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Supplementary Information for

Human Cytomegalovirus lytic infection inhibits replication-dependent histone synthesis and requires stem loop binding protein function

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Materials and Methods

Cells and Viruses. De-identified human foreskin fibroblasts (HFFs) were maintained in Dulbecco's Modified Eagle Medium (DMEM; Sigma) supplemented with 10% fetal bovine serum (FBS; Sigma) and 100 U/ml penicillin, 0.1 mg/ml streptomycin and 2mM L-glutamine (PSG; Sigma). For serum starvation, HFF cells were seeded at 1.2 x 10^4 cells/cm^2 in complete media. After 24 hrs, media was removed and cells were washed twice with phosphate-buffered saline (PBS; ThermoFisher) followed by addition of low serum media (0.1% FBS in DMEM + PSG) for 48 hrs. Serum stimulation was performed by addition of media containing 10% FBS for 24 hrs. Unless otherwise indicated, cells were infected with HCMV strain AD169 at an MOI of 1 pfu/cell in low serum media (resulting in ~60-70% of cells being infected, based on UL44 expression by 48hpi). Infections were carried out by incubating cells with virus in minimal volume at 4°C for 45 min to allow for attachment followed by incubation at 37°C for 15 min to allow for viral entry. Inoculum was then removed and replaced with fresh, low serum media at normal culture volumes and incubated for the indicated amount of time. Where indicated, phosphonoacetic acid (PAA; Sigma cat. no. 284270) dissolved in water was added at the time of infection (final concentration 100ug/ml).

Western Blots. Cells were lysed in SDS lysis buffer (1% SDS, 2% b-mercaptoethanol), boiled, and equivalent amounts of total protein were separated by SDS-PAGE and transferred to Optitran membranes (GE Healthcare). Membranes were blocked with 5% BSA in TBST (10mM Tris pH 8.0, 150mM NaCl, 0.05% Tween-20) and incubated with primary antibody diluted in blocking buffer. Membranes were then washed 3 times in TBST followed by incubation with IR-dye conjugated secondary antibody diluted in blocking buffer and then washed 3 times in TBST prior to imaging on an Odyssey Fc imager using Image Studio v2.1.10 software (LI-COR). Primary and secondary antibodies used are listed in Supplemental Table 2.

Immunofluorescence. For indirect immunofluorescence, cells were cultured and infected on glass coverslips. Coverslips were washed with phosphate buffered saline (PBS) and fixed with 1%

paraformaldehyde in PBS. Cells were permeabilized with PBST (0.1% Triton X-100, 0.05% Tween-20 in PBS) and blocked with blocking buffer (0.5% BSA, 5% goat serum in PBST). Coverslips were incubated with primary antibody diluted in blocking buffer, washed 3 times with PBST, incubated with secondary antibody diluted in blocking buffer, and washed 3 times with PBST. Coverslips were subsequently rinsed with ddH2O and counterstained with Hoechst 33342 prior to being mounted on slides with Fluoromount-G (Invitrogen cat. no. 00-4958-02). Images were acquired using a Nikon confocal laser scanning microscope equipped with Prairie View software and images were processed and analyzed using FIJI software (1) with the Intensity Ratio Nuclei Cytoplasm Tool (RRID:SCR_018573) and Plot Profile plugin. Primary and secondary antibodies used are listed in Supplemental Table 2.

RNA extraction and RT-qPCR. Total RNA was extracted using an IBI total RNA minikit (IBI Scientific cat no. IB145323) according to the manufacturer's directions. Equal amounts of total RNA were treated with dsDNase and converted to cDNA using the Maxima H Minus Supermix with dsDNase system (ThermoScientific cat no. M1682), according to the manufacturer's instructions. qPCR was performed using iTaq SYBR green supermix (Bio-Rad cat. no. 172-5124) and run on an ABI 7900HT real-time PCR system with SDS2.4 software (Applied Biosystems). Primer set sequences, targets, amplicon size, and amplification efficiencies are listed in Supplemental Table 3. Standard curves were run with each plate and primer set to determine primer amplification efficiency and ensure sample Ct values were within the linear range of the assay. Melting curve analysis ensured the presence of a single amplification product of the expected melting temperature. Unless otherwise indicated, transcript levels were normalized to cellular GAPDH levels and expression levels relative to serum-stimulated or siScr knockdown controls were calculated with the comparative Ct method (2).

For selection of polyadenylated RNAs, 75ug of total RNA was used for polyA isolation using the PolyATract mRNA isolation IV kit (Promega cat. no. Z5310) according to the manufacturer's instructions prior to cDNA synthesis and quantitation by qPCR as described above. Equal amounts of total RNA or polyA-selected RNA were converted to cDNA and relative levels of histone

transcripts normalized to GAPDH in each sample were calculated using the comparative Ct method.

Northern blots. RNA (1 ug) was denatured using urea, run on a 5% TBE-urea gel (Bio-Rad cat. no. 3450086), electroblotted to a nylon membrane, and hybridized to 32P-labeled RNA probes complementary to histone RNAs. Equal loading and transfer was confirmed by methylene blue staining of membranes. Histone probes were derived from their entire open reading frames (H2AC18:NM_003516.3:53-445; H2BC11:NM_021058.4:46-426; H3C1: NM_003529.3:40-450; H4C14:NM_003548.2:29-340) which were synthesized as gene block fragments (Integrated DNA Technologies) and cloned into pCR4Blunt-TOPO (Thermo Fisher cat. no. K2875J10). RNA probes were in vitro transcribed by T3 or T7 polymerase (NEB cat. no. M0378S and M0251S) in the presence of [alpha-32P] UTP (Perkin Elmer cat. no. BLU507X250UC). Northern blots were imaged on a Typhoon 9200 imager (GE Healthcare Life Sciences).

siRNA transfection. – For knockdown experiments, serum starved fibroblasts were transfected with ON-TARGETplus siRNAs (Dharmacon) targeting SLBP (siSLBP#1: J-012286-07, CGGCUGACUUUGAGACAGA; siSLBP#2: J-012286-08, GACAGAAGCAGAUCAACUA) or a non-targeting control (siScr: D-001810-10) at 20 pmol of siRNA per one million cells by electroporation using an Amaxa Human Dermal Nucleofector Kit (Lonza cat. no. VPD-1001) according to the manufacturer's directions. Twenty-four hours post transfection, cells were infected with HCMV at the indicated MOI and harvested at the indicated time post infection for various readouts. For determining virus titer, supernatant and cells were collected and viral titers were determined by plaque assay on naïve fibroblasts.

Quantification of histones using mass spectrometry. Changes in histone levels were determined using stable isotope labeling by amino acids in cell culture (SILAC) and mass spectrometry. SILAC media was made using DMEM (ThermoFisher Scientific, Waltham, MA) for SILAC with 10% dialyzed FBS (Sigma-Aldrich, St. Louis, MO) and 1% streptomycin/penicillin

(ThermoFisher Scientific). The medium was supplemented with $^{13}C_6{}^{15}N_4$ L-arginine and $^{13}C_6{}^{15}N_2$-L-lysine or normal L-arginine and L-lysine (Sigma-Aldrich) to produce heavy (H) or light (L) SILAC medium, respectively. HFFs were propagated in H media for eight cell doublings with media replaced every 24 hr. Cells were cultured in H-media with 0.5% FBS for 24 hr to induce a G0/G1 arrest and then either infected with TB40/E at 3.0 IU/cell in prewarmed L medium or stimulated using 7% FBS in prewarmed L medium. Samples were collected at 24 hr post serum stimulation, 24 hpi and 72 hpi followed by histones isolation using histone purification kit (Active Motif, Carlsbad, CA). The resulting proteins were then precipitated by chloroform–methanol extraction, resuspended in 50 mM ammonium bicarbonate, reduced with 10 mM DTT for 30 min at 37°C, and alkylated with 50 mM iodoacetamide for 30 minutes at room temperature. Excess alkylating agent was removed by the addition of 10 mM DTT and samples were digested with Trypsin gold, MS grade (Promega, Madison, WI) at an enzyme-to-substrate ratio of 1:50 overnight at 37°C. Reactions were stopped by the addition of trifluoroacetic acid to a final concentration of 0.1% and desalted using Zip-Tip C18 columns (EMD Millipore Billerica, MA). For each sample, approximately 5 µg of tryptic peptides were analyzed using an LTQ-Orbitrap Velos hybrid mass spectrometer (ThermoScientific) as previously described (3) in technical triplicate for two independent biological experiments.

Protein identification and quantification were performed using MaxQuant 1.2.2.5 (61) and UniProtKB Homo sapiens reference proteome database containing 70,136 canonical and isoform sequences (retrieved 02/2013) through MaxQuant's built-in Andromeda search engine. HCMV reference proteome for strains TB40/E, FIX, AD169, and Toledo was added and used for HCMV peptide identification. Default parameters in MaxQuant were used wherever applicable. Briefly, variable modifications included Lys8 and Arg10, protein N-terminus acetylation, and oxidized methionine. Carbamidomethylated cysteine was the only fixed modification specified. Full trypsin specificity was selected as digestion mode and maximum missed cleavages were set to 2. Peptides with lengths of a minimum of seven amino acids were considered, with both the peptide and protein FDRs set to 1%. Precursor mass tolerance was set to 20 ppm for the first search and 4.5 ppm for the main search. Product ions were searched with a mass tolerance of 20 ppm. Protein

identification required a minimum of two peptides with at least one razor or unique peptide. Relative ratio quantification was performed using quantities of unique and razor peptides and required a minimum of two peptides. Identified proteins that could be reconstructed from a set of peptides are "grouped" and termed protein groups. The top matched or leading protein in a protein group is defined as the protein with the greatest number of identified peptides within the group.

Nuclear/cytoplasmic fractionation. For nuclear/cytoplasmic fractionation experiments, cells were resuspended in hypotonic buffer A (10mM HEPES pH 7.9, 10mM KCl, 1.5mM MgCl2, 1mM DTT, 0.2mM PMSF) supplemented with protease inhibitors and 20 U/ml RNasin (Promega cat. no. N2511) and allowed to swell on ice for 20 minutes prior to being lysed with 0.25% NP-40 on ice for 10 min. Nuclei (pellet) and cytoplasmic (supernatant) fractions were separated by centrifugation and nuclei were washed 3 times with hypotonic buffer A prior to being resuspended in extraction buffer (20mM HEPES, pH 7.9, 0.45M NaCl, 1.5mM MgCl2, 10mM DTT, 0.2mM PMSF, 0.2mM EDTA) supplemented with protease inhibitors and 20 U/ml RNasin. Cytoplasmic and nuclear fractions were then cleared by centrifugation. For RNA analysis, RNA was extracted from equal percentages (25%) of each fraction, converted to cDNA and quantitated by qPCR as described above. For protein analysis, equal percentages (15%) of each fraction were analyzed by western blot as described above along with the equivalent of 7.5% input of unfractionated cells lysed in 1% SDS lysis buffer.

Genomic DNA extraction and qPCR. For quantification of viral genome replication, total genomic DNA was extracted using an IBI Genomic DNA Mini Kit (IBI Scientific cat. no. IB47202) according to the manufacturer's directions. Equal amounts of total DNA were analyzed by qPCR using iTaq SYBR green supermix (Bio-Rad cat. no. 172-5124) and run on an ABI 7900HT real-time PCR system with SDS2.4 software (Applied Biosystems). Primer sequences, targets, and amplification efficiencies are listed in Supplemental Table 3. Standard curves were run with each plate and primer set to determine primer amplification efficiency and ensure sample Ct values were within the linear range of the assay. Melting curve analysis ensured the presence of a single amplification

product of the expected melting temperature. Relative levels of viral genomes (UL123 gene) normalized to cellular genomes (GAPDH) in each sample were calculated using the comparative Ct method.

For quantification of viral genomes contained in virions produced in siScr or siSLBP treated fibroblasts, equal volume or equal PFU of virus stocks were treated with micrococcal nuclease (New England Biolabs, cat no. M0247S) to remove nucleic acids outside of virus capsids. Virus stocks were then treated with DNAzol (Thermofisher, cat no. 10503027) to isolate capsid-contained viral DNA, according to manufacturer's instructions. Residual DNAzol in isolated DNA was removed by using a PCR clean-up kit (IBI Scientific, cat. no. IB47030) and resulting DNA was analyzed by qPCR as described above.

Data presentation and statistical analysis. Unless otherwise indicated, all bar graphs represent the mean ± SEM from at least three biological replicates. Blots and micrographs shown are representative images from at least three biological replicates. Statistical significance was calculated by two-tailed Student's t-test with *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ns: $p>0.1$.

Supplementary Figures and Tables

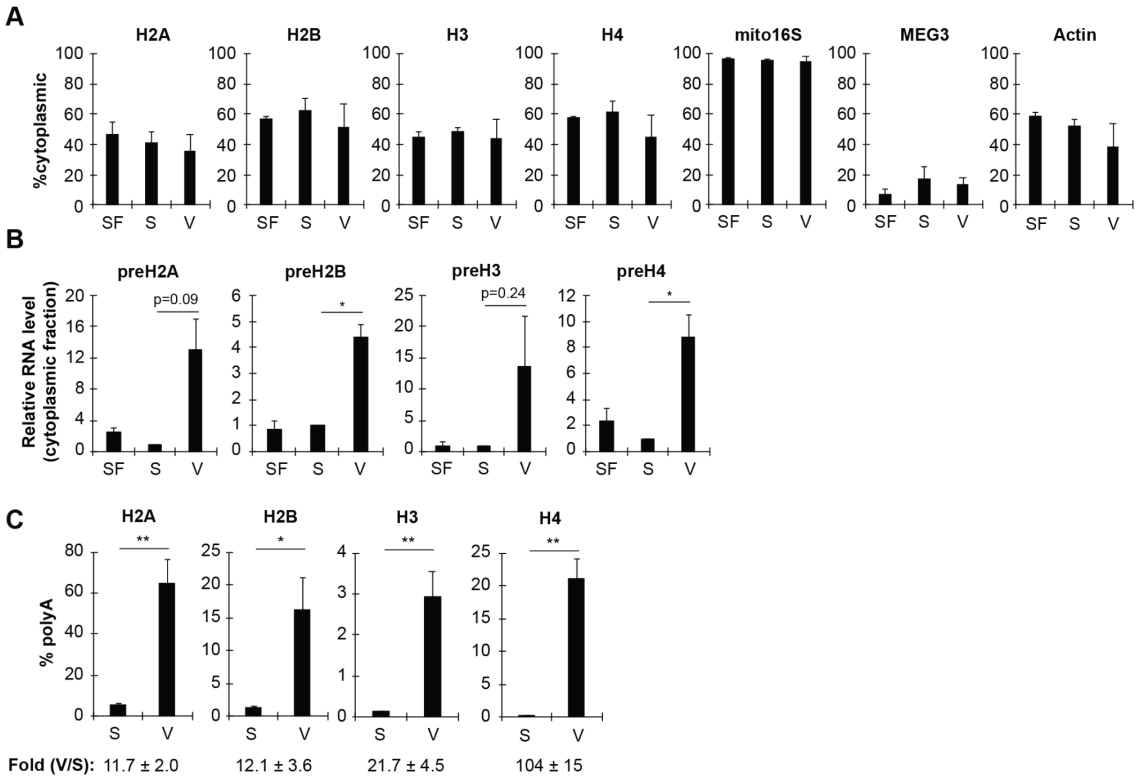

Figure S1. RD histone mRNAs are more likely to be unprocessed and poly-adenylated in HCMV infected cells as compared to S phase cells. A. Serum-starved fibroblasts were left untreated (SF), stimulated with serum (S), or infected with HCMV (V) for 24 hrs and then subjected to nuclear/cytoplasmic fractionation and RNA from equal percentages of each fraction was analyzed by RT-qPCR. The percent of each transcript detected in the cytoplasmic fraction is shown. Cellular mitochondrial 16S rRNA (mito16S) and the MEG3 nuclear long non-coding RNA serve as controls for cytoplasmic and nuclear fractions, respectively. **B.** RNA from cytoplasmic fractions of RNA from panel A was analyzed by RT-qPCR using primers for unprocessed precursor histone RNAs. Levels of the indicated precursor histone RNA were normalized to cellular Actin RNA and are shown relative to the serum-stimulated sample from the same experiment. **C.** Serum-starved fibroblasts were stimulated with serum for 24 hrs (S) or infected with HCMV (V) for 48 hrs. RNA was extracted and poly-adenylated RNA was isolated using poly(dT) beads. Total and polyA-selected RNA were analyzed by RT-qPCR and histone RNAs were normalized to cellular GAPDH in each sample. The ratio of polyadenylated to total transcripts (x100) for the indicated transcript is

shown relative to serum-stimulated cells from the same experiment. The mean fold difference between infected (V) and serum-stimulated (S) samples +/- SEM is indicated below the bar graphs. Bar graphs show the mean ± SEM from three biological replicates.

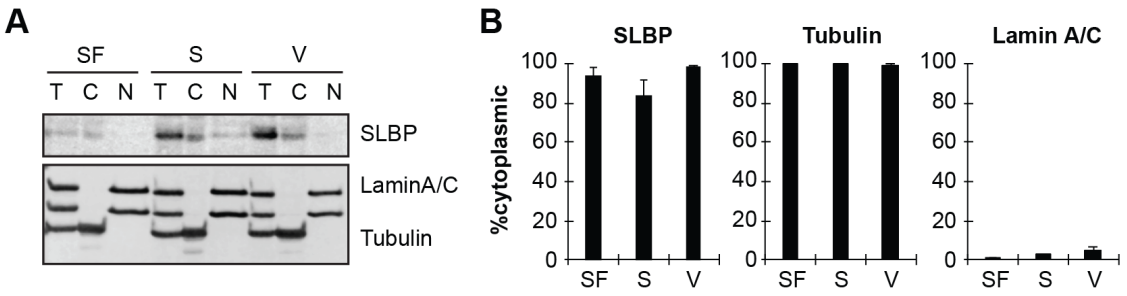

Figure S2. SLBP protein is cytoplasmic in HCMV-infected cells. Serum-starved fibroblasts were left untreated (SF) or were stimulated with serum (S) or infected with HCMV (V) for 24 hrs and then subjected to nuclear/cytoplasmic fractionation as in Fig. S1. **A.** Unfractionated (Total; T) or equal percentages of cytoplasmic (C) or nuclear (N) fractions were analyzed by western blot with the indicated antibodies. Tubulin and Lamin A/C serve as controls for cytoplasmic and nuclear fractions, respectively. **B.** Quantitation of protein levels in panel A. Bar graphs show the mean ± SEM from three biological replicates.

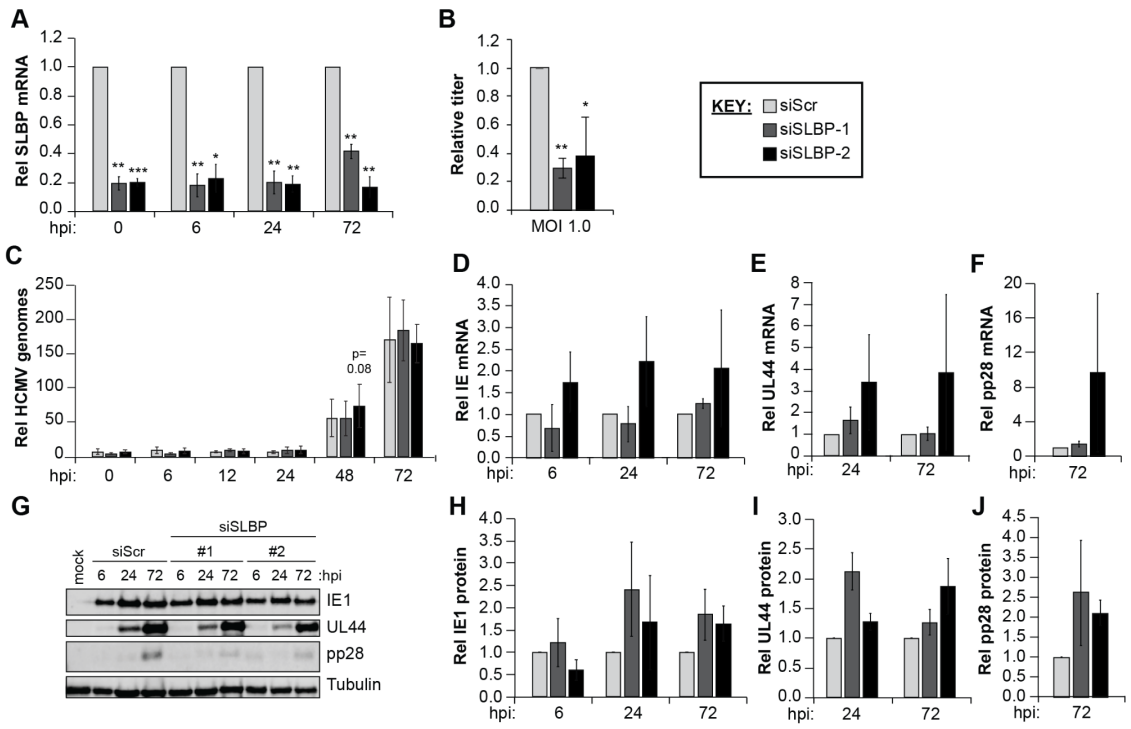

Figure S3. SLBP knockdown does not inhibit the accumulation of viral nucleic acids or proteins after an HCMV infection (MOI = 1.0). A. Serum-starved fibroblasts were transfected with one of two different siRNAs against SLBP (siSLBP) or a scrambled non-targeting control siRNA (siScr), then infected with HCMV strain AD169 at an MOI = 1.0. Infected cells were harvested at the indicated hours post infection (hpi) and SLBP transcript levels were quantified by RT-qPCR, normalized to GAPDH and plotted relative to siScr samples from the same experiment. **B.** siRNA transfected cells were infected with HCMV strain AD169 at an MOI = 1.0 for 6 days after which cell-free and cell associated progeny virus was collected and quantitated by plaque assay on naïve fibroblasts. Titers are shown relative to control siRNA transfected cells from the same experiment. **C.** Serum-starved fibroblasts were transfected, infected, and harvested at indicated time points (hpi) as in panel A. Genomic DNA was isolated and viral DNA was quantified by qPCR using primers for UL122/123. Viral genomes were quantified relative to cellular DNA (GAPDH). **D-F.** Serum-starved fibroblasts were treated as in panel A. Infected cells were harvested at indicated time points (hpi), and transcripts for IE1/2 (**D**), UL44 (**E**), and pp28 (**F**) were quantified by RT-qPCR, normalized to cellular GAPDH, and plotted relative to siScr samples from the same experiment. **G.** Serum-starved fibroblasts were treated as in panel A. Infected cells were harvested at indicated

time points (hpi), lysed in SDS lysis buffer and analyzed by Western blot with the indicated antibodies. Tubulin serves as a loading control. **H-J.** Quantitation of Western blots in panel G normalized to tubulin, and plotted relative to siScr samples from the same experiment. Bar graphs show the mean ± SEM from three biological replicates.

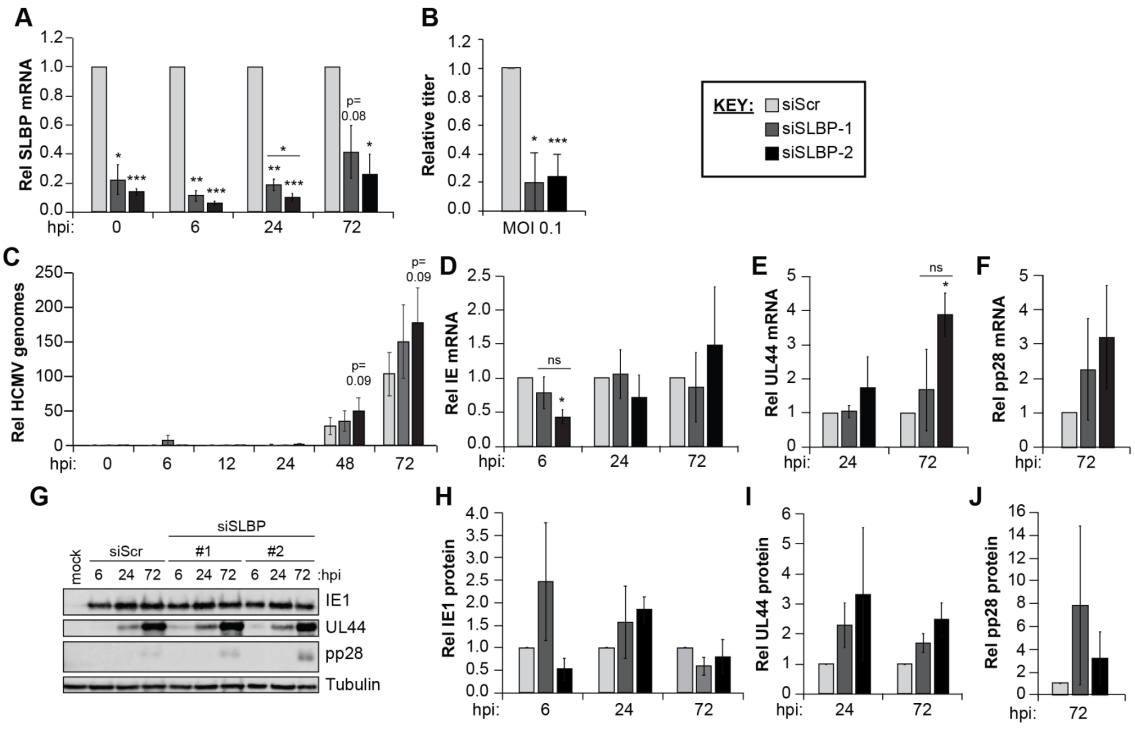

Figure S4. SLBP knockdown does not inhibit the accumulation of viral nucleic acids or proteins after an HCMV infection (MOI = 0.1). A. Serum-starved fibroblasts were transfected with one of two different siRNAs against SLBP (siSLBP) or a scrambled non-targeting control siRNA (siScr), then infected with HCMV strain AD169 at an MOI = 0.1. Infected cells were harvested at indicated hours post infection (hpi) and transcripts for SLBP were quantified by RT-qPCR, normalized to GAPDH levels, and plotted relative to siScr samples from the same experiment. **B.** siRNA transfected cells were infected with HCMV strain AD169 at an MOI = 0.1 for 6 days after which cell-free and cell associated progeny virus was collected and quantitated by plaque assay on naïve fibroblasts. Titers are shown relative to control siRNA transfected cells from the same experiment. **C.** Serum-starved fibroblasts were transfected, infected, and harvested at indicated time points (hpi) as in panel A. Genomic DNA was isolated and quantified by qPCR using primers for UL122/123. Viral genomes were quantified relative to cellular DNA (GAPDH). **D-F.** Serum-starved fibroblasts were treated as in panel A. Infected cells were harvested at indicated time points (hpi), and total RNA was isolated. Transcripts for, IE1/2 (**D**), UL44 (**E**), and pp28 (**F**) were quantified

by RT-qPCR, normalized to GAPDH levels, and plotted relative to siScr samples from the same experiment. **G.** Serum-starved fibroblasts were treated as in panel A. Infected cells were harvested at indicated time points (hpi), lysed in SDS lysis buffer and analyzed by Western blot with the indicated antibodies. Tubulin serving as a loading control. **H-J.** Quantitation of Western blot in panel G normalized to tubulin, and plotted relative to siScr samples from the same experiment. Bar graphs show the mean ± SEM from three biological replicates.

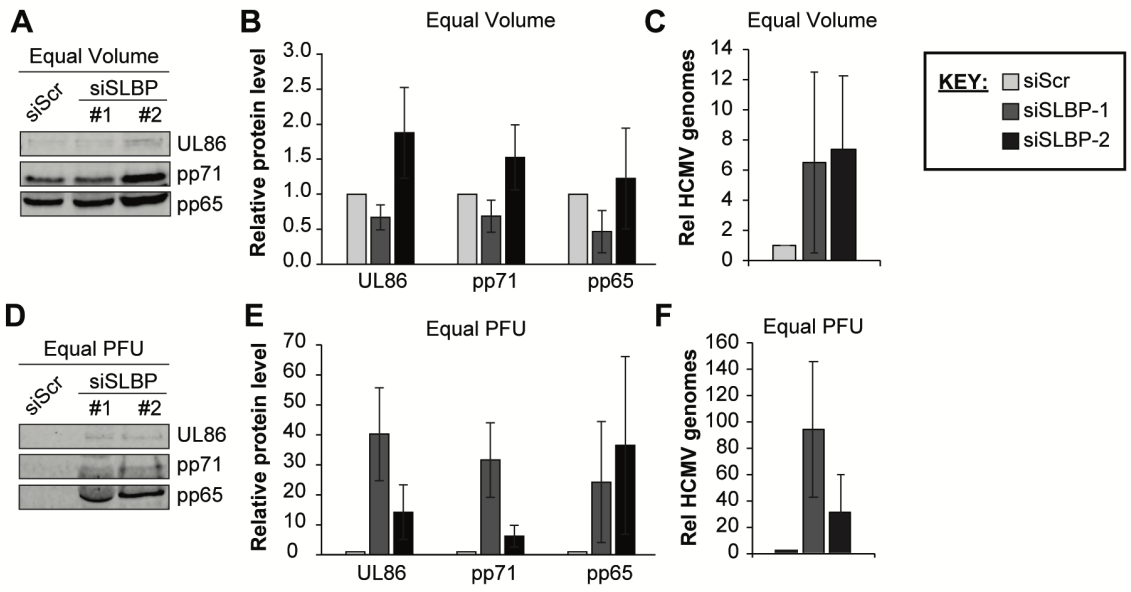

Figure S5. SLBP is required for the efficient production of infectious HCMV virions at MOI = 0.1. A. Serum-starved fibroblasts were transfected with one of two different siRNAs against SLBP (siSLBP) or a scrambled non-targeting control siRNA (siScr). siRNA transfected cells were infected with HCMV strain AD169 at an MOI = 0.1 for 6 days after which cell-free and cell associated progeny virus was collected and quantitated by plaque assay on naïve fibroblasts. Equal volume of collected virus from each condition was analyzed for virion proteins by Western blot with the indicated antibodies. **B.** Quantitation of Western blot in panel A, plotted relative to siScr samples from the same experiment. **C.** Genomic DNA was isolated from equal volumes of virus derived from experiments described in panel A and quantified by qPCR for UL122/123. Results are plotted relative to siScr samples from the same experiment. **D.** Based on the determined titers of virus collected in panel A, equal plaque forming units (PFU) of each virus stock was analyzed for virion proteins by Western blot with the indicated antibodies. **E.** Quantitation of Western blot in panel D, plotted relative to siScr samples from the same experiment. **F.** Genomic DNA was isolated from equal PFU of virus derived from experiments described in panel A, and quantified by qPCR for UL122/123. Results are plotted relative to siScr samples from the same experiment. Bar graphs show the mean ± SEM from three biological replicates.

Supplementary Table 1. SILAC qantification of protein changes between HCMV infection and serum stimulation.

Lowest (H/L Serum) / (H/L HCMV)								Highest (H/L Serum) / (H/L HCMV)							
Names	UniProt	24 hpi			72 hpi			Names	UniProt	24 hpi			72 hpi		
H1.3	P16402	0.161	±	0.006	0.224	±	0.008	HSPA1	P08107	9.330	±	1.028	8.333	±	0.756
H1.5	P16401	0.228	±	0.009	0.150	±	0.009	MDU1	J3KPF3	3.062			9.671		
H1.2	P16403	0.285	±	0.012	0.645	±	0.074	GRP75	P38646	2.370	±	1.166	3.254	±	1.602
H2A (2-A)	Q6FI13	0.309			0.237			ATP6E	P36543	2.273	±	0.135	2.282	±	0.136
H4	P62805	0.316	±	0.014	0.183	±	0.006	ATP6A1	P38606	2.152	±	0.074	2.984	±	0.102
H2B (1-M)	Q99879	0.316			0.232			ATP6B2	P21281	2.120	±	0.022	4.482	±	0.045
H3.1	P68431	0.347	±	0.011	0.221	±	0.008	HSC70	P11142	1.990	±	0.066	3.725	±	0.165
H2A (1-B/E)	P04908	0.365	±	0.020	0.253	±	0.016	DDX5	J3KTA4	1.961	±	0.755	3.293	±	1.303
H1.4	P10412	0.384	±	0.001	0.580	±	0.000	HSP90AB1	P08238	1.934	±	0.109	3.021	±	0.177
TPM4	P67936	0.561	±	0.154	1.091	±	0.339	PAGA	Q06830	1.881	±	0.010	2.505	±	0.021
ABPL	Q14315	0.563	±	0.044	0.864	±	0.067	ERBA2L	P07237	1.821	±	0.178	2.043	±	0.200
NEXN	Q0ZGT2	0.567	±	0.053	0.381	±	0.036	GLIF	P14174	1.803			1.824		
VCL	P18206	0.603			0.709			SRP14	P37108	1.800	±	0.158	1.180	±	0.104
LMN2	P20700	0.622	±	0.008	0.653	±	0.010	C22orf28	Q9Y3I0	1.783			2.734		
AHNAK	Q09666	0.635	±	0.005	1.085	±	0.008	C21orf112	P50990	1.756	±	0.138	3.626	±	0.284
VIM	P08670	0.638	±	0.014	0.448	±	0.008	PAB1	P11940	1.739	±	0.090	2.470	±	0.159
CORO1C	A7MAP1	0.648	±	0.022	0.808	±	0.031	LRRC59	Q96AG4	1.706	±	0.058	2.058	±	0.070
ACTB	P63261	0.667	±	0.020	0.496	±	0.016	GRP78	P11021	1.662	±	0.004	2.572	±	0.006
CD44	P16070	0.686	±	0.077	0.980	±	0.110	COX5A	P20674	1.643	±	0.120	2.666	±	0.194
LMN2	J9JID7	0.716	±	0.069	0.605	±	0.055	BAT1	F8VQ10	1.631	±	0.069	2.131	±	0.097
ACTN1	P12814	0.729			0.600			CLIM1	O00151	1.600	±	0.051	2.346	±	0.014
FKSG13	Q6NZI2	0.742	±	0.044	0.560	±	0.036	DDX48	P38919	1.592			2.440		
KIAA1027	Q9Y490	0.754	±	0.058	1.213	±	0.094	PSMC6	P62333	1.586			3.774		
PLEC1	Q15149	0.764	±	0.006	1.141	±	0.001	HSP90A	P07900	1.583			1.921		
INO80H	Q9Y265	0.765	±	0.055	1.170	±	0.084	HSP60	P10809	1.578	±	0.163	2.595	±	0.236
IGF2BP2	F8W930	0.768			2.164			PCBP2	Q15366	1.572	±	0.002	2.412	±	0.155
CBX5	P45973	0.774	±	0.045	3.158	±	0.182	UCHL1	P09936	1.552			3.022		
ANX2	P07355	0.784	±	0.014	0.989	±	0.017	CPSD	P07339	1.547	±	0.140	2.243	±	1.793
LDHA	P00338	0.785	±	0.042	0.633	±	0.006	CATX11	O76021	1.543	±	0.092	2.930	±	0.175
MSN	P26038	0.789	±	0.021	1.052	±	0.002	GNAS	Q5JWF2	1.531			3.986		
KPNA2	P52292	0.791	±	0.017	0.530	±	0.011	HCC1	P82979	1.522	±	0.076	2.046	±	0.069
ACTB	P60709	0.797	±	0.063	0.750	±	0.059	HNRNPC	P07910	1.499	±	0.021	2.458	±	0.061
MYH9	P35579	0.798	±	0.033	0.758	±	0.035	NCL	P19338	1.486	±	0.061	2.361	±	0.110
GNB2	P62879	0.804	±	0.128	2.677	±	0.255	C1orf77	Q9Y3Y2	1.478			2.189		
ARPC4	C9JWM7	0.808	±	0.074	0.771	±	0.070	DDX21	Q9NR30	1.477	±	0.140	1.935	±	0.184
CFL2	Q9Y281	0.811	±	0.046	1.466	±	0.082	FAEES3	P09211	1.475			1.691		
MYO1C	O00159	0.813	±	0.001	1.418	±	0.001	ERH	P84090	1.468	±	0.039	2.091	±	0.056
EEF1D	E9PRY8	0.814			0.977			PCBP1	Q15365	1.459	±	0.046	2.472	±	0.163
KPNB1	Q14974	0.814	±	0.048	1.596	±	0.094	MIG5	P63000	1.449	±	0.024	1.870	±	0.040
ANX1	P04083	0.816	±	0.057	1.000	±	0.093	C12orf8	P30040	1.449	±	0.005	2.917	±	0.010
ANPEP	P15144	0.816	±	0.143	0.644	±	0.099	CRMP2	Q16555	1.448			0.975		
EBP1	Q9UQ80	0.827			1.395			MDH1	F5H098	1.435			2.390		
FLN	P21333	0.831	±	0.001	0.539	±	0.000	CYPB	P23284	1.427	±	0.047	1.661	±	0.021
RPS11	P62280	0.834	±	0.004	1.181	±	0.009	PGAM1	P18669	1.424			1.396		
EHD2	Q9NZN4	0.852	±	0.130	1.467	±	0.220	TKT	E7EPA7	1.420	±	0.064	2.428	±	0.165
ALDA	P04075	0.853	±	0.223	1.414	±	0.289	THRAP3	Q9Y2W1	1.388			3.229		
OK/SW-cl.83	P62241	0.866	±	0.002	0.650	±	0.031	ATP1A1	F5H3A1	1.383			2.902		
Nbla00170	P48681	0.866			0.921			PSMC3	P17980	1.374			2.871		
LAMBR	P08865	0.877	±	0.036	0.863	±	0.036	NHP2L1	B1AHD1	1.372			1.394		
HSP27	P04792	0.879	±	0.012	1.087	±	0.014	TPI	P60174	1.355	±	0.050	1.513	±	0.055
PRKCDBP	E9PIE3	0.884	±	0.062	0.502	±	0.035	H1x	Q92522	1.349	±	0.060	2.790	±	0.262
								H1.0	P07305	1.238	±	0.213	2.748	±	0.472
								macro-H2A.1	O75367	1.271	±	0.046	1.723	±	0.054
								H2A.Z	P0C0S5	1.241	±	0.057	0.506	±	0.132

Supplemental Table 2: Antibodies used in this study

Antibody	Source	Identifier	Use
anti-GAPDH (clone 6C5)	Invitrogen	Cat#AM4300	WB
anti-Tubulin (clone DM1A)	Sigma	Cat#T9026	WB
anti-LaminA/C (clone 636)	Santa Cruz Biotechnology	Cat#sc-7292	WB
anti-p107 (clone C-18)	Santa Cruz Biotechnology	Cat#sc-318	WB
anti-UL44 (clone 10D8)	Virusys	Cat#CA006-100	WB, IF
anti-IE1 (clone 1B12)	Ref. (4)	N/A	WB
anti-pp71 (clone 2H10-9)	Ref. (5)	N/A	WB
anti-pp65 (clone 8F5)	Ref. (6)	N/A	WB
anti-pp28 (clone CMV-157)	Ref (6)	N/A	WB
anti-UL86 (clone 28-4-C11)	William Britt (UAB) Ref (7)	N/A	WB
anti-SLBP	William Marzluff (UNC) Ref (8)	N/A	WB
anti-SLBP	Bethyl Laboratories	Cat#A303-968A	IF, WB
IRDye680RD goat anti-mouse IgG secondary antibody	LICOR	Cat#925-68070	WB
IRDye800CW goat anti-rabbit IgG secondary antibody	LICOR	Cat#925-32211	WB
Alexa Fluor 594 goat anti-mouse IgG secondary antibody	Invitrogen	Cat#A-11005	IF
Alexa Fluor 488 goat anti-rabbit IgG secondary antibody	Invitrogen	Cat#A-11008	IF

WB: western blot; IF: indirect immunofluorescence; N/A: not applicable

Supplemental Table 3: Oligonucleotides used in this study

qPCR primers	Sequence (5' to 3')	Target	Amplicon size	Efficiency (%)
H2A coding For	GTC CTC GAG TAT CTG ACC GC	H2A coding (H2AC18, H2AC19)	92 bp	96.4 ± 2.2
H2A coding Rev	TGG AGG TGA CGA GGG ATG AT			
H2B coding For	GAA TAG CTC TCC TTG CGG CT	H2B coding (H2BC11)	87 bp	100.4 ± 1.2
H2B coding Rev	CCC GAA AAA GGG CTC CAA GA			
H3 coding For	GGC TCG CAC TAA GCA AAC TG	H3 coding (H3C1)	74 bp	99.4 ± 3.1
H3 coding Rev	CTG CCT TAG TGG CCA ACT GT			
H4 coding For	GGC GGA AAA GGC TTA GGC AA	H4 coding (H4C14, H4C15)	128 bp	97.7 ± 4.0
H4 coding Rev	CCA GAG ATC CGC TTA ACG CC			
H1.0 coding For	GAG CGA CGA ACC CAA GAA GT	H1.0 coding (H1-0)	74 bp	104.8 ± 4.7
H1.0 coding Rev	TCT TTG GCG TGG CTA CCT TC			
mH2A coding For	AAA GAT GAC CTA GGA AAC ACG CT	mH2A coding (MACROH2A1)	185 bp	99.3 ± 0.6
mH2A coding Rev	TCA CAC TTG TCT GCA CCC CAA A			
preH2A For	GGC GTC TTG CCT AAC ATC CA	H2A precursor (H2AC18, H2AC19)	197 bp	94.6 ± 4.1
preH2A Rev	TGG CCA GCA TTA ACA ACT CT			

preH2B For	AGG AGG AAT ACA AGC ACC AGC	H2B precursor (H2BC11)	140 bp	102.7 ± 1.8
preH2B Rev	CGT GTC CGA GGG TAC TAA GG			
preH3 For	CAA GCG CGT CAC TAT CAT GC	H3 precursor (H3C1)	148 bp	94.6 ± 1.9
preH3 Rev	GGT TTC TTA CAG CTA CTT TAC TTG A			
preH4 For	GAT GTG GTG TAC GCG CTC AA	H4 precursor (H4C14, H4C15)	148 bp	98.3 ± 3.6
preH4 Rev	ACG CAG TCA AGT GTC CAA CT			
SLBP For	GCG CAG ACC CGA GAG ATA TAA A	SLBP	113 bp	89.8 ± 6.5
SLBP Rev	CAG CCG GCA CAG TAG ACA TA			
Viral IE For	CGA CGT TCC TGC AGA CTA TG	HCMV UL122/123	118 bp	110.6 ± 5.9
Viral IE Rev	TCC TCG GTC ACT TGT TCA AA			
UL44 For	AGC CGC ACT TTT GCT TCT TG	HCMV UL44	104 bp	101.9 ± 5.7
UL44 Rev	TCG CAA CTC CGG CAA TTA CT			
pp28 For	GAC GGC TCC AAG AAA AAC GC	HCMV UL99	150 bp	100.7 ± 11.2
pp28 Rev	TTC GTC TAG GTC GTC CGT CT			
GAPDH For	GAG CCA AAA GGG TCA TC	GAPDH	187 bp	104.5 ± 2.9
GAPDH Rev	GTG GTC ATG AGT CCT TC			
MEG3 For	CTG CCC ATC TAC ACC TCA CG	MEG3	105 bp	98.6 ± 3.6

MEG3 Rev	ATC CTT TGC CAT CCT GGT CC			
Actin For	CGG AAC CGC TCA TTG CC	ACTB	290 bp	95.3 ± 1.2
Actin Rev	ACC CAC ACT GTG CCC ATC TA			
mito16S For	CCG CAA GGG AAA GAT GAA AAA T	mito 16S rRNA	133 bp	97.4 ± 4.0
mito16S Rev	TCG TCT GGT TTC GGG GGT CT			

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