, A. L., Wisner, T. W., Lei, H., Kazlauskas, A. & Johnson, D. C. PDGF receptor-alpha does not promote HCMV entry into epithelial and endothelial cells but increased quantities stimulate entry by an abnormal pathway. *PLoS Pathog* **8**, e1002905 (2012). <https://doi.org/10.1371/journal.ppat.1002905>
- 75 Ryckman, B. J., Jarvis, M. A., Drummond, D. D., Nelson, J. A. & Johnson, D. C. Human cytomegalovirus entry into epithelial and endothelial cells depends on genes UL128 to UL150 and occurs by endocytosis and low-pH fusion. *J Virol* **80**, 710-722 (2006). <https://doi.org/10.1128/JVI.80.2.710-722.2006>
- 76 Wang, D., Yu, Q. C., Schroer, J., Murphy, E. & Shenk, T. Human cytomegalovirus uses two distinct pathways to enter retinal pigmented epithelial cells. *Proc Natl Acad Sci U S A* **104**, 20037-20042 (2007). <https://doi.org/10.1073/pnas.0709704104>
- 77 Vanarsdall, A. L. & Johnson, D. C. Human cytomegalovirus entry into cells. *Curr Opin Virol* **2**, 37-42 (2012). <https://doi.org/10.1016/j.coviro.2012.01.001>
- 78 He, Y. *et al.* Identification of a lysosomal pathway that modulates glucocorticoid signaling and the inflammatory response. *Sci Signal* **4**, ra44 (2011). <https://doi.org/10.1126/scisignal.2001450>
- 79 Fois, G. *et al.* A new role for an old drug: Ambroxol triggers lysosomal exocytosis via pH-dependent Ca(2)(+) release from acidic Ca(2)(+) stores. *Cell Calcium* **58**, 628-637 (2015). <https://doi.org/10.1016/j.ceca.2015.10.002>
- 80 Ziegler, H. K. & Unanue, E. R. Decrease in macrophage antigen catabolism caused by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells. *Proc Natl Acad Sci U S A* **79**, 175-178 (1982). <https://doi.org/10.1073/pnas.79.1.175>
- 81 Xiu, Y. *et al.* Chloroquine reduces osteoclastogenesis in murine osteoporosis by preventing TRAF3 degradation. *J Clin Invest* **124**, 297-310 (2014). <https://doi.org/10.1172/JCI66947>
- 82 Vomero, M. *et al.* Autophagy and Rheumatoid Arthritis: Current Knowledges and Future Perspectives. *Front Immunol* **9**, 1577 (2018). <https://doi.org/10.3389/fimmu.2018.01577>
- 83 Kuznik, A. *et al.* Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines. *J Immunol* **186**, 4794-4804 (2011). <https://doi.org/10.4049/jimmunol.1000702>
- 84 Redmann, M. *et al.* Inhibition of autophagy with bafilomycin and chloroquine decreases mitochondrial quality and bioenergetic function in primary neurons. *Redox Biol* **11**, 73-81 (2017). <https://doi.org/10.1016/j.redox.2016.11.004>
- 85 Kondo, H. *et al.* Mutation in VPS33A affects metabolism of glycosaminoglycans: a new type of mucopolysaccharidosis with severe systemic symptoms. *Hum Mol Genet* **26**, 173-183 (2017). <https://doi.org/10.1093/hmg/ddw377>
- 86 Soyombo, A. A. *et al.* TRP-ML1 regulates lysosomal pH and acidic lysosomal lipid hydrolytic activity. *J Biol Chem* **281**, 7294-7301 (2006). <https://doi.org/10.1074/jbc.M508211200>
- 87 Kogot-Levin, A., Zeigler, M., Ornoy, A. & Bach, G. Mucopolipidosis type IV: the effect of increased lysosomal pH on the abnormal lysosomal storage. *Pediatr Res* **65**, 686-690 (2009). <https://doi.org/10.1203/PDR.0b013e3181a1681a>
- 88 Tang, Q. *et al.* NDST3 deacetylates alpha-tubulin and suppresses V-ATPase assembly and lysosomal acidification. *EMBO J* **40**, e107204 (2021). <https://doi.org/10.15252/emboj.2020107204>
- 89 Coffey, E. E., Beckel, J. M., Laties, A. M. & Mitchell, C. H. Lysosomal alkalization and dysfunction in human fibroblasts with the Alzheimer's disease-linked presenilin 1 A246E mutation can be reversed with cAMP. *Neuroscience* **263**, 111-124 (2014). <https://doi.org/10.1016/j.neuroscience.2014.01.001>
- 90 Vizcaino, J. A. *et al.* 2016 update of the PRIDE database and its related tools. *Nucleic Acids Res* **44**, D447-456 (2016). <https://doi.org/10.1093/nar/gkv1145>
- 91 Menzies, S. A. *et al.* The sterol-responsive RNF145 E3 ubiquitin ligase mediates the degradation of HMG-CoA reductase together with gp78 and Hrd1. *Elife* **7** (2018). <https://doi.org/10.7554/eLife.40009>
- 92 Dolan, A. *et al.* Genetic content of wild-type human cytomegalovirus. *J Gen Virol* **85**, 1301-1312 (2004). <https://doi.org/10.1099/vir.0.79888-0>
- 93 Stanton, R. J. *et al.* Reconstruction of the complete human cytomegalovirus genome in a BAC reveals RL13 to be a potent inhibitor of replication. *Journal of Clinical Investigation* **120**, 3191-3208 (2010). <https://doi.org/10.1172/JCI42955>
- 94 McSharry, B. P., Jones, C. J., Skinner, J. W., Kipling, D. & Wilkinson, G. W. G. Human telomerase reverse transcriptase-immortalized MRC-5 and HCA2 human fibroblasts are fully permissive for human cytomegalovirus. *J Gen Virol* **82**, 855-863 (2001). <https://doi.org/10.1099/0022-1317-82-4-855>
- 95 Stanton, R. J. *et al.* Cytomegalovirus destruction of focal adhesions revealed in a high-throughput Western blot analysis of cellular protein expression. *J Virol* **81**, 7860-7872 (2007). <https://doi.org/10.1128/JVI.02247-06>

- 96 Stanton, R. J., McSharry, B. P., Armstrong, M., Tomasec, P. & Wilkinson, G. W. Re-engineering adenovirus vector systems to enable high-throughput analyses of gene function. *Biotechniques* **45**, 659-662, 664-658 (2008). <https://doi.org:10.2144/000112993>
- 97 Gierasch, W. W. *et al.* Construction and characterization of bacterial artificial chromosomes containing HSV-1 strains 17 and KOS. *J Virol Methods* **135**, 197-206 (2006). <https://doi.org:10.1016/j.jviromet.2006.03.014>
- 98 Tischer, B. K., Smith, G. A. & Osterrieder, N. En passant mutagenesis: a two step markerless red recombination system. *Methods Mol Biol* **634**, 421-430 (2010). https://doi.org:10.1007/978-1-60761-652-8_30
- 99 McAlister, G. C. *et al.* MultiNotch MS3 enables accurate, sensitive, and multiplexed detection of differential expression across cancer cell line proteomes. *Anal Chem* **86**, 7150-7158 (2014). <https://doi.org:10.1021/ac502040v>
- 100 McAlister, G. C. *et al.* Increasing the multiplexing capacity of TMTs using reporter ion isotopologues with isobaric masses. *Anal Chem* **84**, 7469-7478 (2012). <https://doi.org:10.1021/ac301572t>
- 101 Albarnaz, J. D. *et al.* Molecular mimicry of NF-kappaB by vaccinia virus protein enables selective inhibition of antiviral responses. *Nat Microbiol* **7**, 154-168 (2022). <https://doi.org:10.1038/s41564-021-01004-9>
- 102 Stern-Ginossar, N. *et al.* Decoding human cytomegalovirus. *Science* **338**, 1088-1093 (2012). <https://doi.org:10.1126/science.1227919>
- 103 Huttlin, E. L. *et al.* A tissue-specific atlas of mouse protein phosphorylation and expression. *Cell* **143**, 1174-1189 (2010). <https://doi.org:10.1016/j.cell.2010.12.001>
- 104 Cox, J. & Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol* **26**, 1367-1372 (2008). <https://doi.org:10.1038/nbt.1511>
- 105 Huang da, W., Sherman, B. T. & Lempicki, R. A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* **4**, 44-57 (2009). <https://doi.org:10.1038/nprot.2008.211>
- 106 Costes, S. V. *et al.* Automatic and quantitative measurement of protein-protein colocalization in live cells. *Biophys J* **86**, 3993-4003 (2004). <https://doi.org:10.1529/biophysj.103.038422>
- 107 Tunyasuvunakool, K. *et al.* Highly accurate protein structure prediction for the human proteome. *Nature* **596**, 590-596 (2021). <https://doi.org:10.1038/s41586-021-03828-1>
- 108 Jahreis, L., Menzies, F. M. & Rubinsztein, D. C. The itinerary of autophagosomes: from peripheral formation to kiss-and-run fusion with lysosomes. *Traffic* **9**, 574-587 (2008). <https://doi.org:10.1111/j.1600-0854.2008.00701.x>









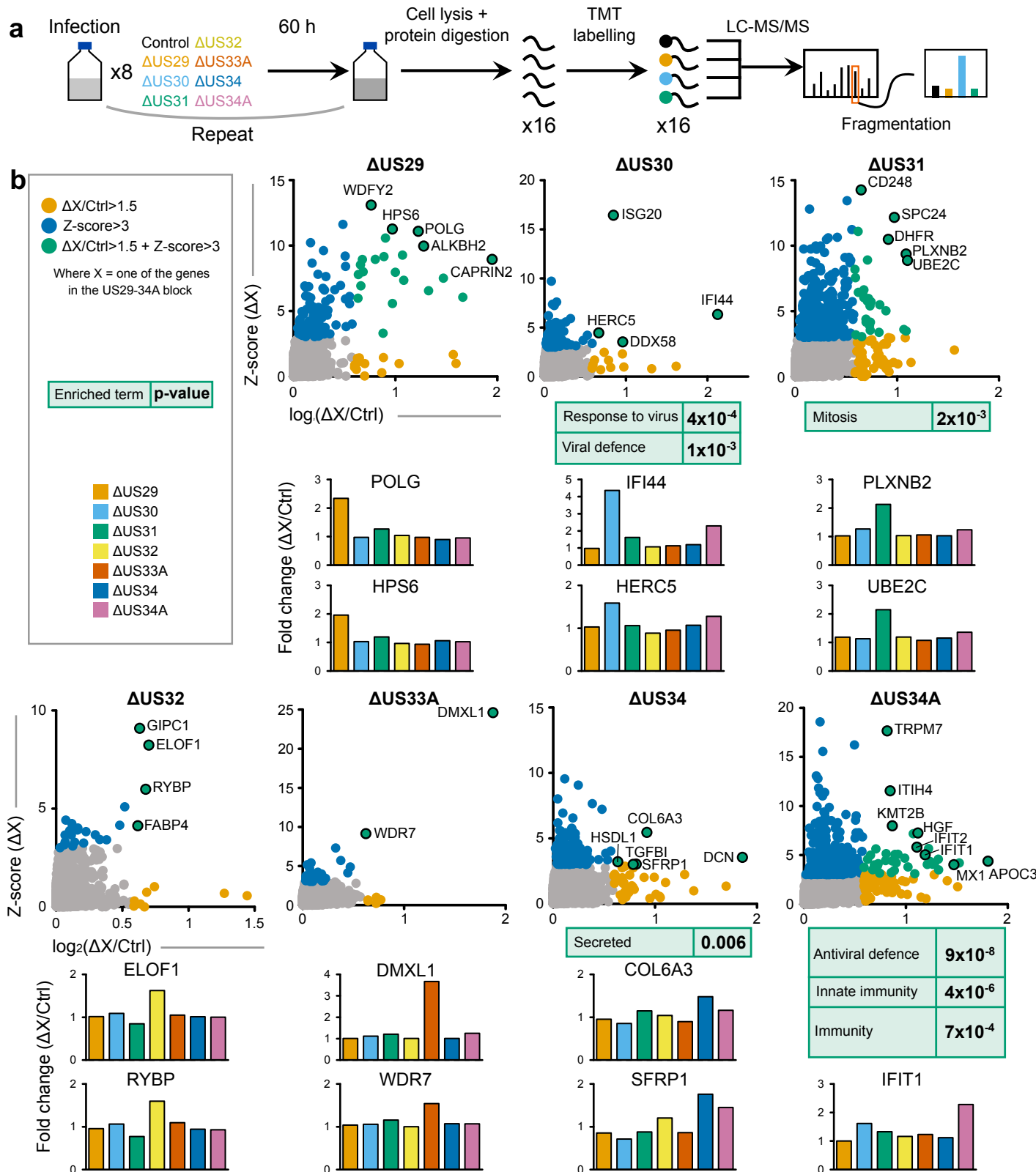


Figure S1. Systematic proteomic analysis of HCMV US29-US34A single-gene deletion mutants reveals functions in host immunity, cell division and protein secretion, related to Figure 1. (a) Schematic of the experiment. (b) Functions of each viral gene. For each quantified protein, signal-to-noise values from the two biological replicates were summed and divided by the summed signal-to-noise for the control sample to give a fold change (FC) [e.g. $(\Delta\text{US29}(1) + \Delta\text{US29}(2)) / (\text{Control}(1) + \text{Control}(2))$]. A Z-score for each protein was calculated from the summed signal-to-noise values. The Z-score is the number of standard deviations by which an observed value (e.g. summed signal:noise for ΔUS29 -infected cells) is above or below the summed signal:noise values for all pairs of samples in the experiment, and represents a measure of how unique rescue of a protein is to a sample infected with a particular deletion virus. Z-scores and FCs for human proteins with a $Z > 0$ and $\text{FC} > 1$ are presented in each scatter plot, to identify proteins that increase in abundance upon deletion of each viral gene. Proteins with $Z > 3$ and $\text{FC} > 1.5$ were subjected to DAVID enrichment analysis, examining GO biological process and UniProt keyword terms. A background of all human proteins quantified was selected, with default settings for functional annotation clustering²². A representative term from each significantly enriched cluster is presented, alongside the Benjamini-Hochberg-adjusted p-value for that term. A list of human proteins targeted by each viral protein in the screen ($\text{FC} > 1.5$, $Z > 3$), and enriched categories from DAVID analysis can be found in **Table S2**. Examples of proteins that were significantly rescued by deletion of a given viral gene are shown below the scatterplots.

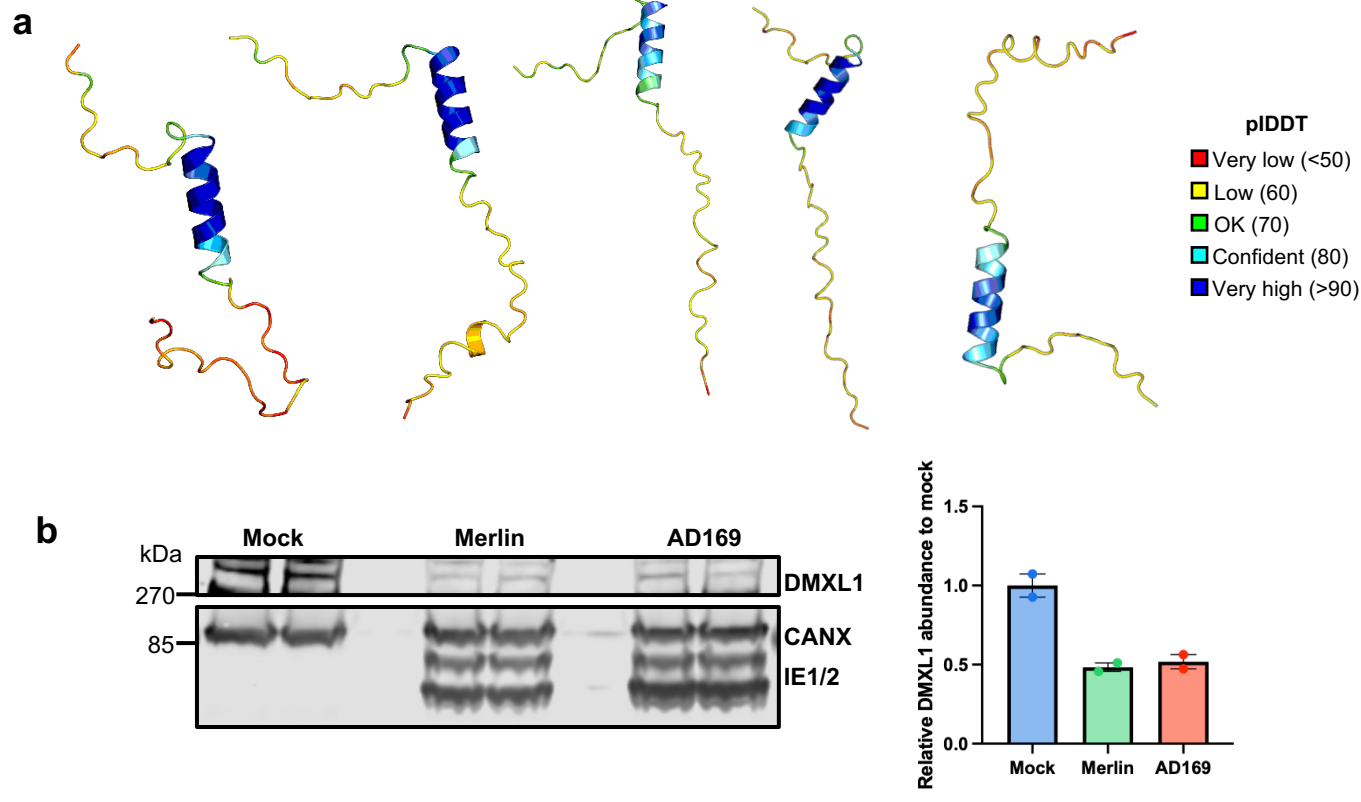


Figure S2. AlphaFold prediction of US33A structure and DMXL1 downregulation by HCMV Merlin and AD169 strains, related to Figure 1. (a) Five unrelaxed AlphaFold models of US33A (UniProt F7V999) from HCMV strain Merlin. A central alpha helix (residues 28-41) with a high confidence pLDDT score was predicted in all five models. Other residues (1-27 and 42-57) were predicted to form unstructured regions. (b) Immunoblot quantifying DMXL1 degradation during infection with HCMV strains Merlin and AD169 in HFFF-TERTs (n=2). Right panel: quantification of DMXL1 shown in the left panel. Values are mean $\pm$ range.

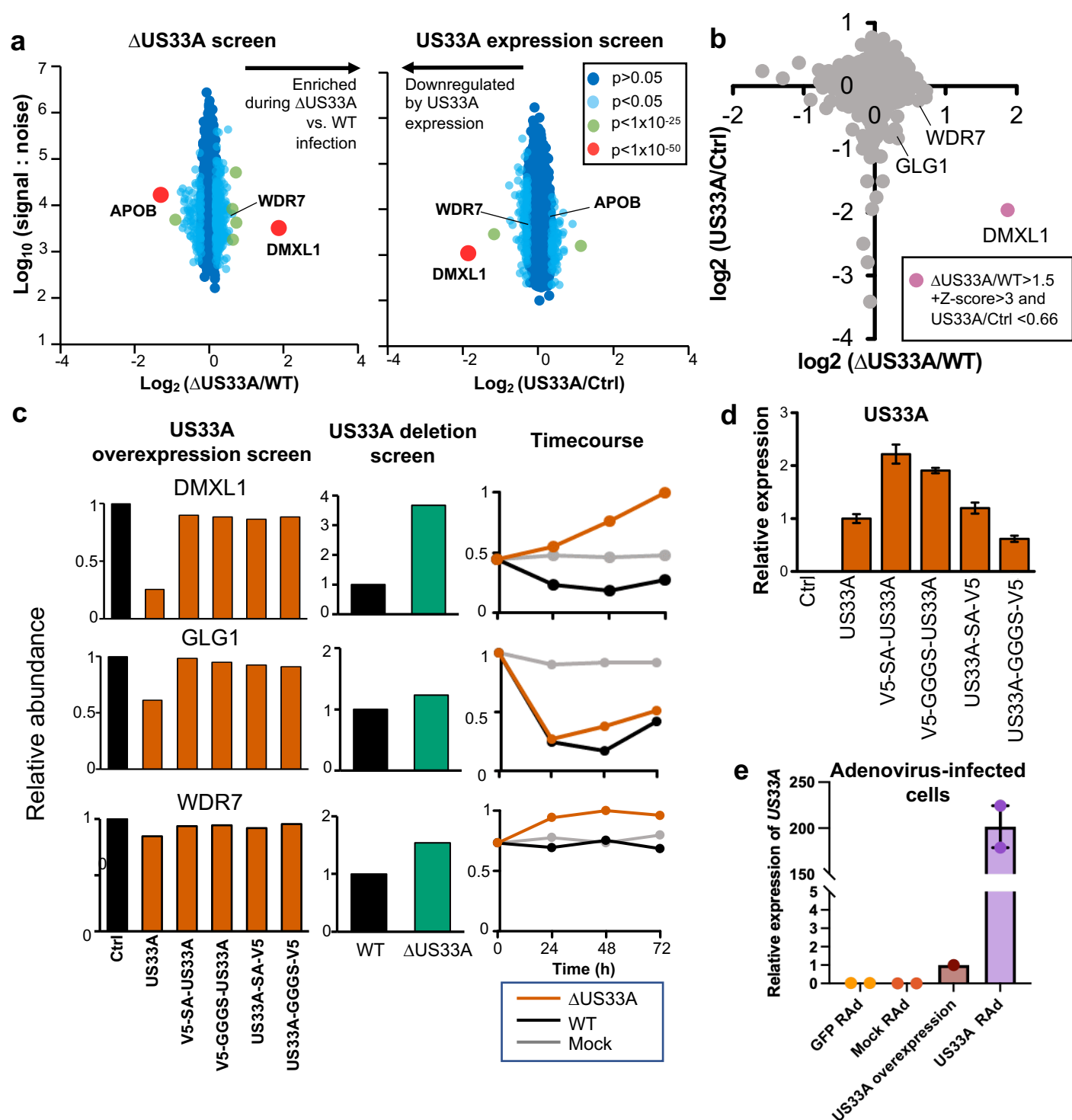


Figure S3. Proteomic analysis of cells overexpressing US33A, V5-tagged US33A, or infected with WT or Δ US33A HCMV, related to Figure 1. (a) Scatterplots showing full data for the US33A deletion screen and the US33A overexpression screen (Figures 1g-h). Of note, whereas a Z-score was employed in Figure 1g (since fold change for Δ US33A/wt could be compared to each other single-gene deletion virus as shown in Figure 1e), no such score could be calculated for US33A overexpression compared to control. p-values were instead estimated using the method of Significance A [S1]. See also Table S1. Although APOB was significantly downregulated during infection with Δ US33A HCMV compared to wt, it was only very subtly upregulated during stable US33A expression (ratio US33A/control = 1.09). It may not therefore represent a bona fide US33A target, and might potentially be a contaminant. (b) Full data for Figure 1h: overlap of fold change data for the US33A deletion and overexpression screens, shown individually in Figure S3A. (c) Data for individual proteins from all proteomic screens. Left panels: Data from overexpression of US33A or various N- or C-terminally V5-tagged US33A variants (as shown in Figures 1h, S3a and Table S1). Middle panels: cells infected with WT or Δ US33A HCMV at MOI 10 at 48 h (as shown in Figures 1g, S3a and Table S1). Right panels: timecourse data, as shown in Figure 1i. (d) qPCR measurement of *US33A* expression in cells stably expressing US33A or various N- or C-terminally V5-tagged US33A variants. (e) qPCR measurement of *US33A* expression in HFFF-TERTs infected with adenovirus expressing GFP or US33A. The relative US33A expression level was calculated by normalising the $\Delta\Delta$ Ct for each condition to cells stably expressing US33A. Shown are mean $\pm$ range of two independent experiments.

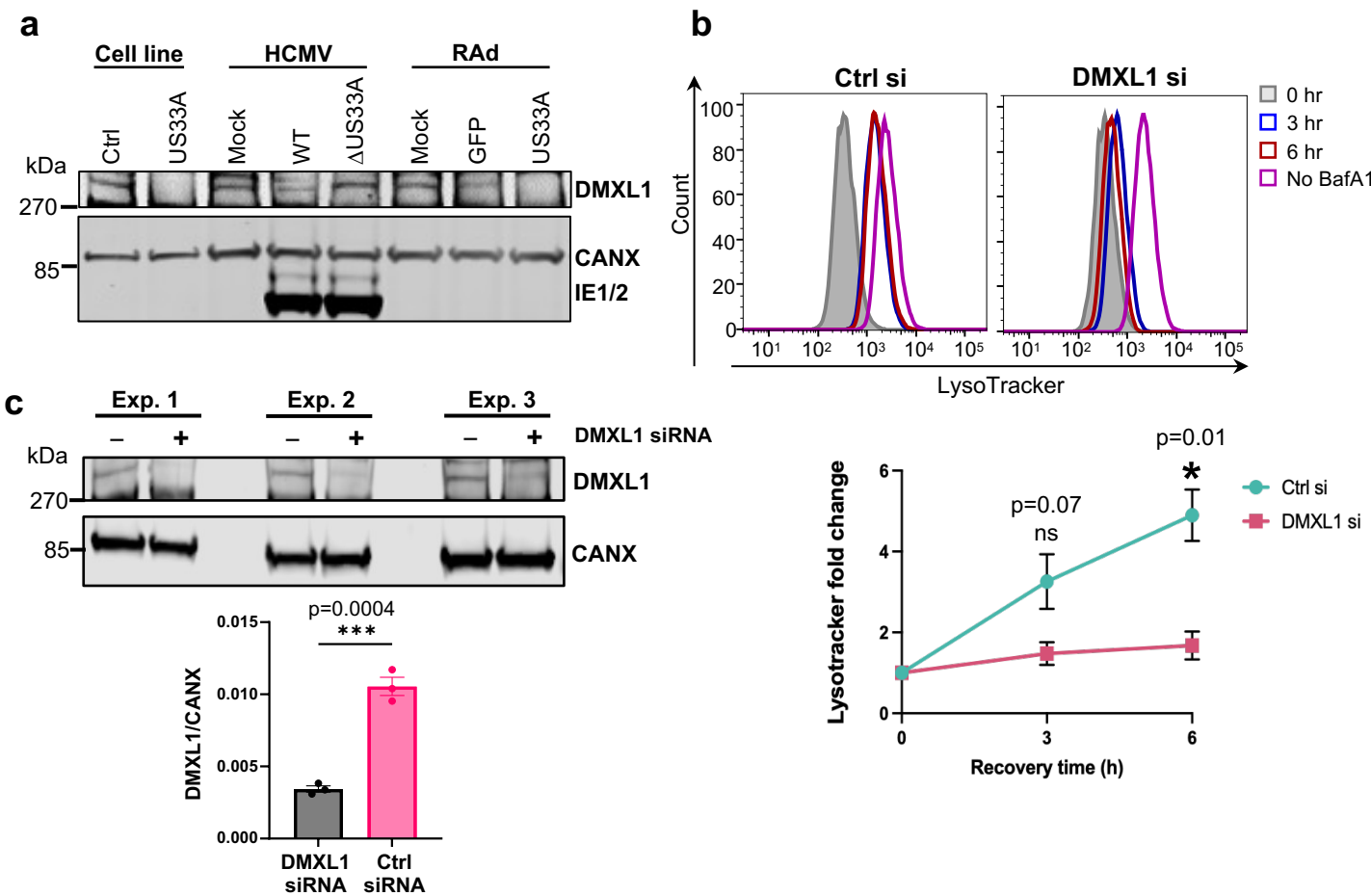


Figure S4. Inhibition of lysosomal acidification after DMXL1 downregulation by US33A or siRNA, related to Figure 2. (a) Immunoblot showing DMXL1 degradation in HFFF-TERTs stably expressing US33A or control; HFFF-TERTs infected with WT or Δ US33A HCMV at MOI 5 for 24 h; and HFFF-CARs infected with adenovirus expressing US33A or GFP at MOI 5 for 72 h. (b) siRNA-based DMXL1 knockdown inhibits lysosomal re-acidification (after 400 nM BafA1 treatment for 1 h). Top panels: representative flow cytometry plots of LysoTracker staining of HFFF-TERTs treated with DMXL1 or control siRNAs. Bottom panel: quantification of lysosomal acidity via LysoTracker staining in HFFF-TERTs. Values shown are mean $\pm$ SEM (n=3 independent experiments). P-values were estimated using an unpaired two-tailed t-test. *p<0.05. (c) Immunoblot and its quantitation, showing DMXL1 knockdown efficiency by siRNA in HFFF-TERTs for the experiment shown in (b).

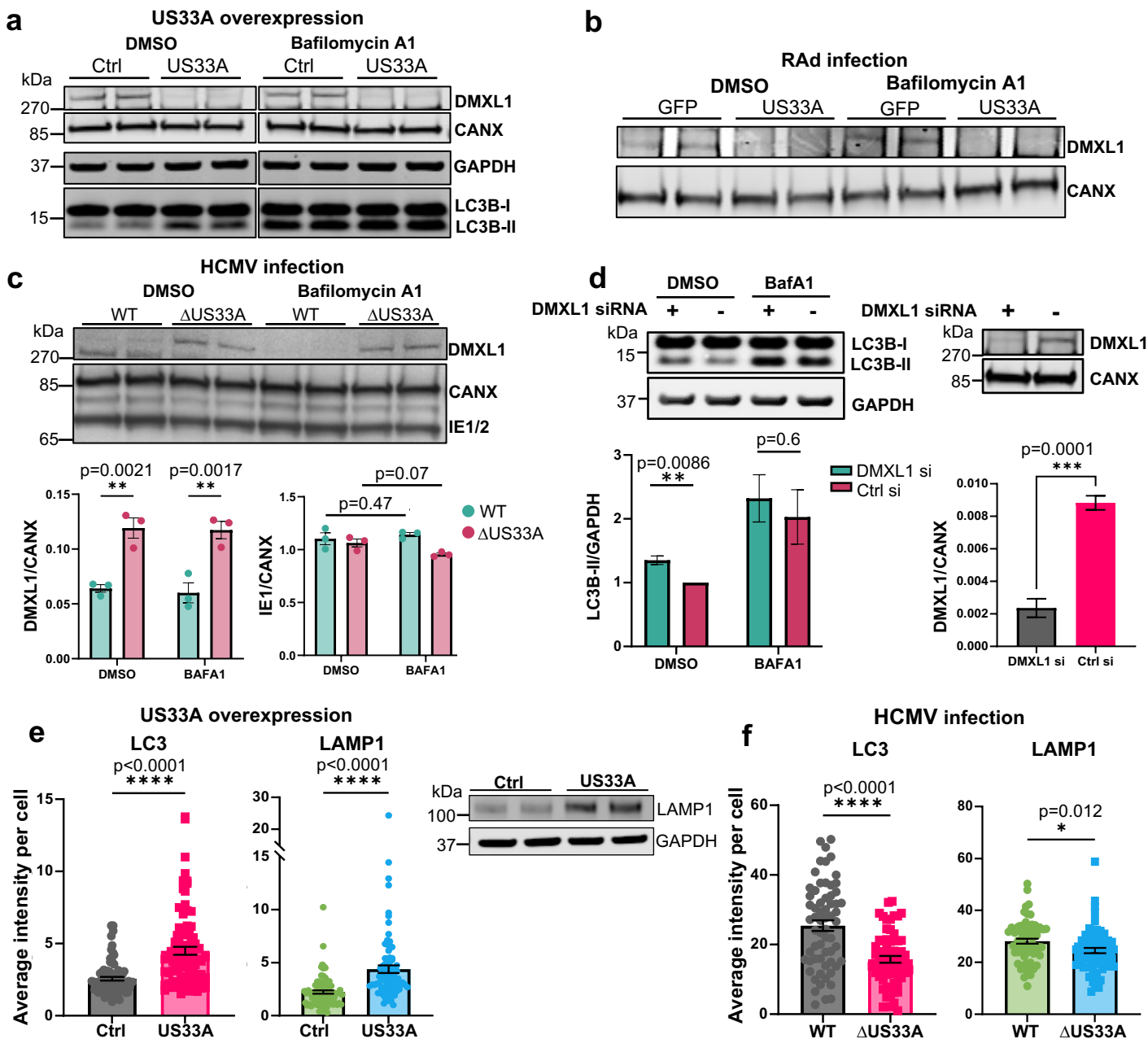


Figure S5. Increased LC3 and LAMP1 upon DMXL1 downregulation in cells overexpressing US33A, infected with RAD expressing US33A, or infected with HCMV, related to Figure 3. (a) DMXL1 degradation and LC3B-II accumulation in HFFF-TERTs stably expressing US33A or control. This is a direct repeat of the experiment shown in Figure 3b, in order to quantify DMXL1 in addition to LC3. (b) DMXL1 degradation in HFFF-CARs infected with adenovirus expressing GFP or US33A. The blot shown is derived from the same lysates shown in Figure 3c. (c) Top panel: DMXL1 degradation in HFFF-TERTs infected with WT or Δ US33A HCMV at MOI=25 for 48 h. Cells were treated with BafA1 or DMSO (vehicle control) for 4 h before harvest. Bottom panel: quantification of DMXL1 and IE1, adjusted for loading using calnexin (CANX). For IE1, the total intensity of both bands shown in the immunoblot was quantified. Plots show mean $\pm$ SEM (n=3; blot shown is derived from the same lysates shown in Figure 3d). P values were estimated using two-way ANOVA. **p<0.01. (d) DMXL1 knockdown in HFFF-TERTs inhibits LC3B-II degradation. Top panel: representative immunoblot. Bottom panel: quantification of LC3B-II (left) and DMXL1 (right). Values show mean $\pm$ SEM (n=4). For LC3B-II quantification, p-values were examined using a ratio paired t-test **p<0.01; for DMXL1, p-values were examined using a two-tailed unpaired t-test. ***p<0.001. (e) Left panel: Quantification of the average intensity of LC3 and LAMP1 in HFFF-TERTs stably expressing US33A or control. Values are mean $\pm$ SEM (n= 79 for control, and n=87 for US33A from two independent replicates). Right: Immunoblot showing LAMP1 abundance in HFFF-TERTs stably expressing US33A or control (n=2). (f) Quantification of the average intensity of LC3 and LAMP1 in HFFF-TERTs infected with WT or Δ US33A HCMV. The images shown in **Figure 3h** were examined to generate this plot. Values are mean $\pm$ SEM (n=63 for both WT and Δ US33A HCMV infection). P values were estimated using an unpaired t-test. *p<0.05, ****p<0.0001.

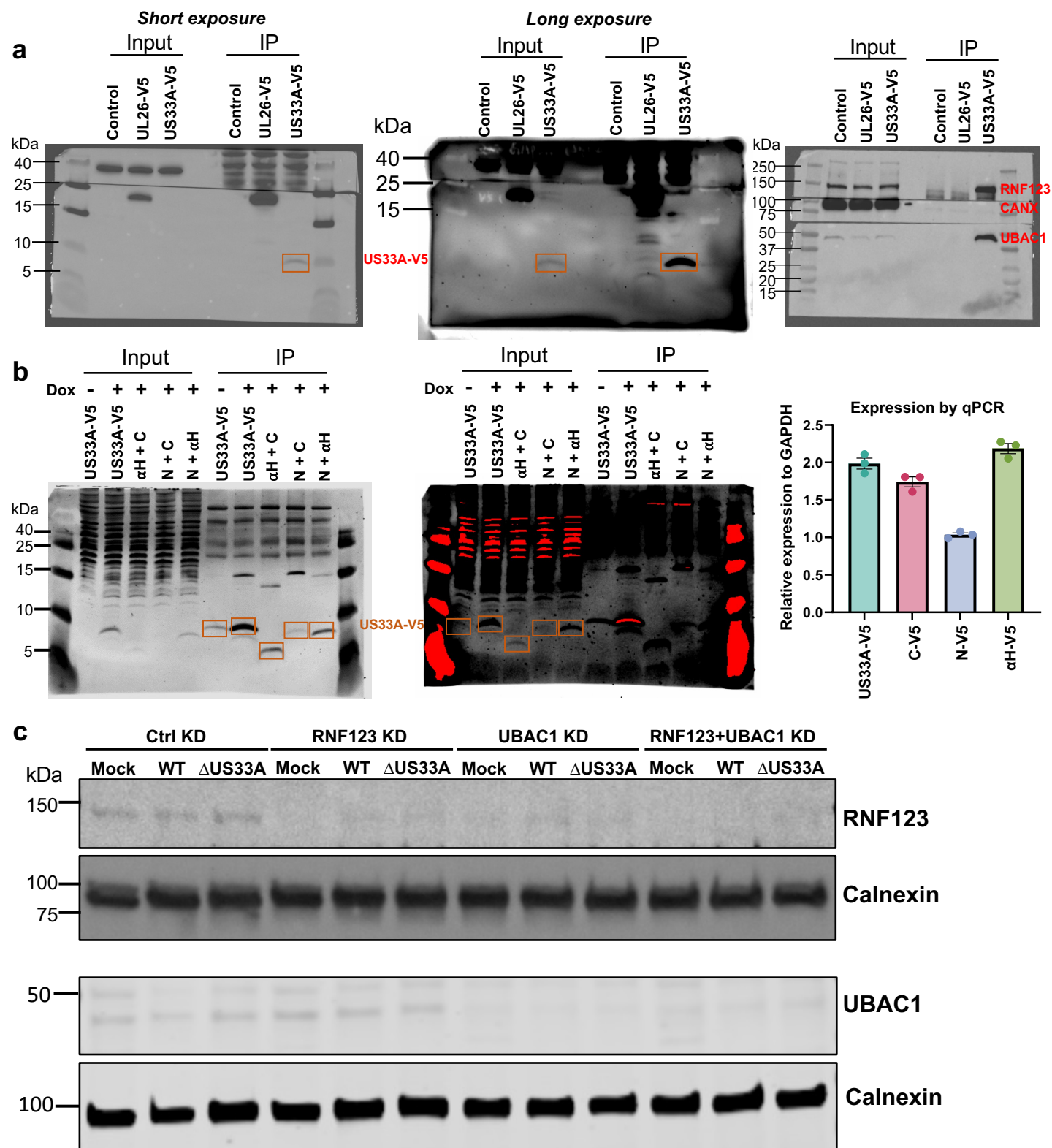


Figure S6. The N-terminal region of US33A is required for KPC recruitment, related to Figure 4. (a) Full immunoblot images with short exposure (shown in Figure 4b), and long exposure, demonstrating expression of US33A-V5 in both input and IP samples. (b) Left and middle: Full immunoblot images with short exposure (shown in Figure 4c), and long exposure, demonstrating the expression of US33A-V5 truncation constructs in input and IP samples. Right panel: qPCR validation of expression of the US33A N-terminus, alpha-helix and C-terminus in isolation; these mutants were of insufficient size to detect by immunoblot. (c) Immunoblots demonstrating knockdown of RNF123 and UBAC1 in polyclonal CRISPR populations used for Figure 4d. UBAC1 is known to be required for the stability of RNF123, explaining the decrease in RNF123 expression in cells expressing UBAC1 gRNAs [S2].

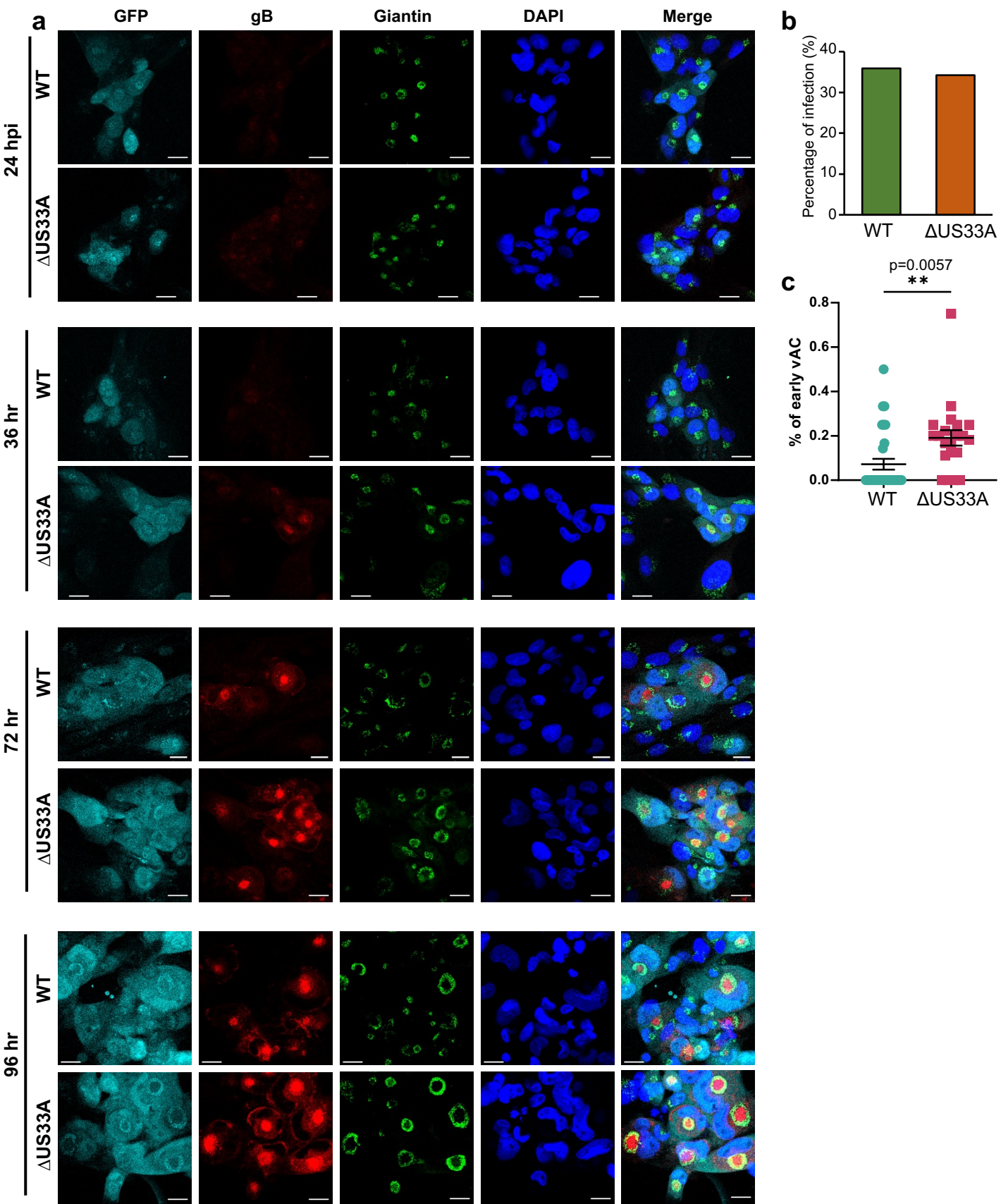
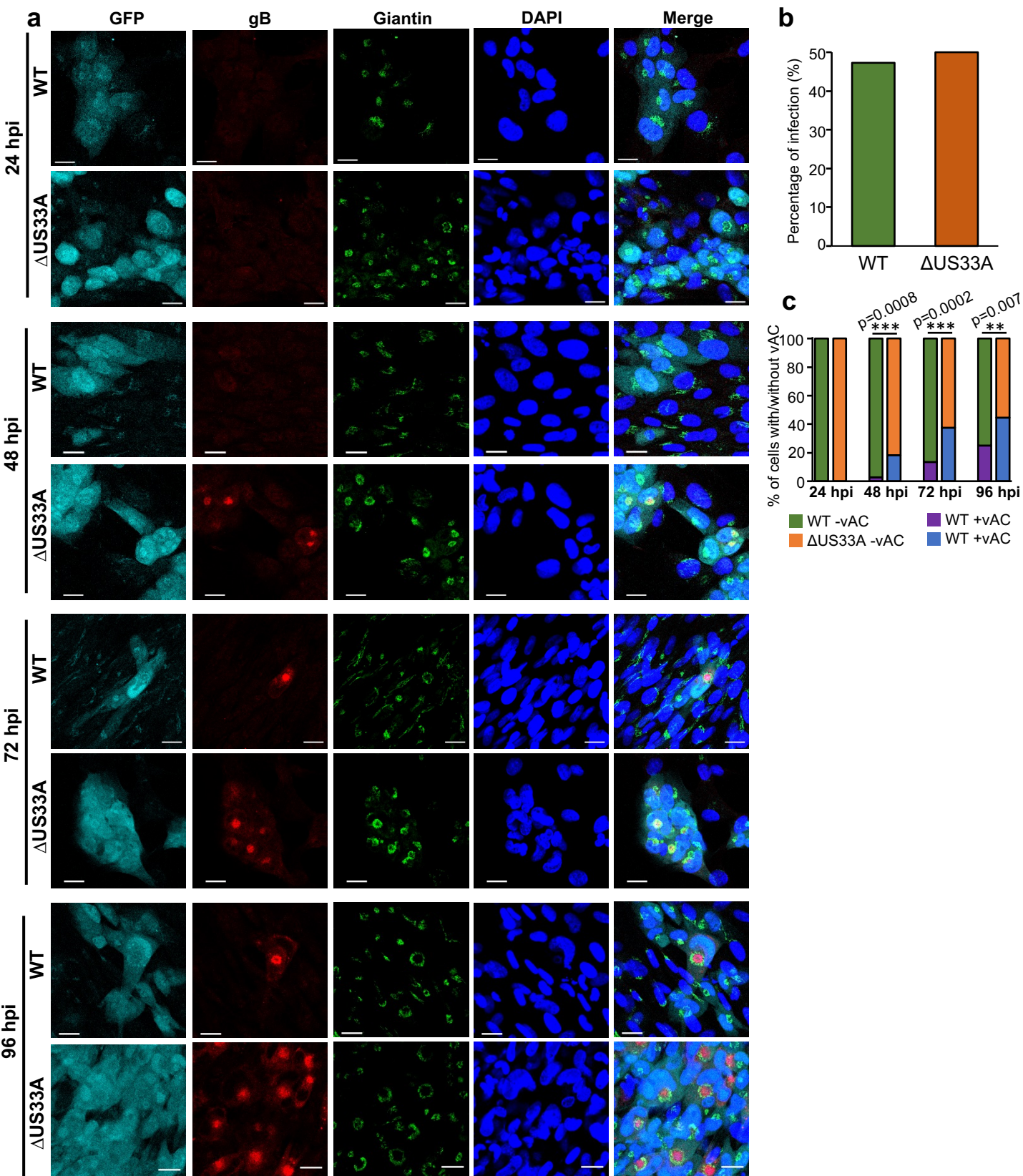


Figure S7. vAC formation timecourse in HFFF-TERTs infected for 24, 36, 72, and 96 h with WT or Δ US33A HCMV, related to Figure 4. (a) Representative images of vAC formation in HFFFs infected with WT or Δ US33A HCMV at 24, 36, 72, and 96 hpi. Scale bar: 20 μ m. (b) Percentage of cells infected with WT or Δ US33A HCMV at 24 hpi. Infected cells were identified by GFP expression. For WT HCMV infection, 112 cells were counted, and for Δ US33A HCMV infection, 115 cells were counted to calculate the percentage of infected cells. (c) Percentage of infected cells with immature/early vACs at 36 h. Values are mean $\pm$ SEM (n=31 images with 122 infected cells for WT; n=21 images with 146 infected cells for Δ US33A). P values were examined using two-tailed unpaired t-test. **p<0.01.



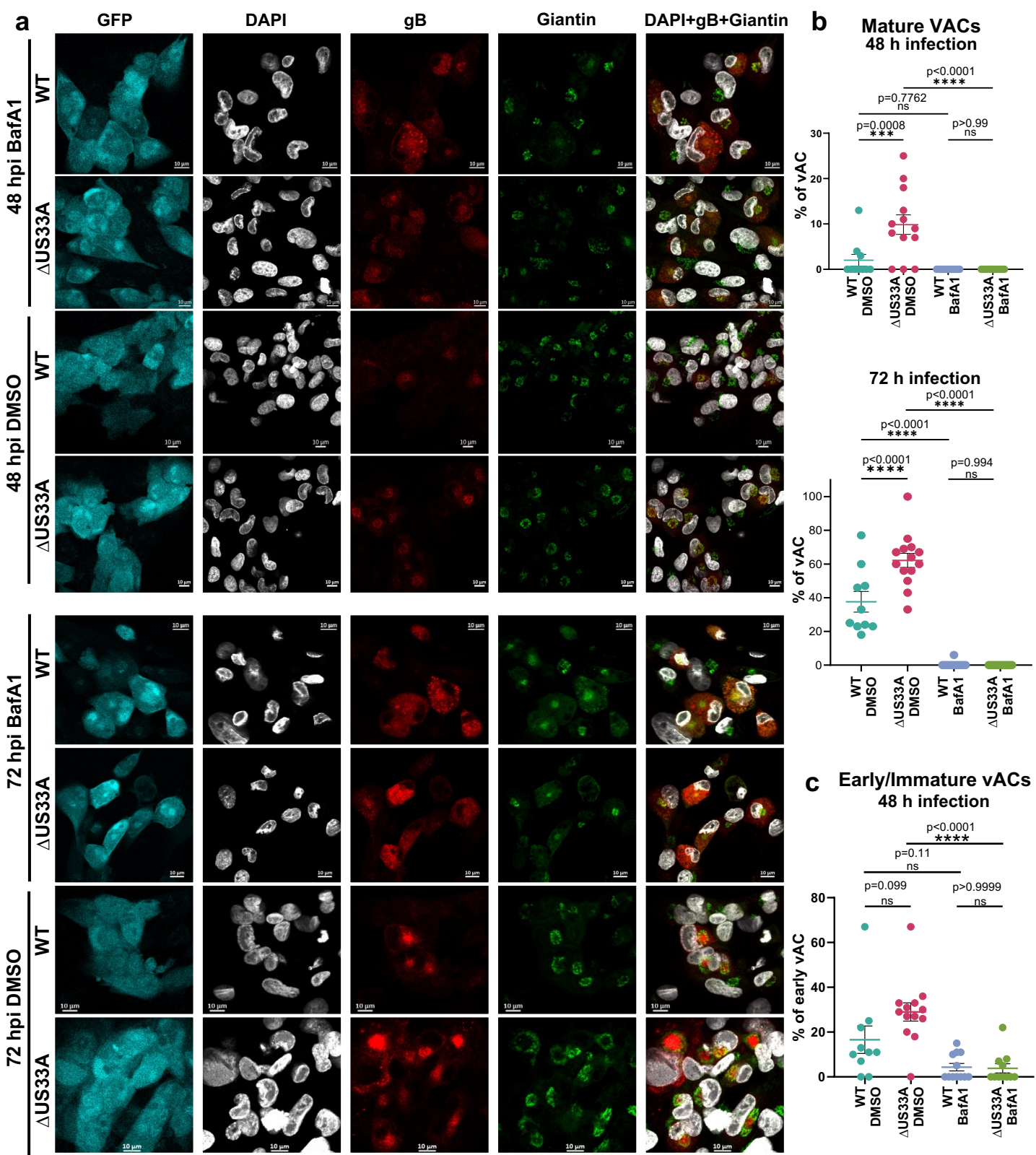


Figure S9. Bafilomycin A1 inhibits vAC formation in cells infected with WT or Δ US33A HCMV, related to Figure 4.

(a) Representative images of vAC formation in HFFs infected with WT or Δ US33A HCMV $\pm$ 10nM BafA1 treatment, at 48 and 72 hpi. Here, the infectious inoculum was replaced after 2 h with media containing BafA1 or control for 48 or 72 h. Scale bar: 10 μ m. **(b)** Percentage of infected cells forming mature vACs at 48 hpi (top panel) or 72 hpi (bottom panel). Infected cells were identified by GFP expression. Top panel: values are mean $\pm$ SEM (n=10 images with 155 infected cells for WT HCMV+DMSO; n=12 images with 164 infected cells for Δ US33A HCMV+DMSO; n=10 images with 127 infected cells for WT HCMV+BafA1; n=10 images with 156 infected cells for Δ US33A HCMV+BafA1). Bottom panel: values are mean $\pm$ SEM (n=11 images with 215 infected cells for WT HCMV+DMSO; n=13 images with 223 infected cells for Δ US33A HCMV+DMSO; n=11 images with 135 infected cells for WT HCMV+BafA1; n=11 images with 136 infected cells for Δ US33A HCMV+BafA1). **(c)** Percentage of infected cells forming early/immature vACs at 48 hpi under each condition. Values are mean $\pm$ SEM, and images used for quantification were the same used in (b) top panel. P values were examined using ordinary one-way ANOVA. *** $p<0.001$, **** $p<0.0001$, ns: non-significant, $p>0.05$.

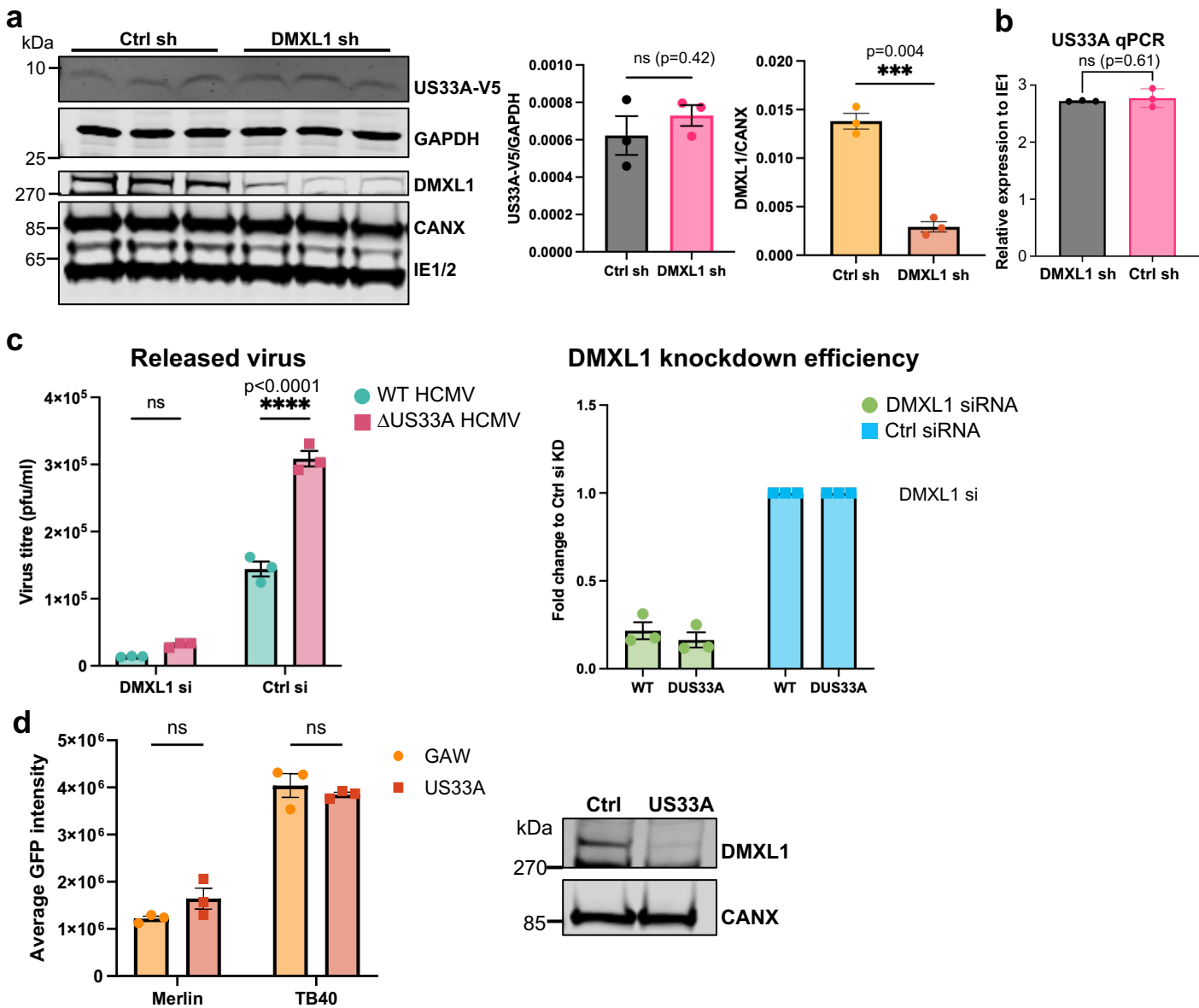


Figure S10. DMXL1 inhibition by shRNA or siRNA prevents viral release in HFFF-TERTs infected with WT HCMV, related to Figure 4. (a) Left panel: Immunoblot showing the expression of US33A-V5 in HFFF-TERTs knocked down for DMXL1 or control using shRNA then infected with US33A-V5 HCMV. Right panel: quantification of US33A-V5 abundance. Values shown are mean $\pm$ SEM (n=3 independent samples). An unpaired t-test was used to calculate p-values. ***p<0.001. (b) qPCR quantification of the expression of US33A transcript in HFFF-TERTs infected with WT HCMV. Values shown are mean $\pm$ SEM (n=3 independent samples). An unpaired t-test was used to calculate p-values. ns: non-significant, p>0.05, ****p<0.0001. (c) Left: quantification of viral release in HFFF-TERTs treated with DMXL1 or control siRNAs then infected with WT or Δ US33A HCMV at MOI=0.01 for 6 days. Values are mean $\pm$ SEM (n=3 independent experiments). A two-way ANOVA test was used to calculate p-values. ****p<0.0001. ns: non-significant, p>0.05. Right: qPCR to estimate DMXL1 siRNA knockdown efficiency. (d) HCMV entry into ARPE19 epithelial cells is not affected by US33A expression. Left panel: quantification of HCMV entry of GFP-expressing Merlin and TB40E HCMV recombinants into ARPE19 cells expressing US33A or control (MOI=1, 48 hpi). Values are mean $\pm$ SEM (n=3). P values were calculated using a two-way ANOVA test. ns: p>0.05. Right panel: immunoblot showing DMXL1 degradation in ARPE19 cells expressing US33A or control.

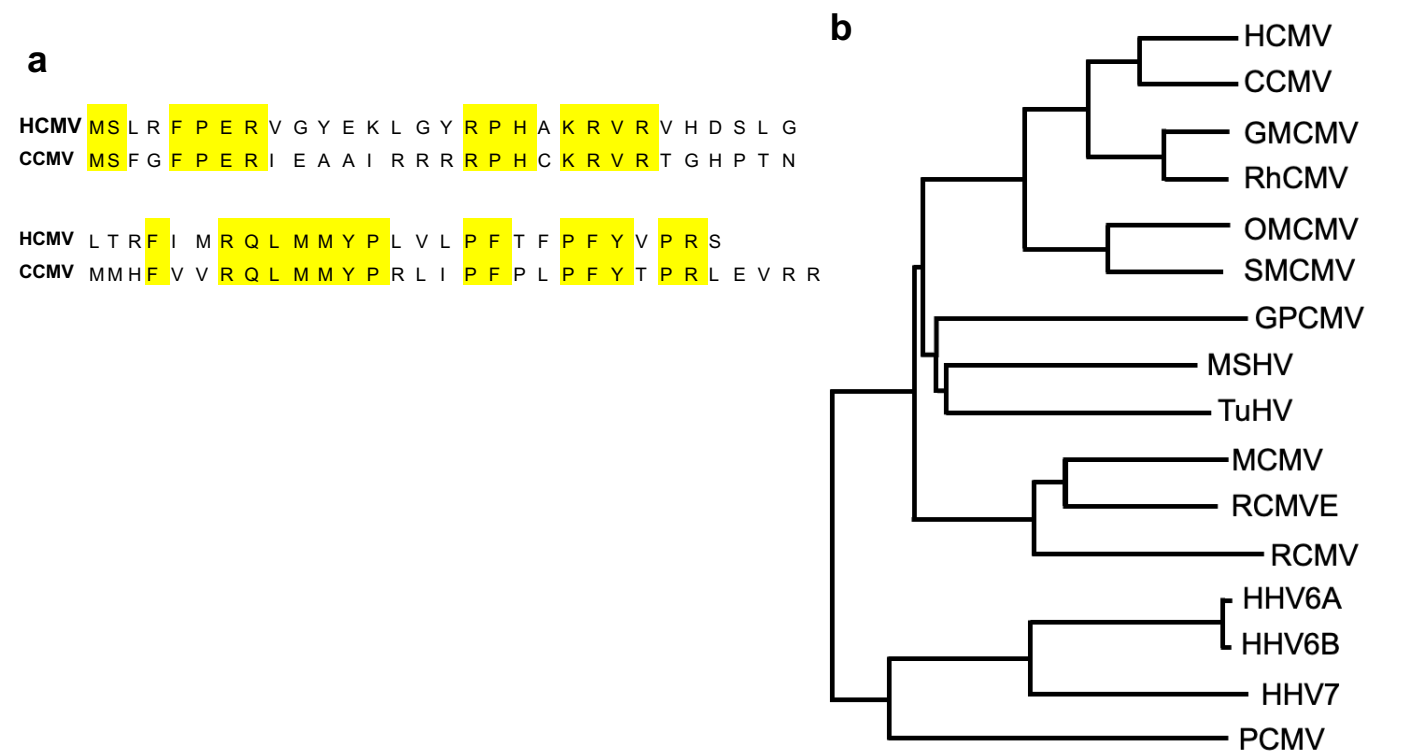


Figure S11. Conservation of US33A and phylogenetic tree of beta-herpesviruses, related to Figures 1 and 4. (a) Amino acid sequence alignments of predicted HCMV and chimpanzee cytomegalovirus proteins. Conserved residues are shaded yellow. **(b) Phylogenetic tree of beta-herpesviruses, re-generated from Wilkie *et al.*, 2014 [S3].** HCMV, human cytomegalovirus; CCMV, chimpanzee cytomegalovirus; GMCMV, green monkey cytomegalovirus; RhCMV, rhesus cytomegalovirus; OMCMV, owl monkey cytomegalovirus; SMCMV, squirrel monkey cytomegalovirus; GPCMV, guinea pig cytomegalovirus; MSHV, *Miniopterus schreibersii* herpesvirus; TuHV, *tupaia*id herpesvirus 1; MCMV, murine cytomegalovirus; RCMVE, rat cytomegalovirus England; RCMV, rat cytomegalovirus; HHV6A, human herpesvirus 6A; HHV6B, human herpesvirus 6B; HHV7, human herpesvirus 7; and PCMV, porcine cytomegalovirus.

Table S2. Known characteristics of proteins encoded by the US29-34A block, related to Figure 1.

Gene	Growth [S4, S5]	Kinetics [S5, S6]	Size (kDa)	Function
US29	D	E/L	51	Enhances replication in epithelial cells [4]. Putative transmembrane protein [S7].
US30	EG/D	E	39	Inhibits replication in fibroblasts [S4]. Putative transmembrane protein [S7].
US31	D	N/A	19	May activate NF-κB2 and stimulate M1 macrophage differentiation [S8].
US32	D	L	22	Might be expressed during latency [S9].
US33A	N/A	N/A	7	N/A
US34	D	N/A	18	Might be expressed during latency [S9]. Putative secreted protein [S7].
US34A	D	N/A	8	Putative transmembrane protein [S7]. Potential target of SUMO [S10].

Data S1. Conservation of US33A across 264 strains of HCMV, related to Figures 1 and 4.

US33A_NCBI-AY446894.2	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-GQ221975.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-GQ396662.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-GU179289.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-GU179290.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-HQ380895.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-JX512197.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-JX512199.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-JX512200.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-JX512203.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-JX512204.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-JX512205.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-JX512208.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KC519320.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KC519323.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KF297339.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361946.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361948.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361949.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361951.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361952.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361953.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361956.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361959.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361963.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361966.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ361969.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ872539.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KJ872540.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KP745635.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KP745639.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KP745640.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KP745642.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KP745643.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KP745647.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS
US33A_NCBI-KP745649.1	MSLRFFPERAGYEKLGYPHAKRVVHDEPLGLTRFIMRQLMMYPLVLPFTFFPFYVPRS

[illegible]

[illegible]

US33A_NCBI-MT070140.1	MSLRFPERAGYEKLGYPHAKRVVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MT070141.1	MSLRFPERAGYEKLGYPHAKRVVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MT070142.1	MSLRFPERAGYEKLGYPHAKRVVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MW197155.1	MSLRFPERAGYEKLGYPHAKRVVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-GQ221973.1	MSLRFPERAGYEKLGHRPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-GQ221974.1	MSLRFPERAGYEKLGHRPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-JX512198.1	MSLRFPERAGYEKLGHRPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KC519319.1	MSLRFPERAGYEKLGHRPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361970.1	MSLRFPERAGYEKLGHRPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745657.1	MSLRFPERAGYEKLGHRPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745671.1	MSLRFPERAGYEKLGHRPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745717.1	MSLRFPERAGYEKLGHRPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KT726940.2	MSLRFPERAGYEKLGHRPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490079.1	MSLRFPERAGYEKLGHRPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-GQ466044.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-JX512206.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-JX512207.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KF021605.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361947.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361950.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361954.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361957.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361958.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361960.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361961.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361962.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ426589.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745633.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745645.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745646.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745652.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745659.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745660.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745668.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745673.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745675.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745681.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745694.1	MSLRFPERAGYEKLGYPHAKRVRVHDPGLGLTRFIMRQLMMYPLVLPFTFPFYVPRS

US33A_NCBI-KP745697.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745700.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745701.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745702.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745705.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745712.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745714.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745720.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745722.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KR534198.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KR534199.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KR534201.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KT634296.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KT726941.2	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KT726942.2	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KT726955.2	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY123652.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490068.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490076.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490078.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490080.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490083.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490088.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-LR131940.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MN274568.2	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MT044480.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MT044483.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MT044485.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MT070139.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MW197154.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MW197157.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745641.1	MSLRFPERAGYEKLGYPYAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY123650.1	MSLRFPERAGYEKLGYPHAKRVVHDPLGLTRFIMRQLMMYPLVVPFTFPFYVPRS
US33A_NCBI-KY490072.1	MSLRFPERAGYEKLGYPHAKRVVHDPLGLTRFIMRQLMMYPLVLPFTLPFYVPRS
US33A_NCBI-KJ872542.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLMRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745707.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMIYPLVLPFTFPFYVPRS
US33A_NCBI-KP745727.1	MSLRFPERAGYEKLGYPHAKRVRVHDPLGLTRFIMRQLMIYPLVLPFTFPFYVPRS
US33A_NCBI-GQ396663.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS

US33A_NCBI-GU179291.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-JX512201.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KC519322.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361955.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361964.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361965.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361967.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745637.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745644.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745648.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745655.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745665.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745666.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745670.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745680.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745692.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745693.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745704.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745710.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745711.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745713.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745719.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KR534203.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KR534204.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KT726944.2	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KT726948.2	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KU550090.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY123653.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490063.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490073.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490075.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490081.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490082.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MK290742.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MT044477.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MT044481.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MW197156.1	MSLRFPERAGYEKLGYPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-FJ527563.1	MSLRFPERVGYEKLGYRPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS

US33A_NCBI-KP745636.1	MSLRFPERVGYEKLGYRPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745658.1	MSLRFPERVGYEKLGYRPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745687.1	MSLRFPERVGYEKLGYRPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745706.1	MSLRFPERVGYEKLGYRPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490061.1	MSLRFPERVGYEKLGYRPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490065.1	MSLRFPERVGYEKLGYRPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490084.1	MSLRFPERVGYEKLGYRPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MK290744.1	MSLRFPERVGYEKLGYRPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361971.1	MSLRFPERVGYEKLGYRPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745650.1	MSLRFPERVGYEKLGYRPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745651.1	MSLRFPERVGYEKLGYRPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745698.1	MSLRFPERVGYEKLGYRPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745708.1	MSLRFPERVGYEKLGYRPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KY490071.1	MSLRFPERVGYEKLGYRSHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MK422176.1	MSLKFPERAGYEKLGYRPHAKRVRVHDSLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745696.1	MSLRFPERAGYKKLGYRPHAKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-JX512202.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KC519321.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ361968.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KJ872541.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745634.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745638.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745661.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745663.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745676.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745677.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745682.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745695.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KP745726.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KR534200.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KR534208.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KR534213.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KT726950.2	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KT959235.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KU550087.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-MK290743.1	MSLKFPERVGYEKLGHRPYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-LR131943.1	MSLKFPERVGYEKLGHRSYTKRVRVHDPLGLTRFIMRQLMMYPLVLPFTFPFYVPRS
US33A_NCBI-KT726951.2	MSFRFPERADYEKLGYRPHAKRVRVHDTLGLTRFIMRQLMMYPLVLPFTFPFYVPRS

REFERENCES

- [S1] Cox, J., and Mann, M. (2008). MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol* 26, 1367-1372. 10.1038/nbt.1511.
- [S2] Hara, T. et al. (2005). Role of the UBL-UBA protein KPC2 in degradation of p27 at G1 phase of the cell cycle. *Mol Cell Biol* 25, 9292-9303.
- [S3] Wilkie, G. S. et al. (2014). First fatality associated with elephant endotheliotropic herpesvirus 5 in an Asian elephant: pathological findings and complete viral genome sequence. *Sci Rep* 4, 6299.
- [S4] Dunn, W. et al. (2003). Functional profiling of a human cytomegalovirus genome. *Proc Natl Acad Sci U S A* 100, 14223-14228.
- [S5] Van Damme, E. & Van Loock, M. (2014). Functional annotation of human cytomegalovirus gene products: an update. *Front Microbiol* 5, 218.
- [S6] Chambers, J. et al. (1999). DNA microarrays of the complex human cytomegalovirus genome: profiling kinetic class with drug sensitivity of viral gene expression. *J Virol* 73, 5757-5766.
- [S7] Mocarski ES, S. T., Pass RF. (2007). Cytomegaloviruses. (Lippincott Williams & Wilkins).
- [S8] Guo, G. et al. (2018). The cytomegalovirus protein US31 induces inflammation through mono-macrophages in systemic lupus erythematosus by promoting NF-kappaB2 activation. *Cell Death Dis* 9, 104.
- [S9] Goodrum, F. D., Jordan, C. T., High, K. & Shenk, T. (2002). Human cytomegalovirus gene expression during infection of primary hematopoietic progenitor cells: a model for latency. *Proc Natl Acad Sci U S A* 99, 16255-16260.
- [S10] Mancebo, F. J., Parras-Molto, M., Garcia-Rios, E. & Perez-Romero, P. (2022). Deciphering the Potential Coding of Human Cytomegalovirus: New Predicted Transmembrane Proteome. *Int J Mol Sci* 23.