

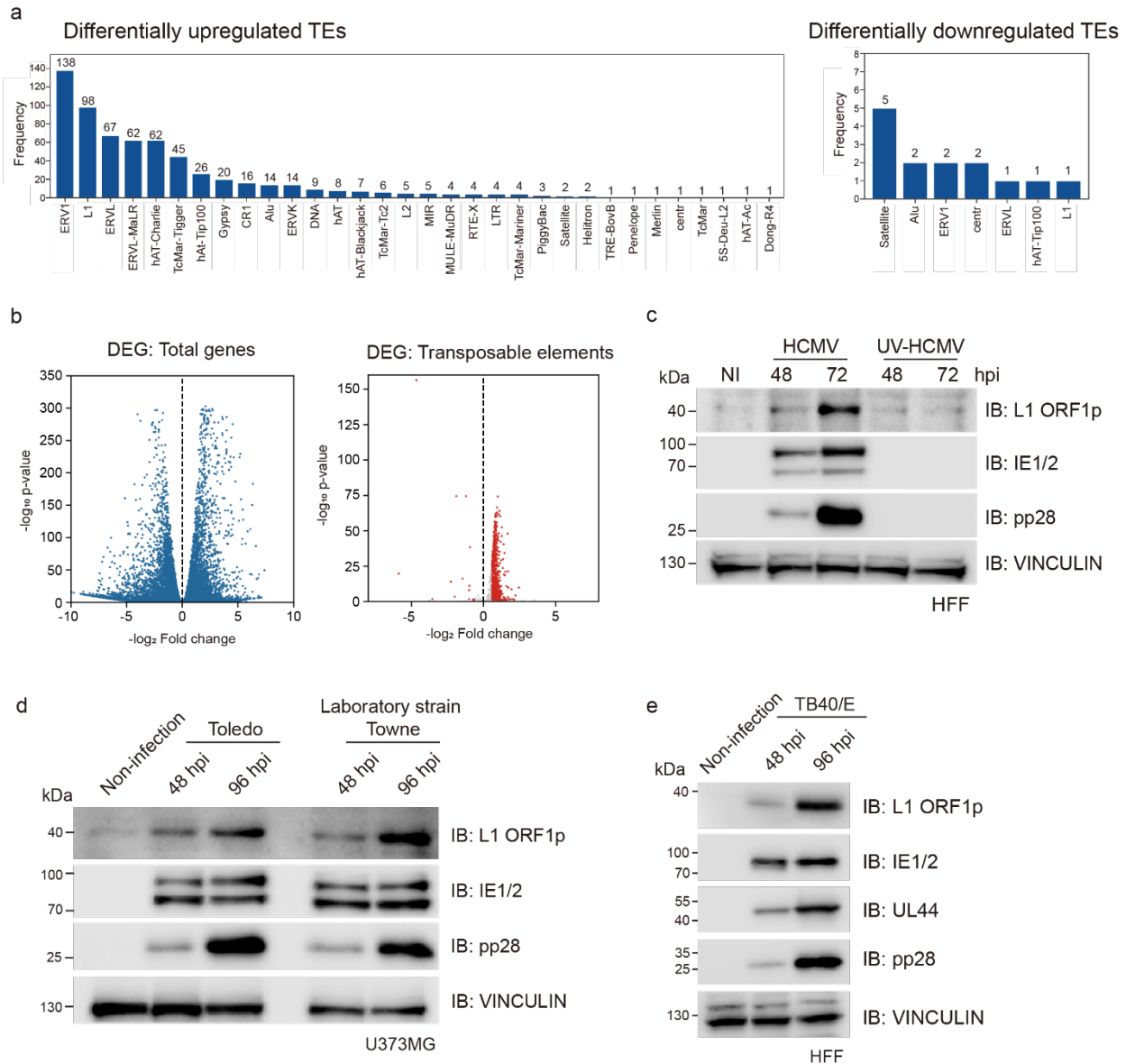
Supplementary Information for

Human cytomegalovirus harnesses host L1 retrotransposon for efficient replication

Sung-Yeon Hwang, Hyewon Kim, Danielle Denisko, Boxun Zhao, Dohoon Lee, Jiseok Jeong, Jinuk Kim, Kiwon Park, Junhyun Park, Dongjoon Jeong, Sehong Park, Hee-Jung Choi, Sun Kim, Eunjung Alice Lee, Kwangseog Ahn

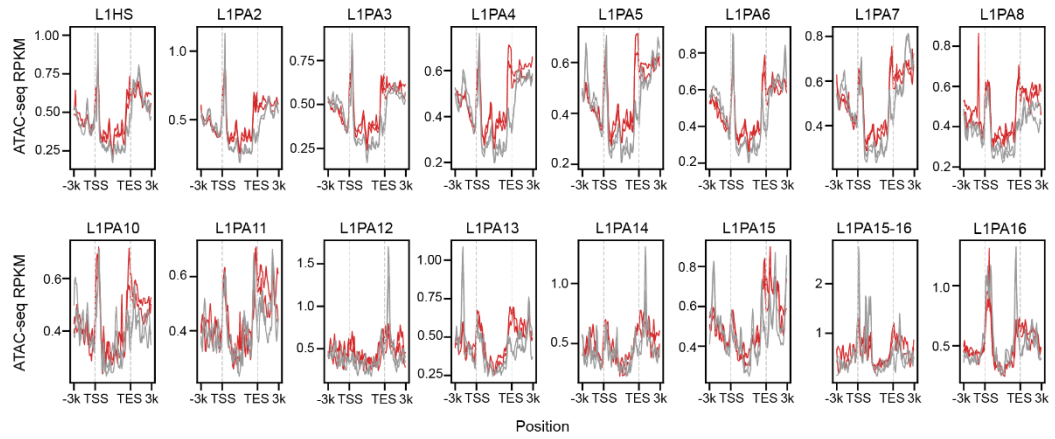
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Supplementary Figure 1. HCMV induces L1 retrotransposon expression (related to Figure 1). (a) Differentially expressed transposable elements (TEs) in HCMV-infected fibroblasts. Frequency of upregulated human TE subfamilies (left) and downregulated TE subfamilies (right). (b) Volcano plot showing differentially expressed genes of the total gene (blue) and TEs (red). The dataset of GSE120890¹ was analyzed for (a) and (b). (c) Immunoblot assays showing that HCMV promotes L1 expression in HFF. Vinculin is the loading control. (d) Immunoblot assays showing the L1-inducing capacity of HCMV Toledo strain and laboratory Towne strain. U373MG were infected with HCMV Toledo or laboratory Towne strain and subjected to immunoblot assay. (e) Immunoblot assays of HCMV clinical strain TB40/E-infected HFFs. (c-e) Representative blot of n=2 independent replicates.

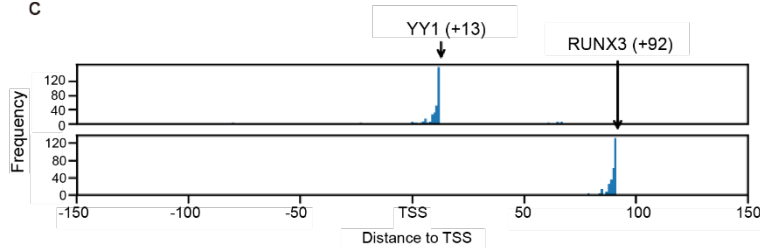
a — Non infection — HCMV infection



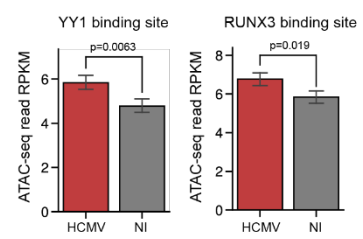
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Rank	Motif	P-value	log P-value	% of Targets	% of Background	STD(Bg STD)	Best Match/Details
1	CGTGAGCGACGC	1e-1068	-2.46E+03	85.24%	0.01%	11.4bp (0.0bp)	z/dmmpmm(Bigfoot)/fly(0.609)
2	CTGCATTTCCAT	1e-939	-2.16E+03	93.37%	0.07%	15.2bp (78.1bp)	CST6(MacIsaac)/Yeast(0.758)
3	ATCTGAGGTA	1e-858	-1.98E+03	90.96%	0.11%	12.6bp (102.1bp)	RUNX3/MA0684.2/Jaspar(0.687)
4	TGGCGAATAGG	1e-825	-1.90E+03	93.98%	0.18%	27.8bp (51.1bp)	br-Z2/dmmpmm(Bigfoot)/fly(0.627)
5	CGGTCTACAGCT	1e-802	-1.85E+03	88.86%	0.13%	9.9bp (52.1bp)	bZIP52(bZIP)/colamp-bZIP52-DAP-Seq(GSE60143)/Homer(0.647)
6	GATTTCTGCA	1e-794	-1.83E+03	93.37%	0.21%	14.4bp (74.5bp)	PB0145.1_MafB_2/Jaspar(0.732)
7	GATGGATCSAAAT	1e-792	-1.83E+03	93.07%	0.21%	9.6bp (78.1bp)	MAC1(MacIsaac)/Yeast(0.658)
8	TAGGAACAGC	1e-783	-1.80E+03	93.37%	0.23%	11.1bp (89.1bp)	ZNF341(Zf)/EBV-ZNF341-ChIP-Seq(GSE113194)/Homer(0.853)
9	CCCAGCGTGAGG	1e-776	-1.79E+03	90.66%	0.19%	20.8bp (89.1bp)	PB0206.1_Zic2_2/Jaspar(0.711)
10	TTCCATCTGA	1e-758	-1.75E+03	95.48%	0.34%	37.7bp (76.7bp)	NEUROG2(var.2)/MA1642.1/Jaspar(0.744)
11	CTCCGTCTACA	1e-738	-1.70E+03	93.07%	0.31%	18.8bp (94.0bp)	ZSCAN29/MA1602.1/Jaspar(0.683)
12	GATGGGTGATT	1e-631	-1.45E+03	76.20%	0.16%	19.8bp (120.6bp)	PH0037.1_Hdx/Jaspar(0.660)
13	CCAGCCAA	1e-557	-1.28E+03	86.75%	0.75%	9.8bp (67.2bp)	NFIX/MA0671.1/Jaspar(0.762)
14	AAGATGGC	1e-486	-1.12E+03	93.37%	2.09%	37.6bp (79.9bp)	YY1/MA0095.2/Jaspar(0.888)

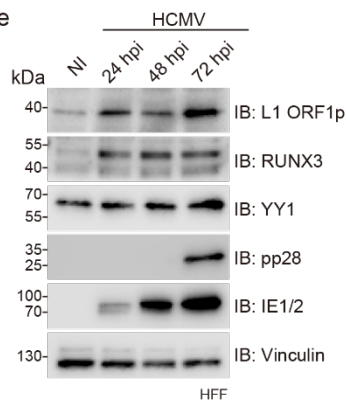
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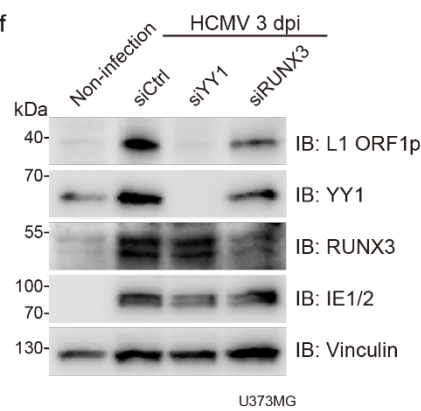
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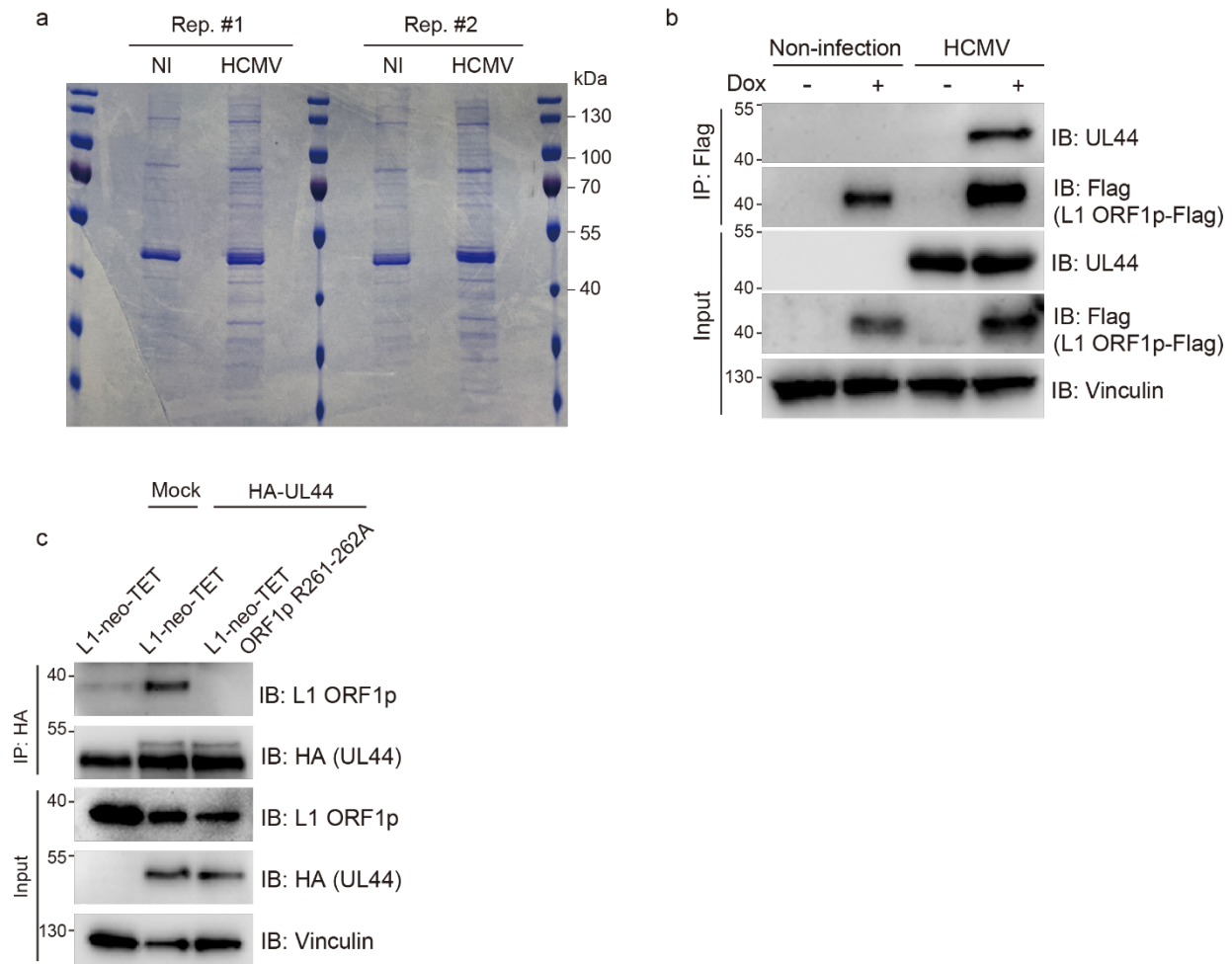
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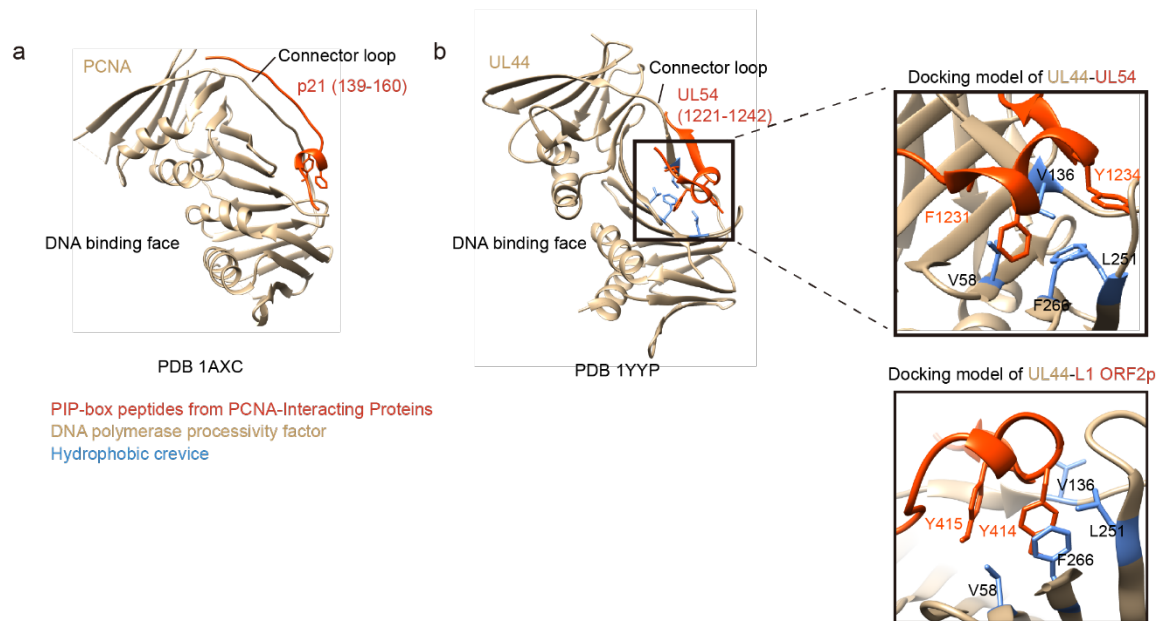
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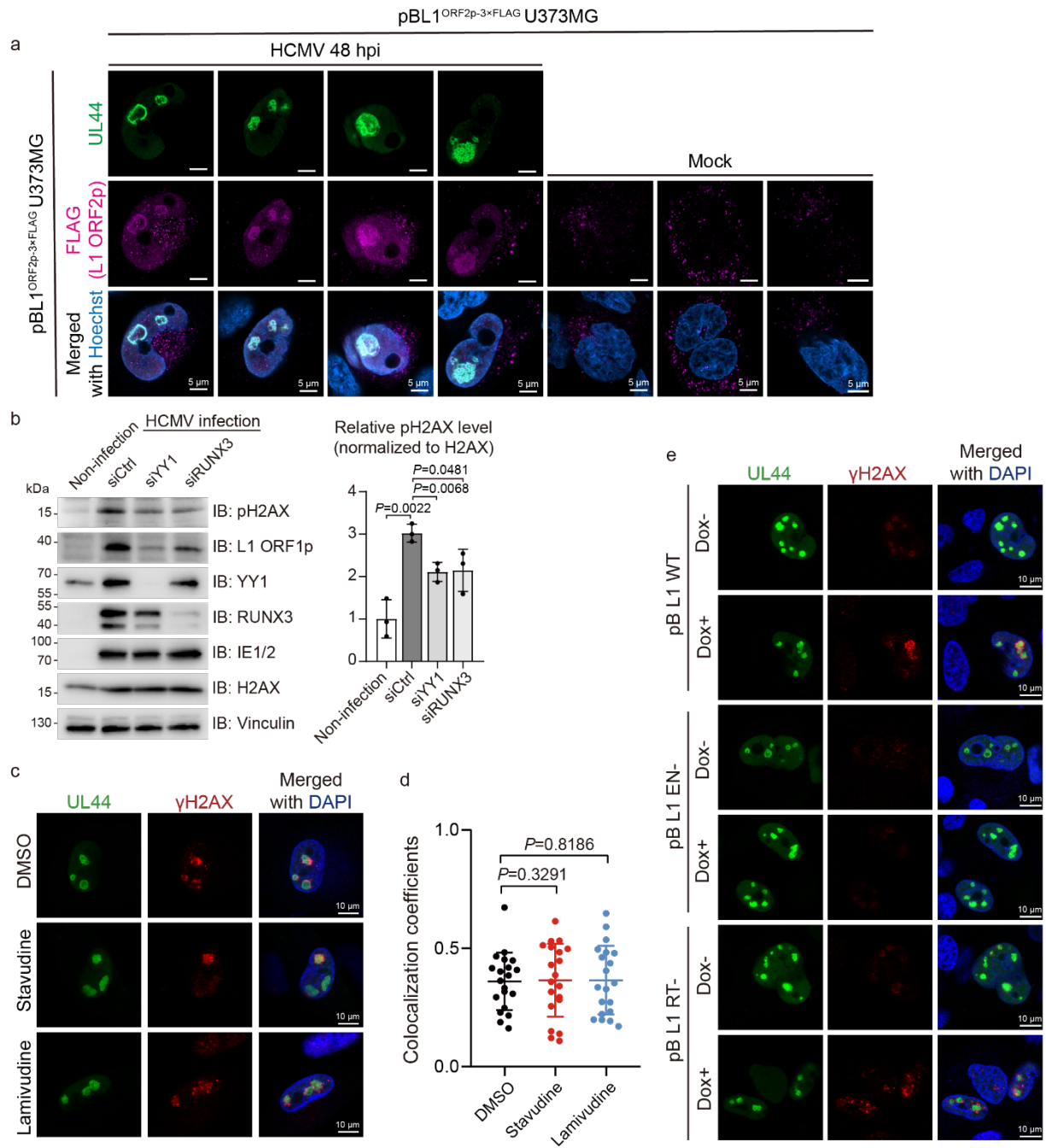
Supplementary Figure 2. HCMV enhances chromatin accessibility of evolutionarily young L1s via upregulation of YY1 and RUNX3 (related to Figure 1). (a) Map of ATAC-seq signals in L1 subfamilies according to evolutionary age. Read coverage is normalized to the total number of reads mapped to the genomic regions of L1. The plot presents HCMV infection in red and non-infection in gray. TSS; transcription start site, TES; transcription end site. (b) L1Hs promoter motif enrichment analysis using HOMER. Full-length L1Hs sequence (> 5kb) was sorted for this assay. Red boxes indicate YY1 and RUNX3 binding motifs. The nucleotide height indicates the frequency of the corresponding nucleotide. (c) Map of YY1- or RUNX3-consensus motif in L1H promoter region. (d) The levels of ATAC-seq reads mapped on YY1 or RUNX3 binding sites of L1 TSS. (e) Immunoblot assays showing expressions of YY1 and RUNX3 in HCMV-infected HFF. Representative blot of n = 2 independent replicates. (f) Immunoblot assays showing the effect of YY1 and RUNX3 knockdown in HCMV-infected U373MG. Representative blot of n = 2 independent replicates.



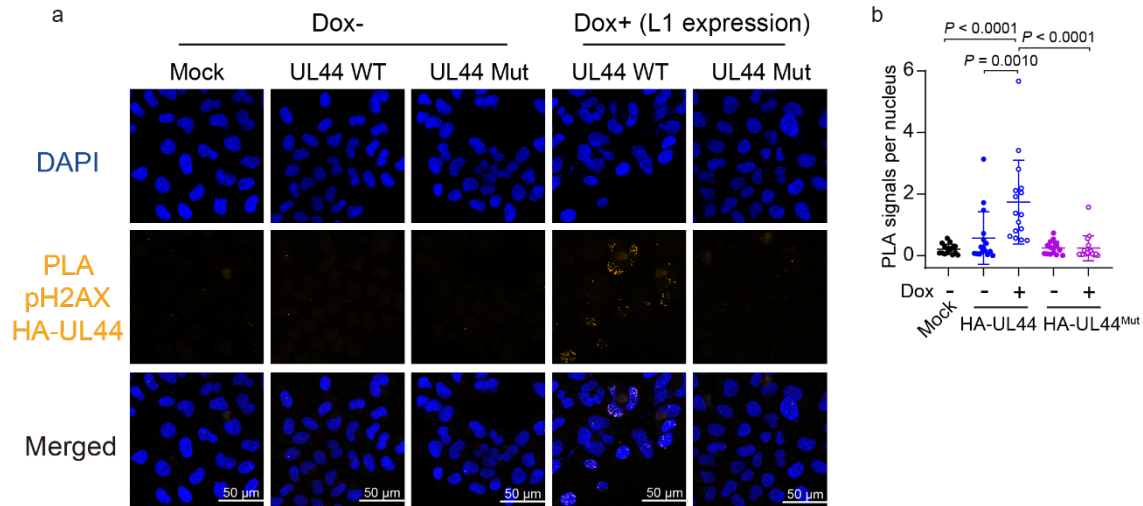
Supplementary Figure 3. HCMV UL44 interacts with L1 RNP (related to Figure 3). (a) SDS-PAGE of L1 RNP immunoprecipitation eluates. pBL1^{ORF1p-FLAG} U373MG were infected with HCMV and treated with 50 ng/mL dox. Immunoprecipitation was performed as in Fig. 5a. The gel was stained with Coomassie blue, and emerged protein bands were cut and further analyzed by LC/MS. (b) Co-IP assays using HCMV-infected pBL1^{ORF1p-FLAG} U373MG. Dox treatment and HCMV infection are indicated. Representative blot of n = 2 independent replicates. (c) Co-IP assays showing RNA-binding dependent interaction between L1 ORF1p and UL44. HeLa cells were transfected with L1-neo-TET and/or HA-UL44. RNA binding defective L1 ORF1p mutations (R261-262A) are indicated. Representative blot of n = 2 independent replicates.



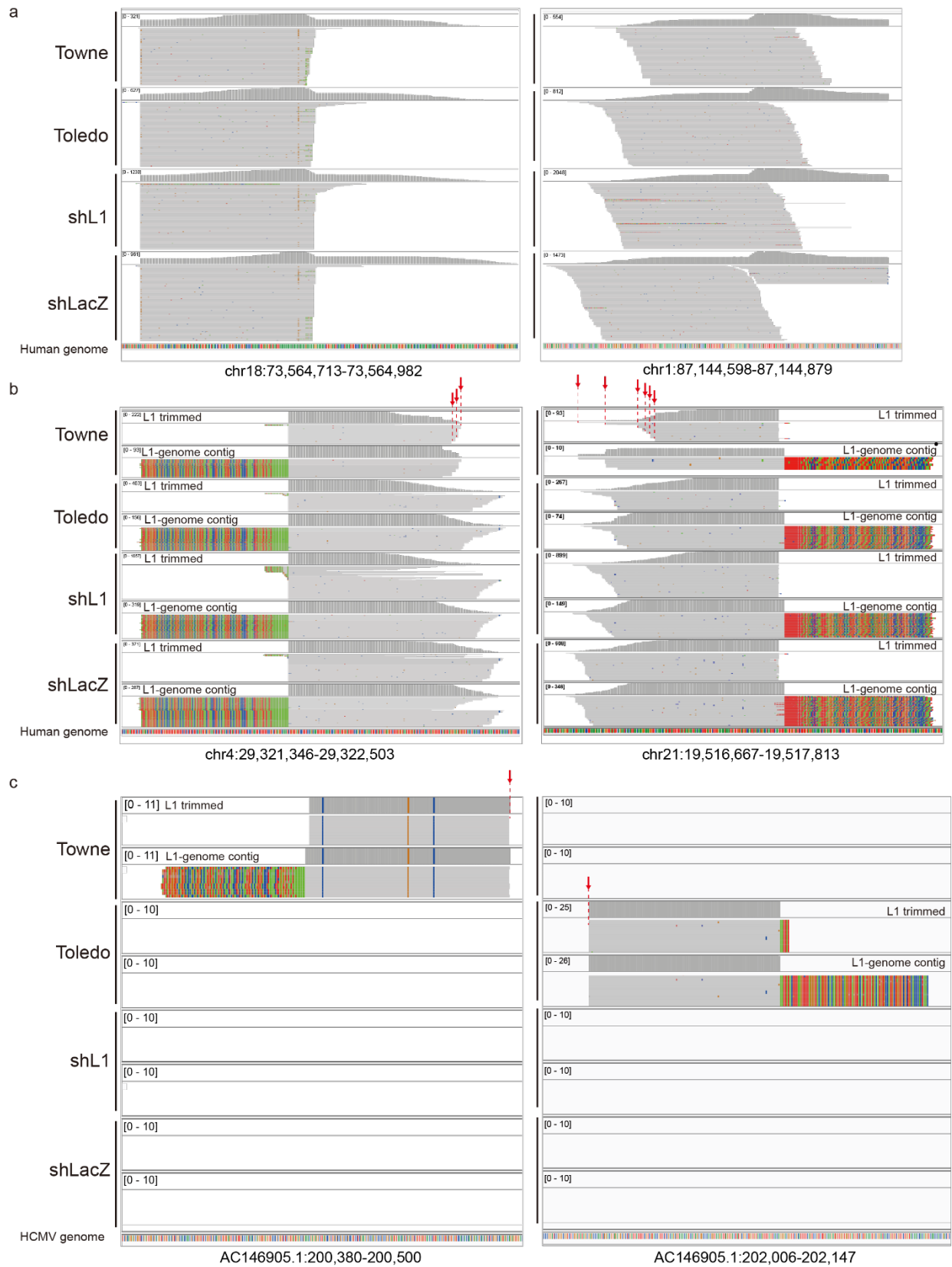
Supplementary Figure 4. Structural similarity between HCMV UL44 and PCNA (related to Figure 3). (a) Ribbon diagram of PCNA (gold) and its PIP-box peptide of p21 (139–160, red)². (b) Ribbon diagram of UL44 (gold) with UL54 C-terminal peptide (1221–1242, red)^{3,4}. UL44 hydrophobic crevice composed of Val⁵⁸, Val¹³⁶, Leu²⁵¹, and Phe²⁶⁶ (light blue) and UL54 hydrophobic plug peptide (red) are shown in the upper panel. For comparison, the model of the UL44 hydrophobic crevice and L1 ORF2p docking plug are presented in the lower panel.



Supplementary Figure 5. HCMV UL44 interacts with L1 proteins and recruits ORF2p to the replication complex (related to Figure 4). (a) Representative images (n = 2 independent replicates) of immunofluorescence assay using non-infected or HCMV-infected (48 hpi) pBL1^{ORF2p-3×FLAG} U373MG. At 24 hpi, the cells were treated with 50 ng/mL dox before fixation. UL44 (green), L1 ORF2p-3×FLAG (magenta), and merged with Hoechst (blue) images are shown. The scale bar represents 5 μ m. (b) Immunoblot assay of HCMV-infected HFFs treated with indicated siRNAs. Representative blot of n = 3 independent replicates (left). pH2AX intensities were quantified with normalization to H2AX level (right). Mean $\pm$ SD of n = 3 independent samples. Two-sided student's *t*-test. (c-d) Immunofluorescence assay assessing reverse transcriptase (RT) inhibitor effects in HCMV-induced DNA damage. HFFs were infected with HCMV and treated with indicated RT inhibitors (Stavudin 5 μ M and Lamivudine 50 μ M). At 48 hpi, cells were fixed and stained. UL44 (green), pH2AX (red), and merged with DAPI (blue) images are shown. The scale bar represents 10 μ m. (c) Representative images from n=2 independent replicates. (d) Colocalization coefficients represent the number of UL44 and pH2AX colocalized pixels. Dots with mean (the horizontal line) $\pm$ SD indicate the values from 20 cells per sample from 2 independent experiments. Two-sided Kolmogorov–Smirnov test. (e) Immunofluorescence assay using dox inducible L1 expressing U373MG (pBL1 U373MG) at 48 hpi. Mutations in L1 ORF2 and dox treatment are indicated. Images for UL44 (green), pH2AX (red), and DAPI (blue) are indicated. The scale bar represents 10 μ m.

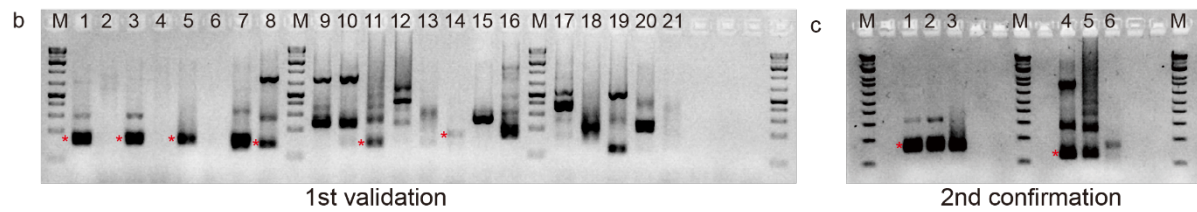
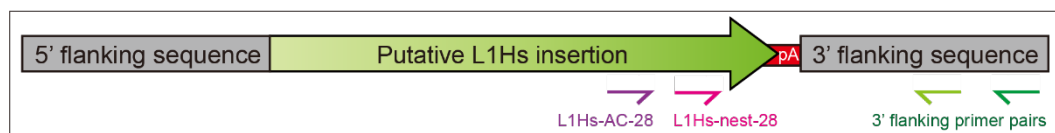


Supplementary Figure 6. Interaction between HCMV UL44 and L1 ORF2p induces DNA breaks (related to Figure 4). (a) PLA assays showing interactions between HA-UL44 and pH2AX upon L1 expression. pBL1^{ORF2p-3×FLAG} HeLa cells were transfected with HA-UL44, and after 2 days, cells were treated with dox for L1 expression. Four days after transfection, cells were fixed and stained with pH2AX rabbit and HA mouse antibodies for PLA. Representative PLA (orange), DAPI (blue), and merged images are shown (n = 2 independent replicates). The scale bar represents 50 μ m. (b) Colocalization coefficients representing the number of pixels from PLA signals that were located within DAPI. Dots with mean $\pm$ SD indicate the values from 16 cells per sample from n = 2 independent replicates. Two-sided Kolmogorov–Smirnov test.

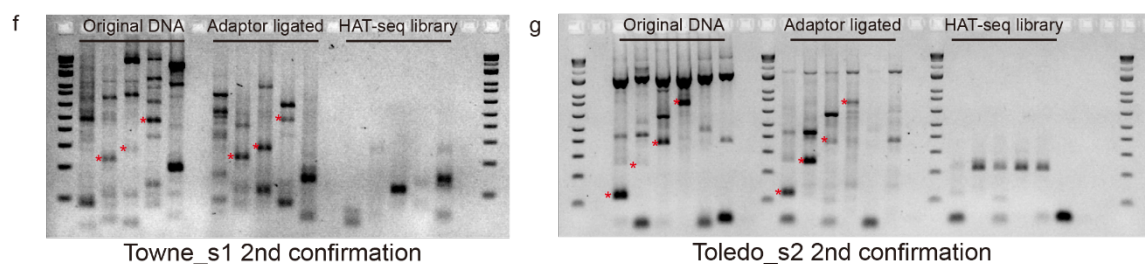
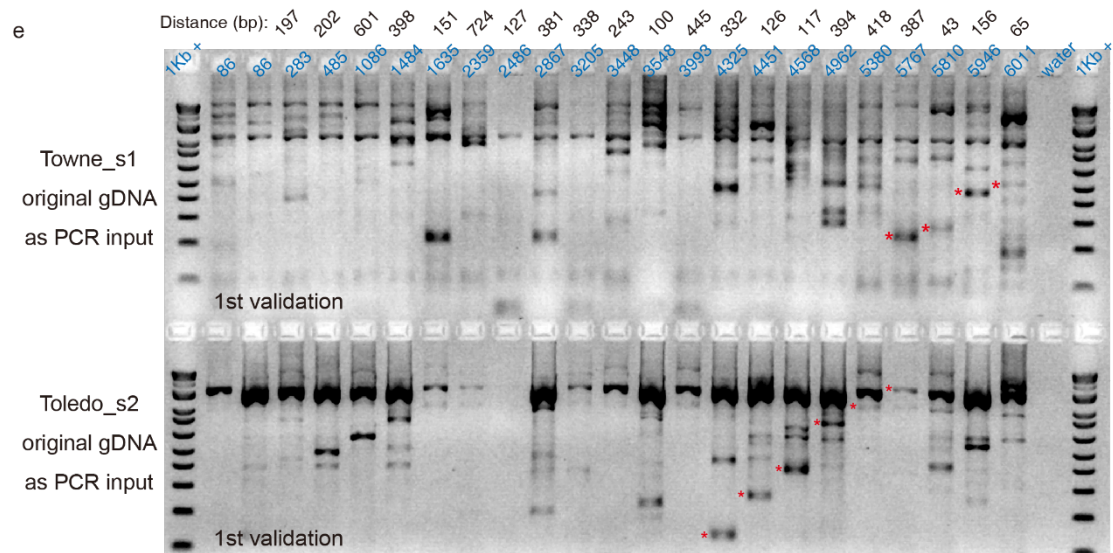
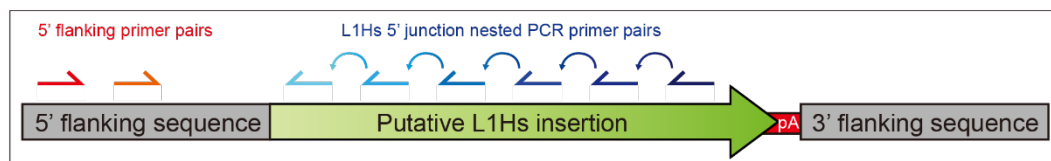


Supplementary Figure 7. Alignment of HAT-seq peaks for L1 insertions in the human and HCMV genomes (related to Figure 5). (a-c) Integrative Genomics Viewer plots of representative L1 insertions in the human and HCMV genomes. Insertions in the sense orientation relative to the genome are on the left and those in the antisense orientation are on the right. (a) Two reference L1H insertions in the human genome supported by uniquely mapped read pairs. (b) Two non-reference L1Hs insertions supported by soft-clipped reads (with colored bases) that span the L1-genome junction. For each library, the upper track displays the alignment of L1-trimmed 3' flanking sequence, while the lower track shows intact but soft-clipped contigs spanning the integration site. (c) Two putative L1 insertions in the HCMV genome supported by a single template with PCR duplicates. The red arrows indicate the start position of supporting contigs, which mark the number of different templates bearing the same insertion.

a Schematic diagram of 3' junction nested PCR



d Schematic diagram of step-wise 5' junction nested PCR



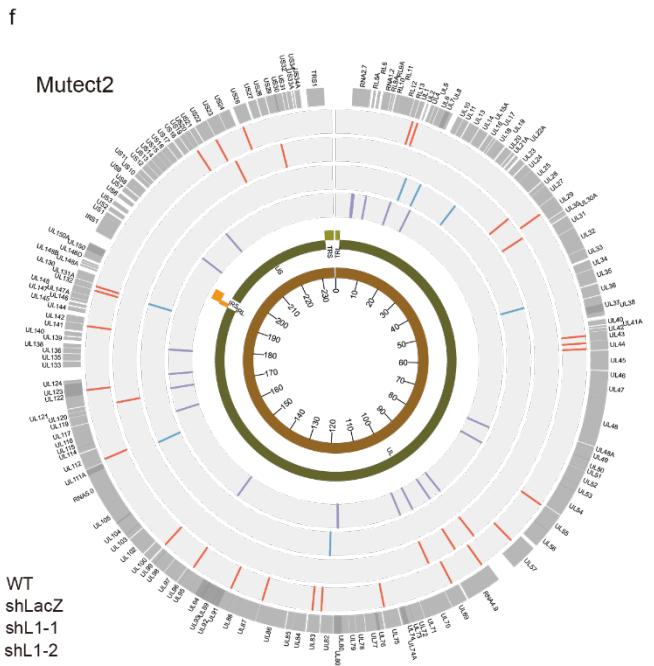
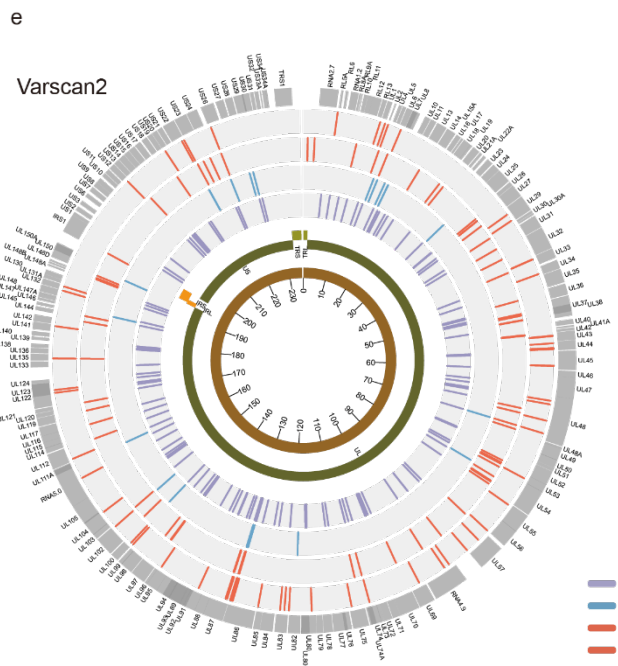
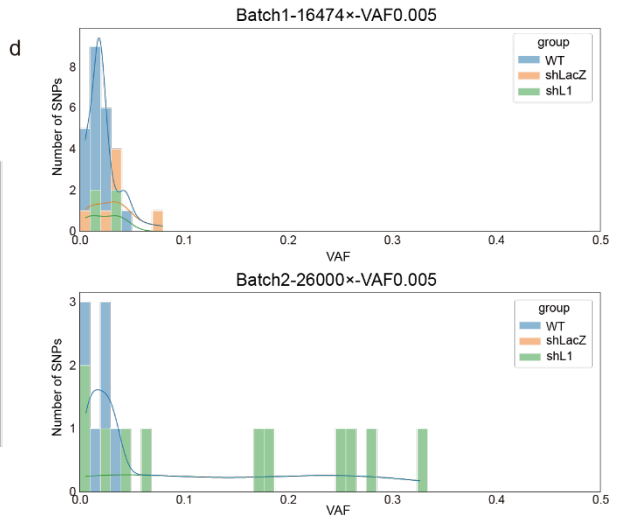
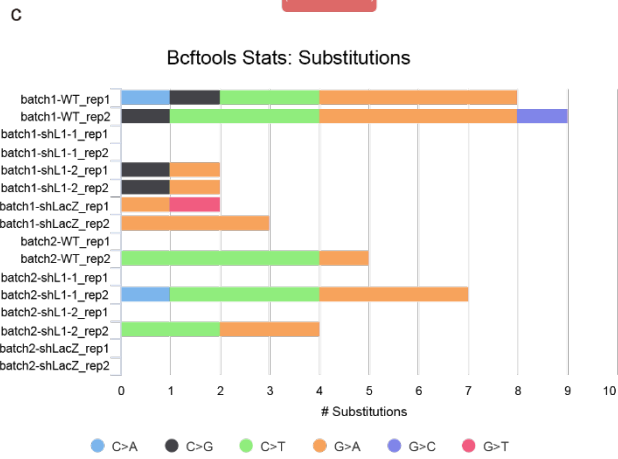
Supplementary Figure 8. Experimental validation on putative low-level L1 insertions in HCMV genomes (related to Figure 5). (a) Schematic diagram of 3' junction nested PCR. (b) Initial round of validation for seven putative L1 insertions. Sample information for each lane is listed in Supplementary Data 5. (c) Confirmation of 3' junction nested PCR results for two validated insertions from the initial round. (d) Schematic diagram of stepwise 5' junction nested PCR. (e) Initial round of validation for insertions from Towne and Toledo by using 3' junction nested PCR. (f–g) Confirmation of 5' junction PCR results for insertions from Towne (f) and Toledo (g). Red asterisks indicate amplicon bands of expected sizes. The bands were excised and subjected to amplicon sequencing to determine internal sequences.



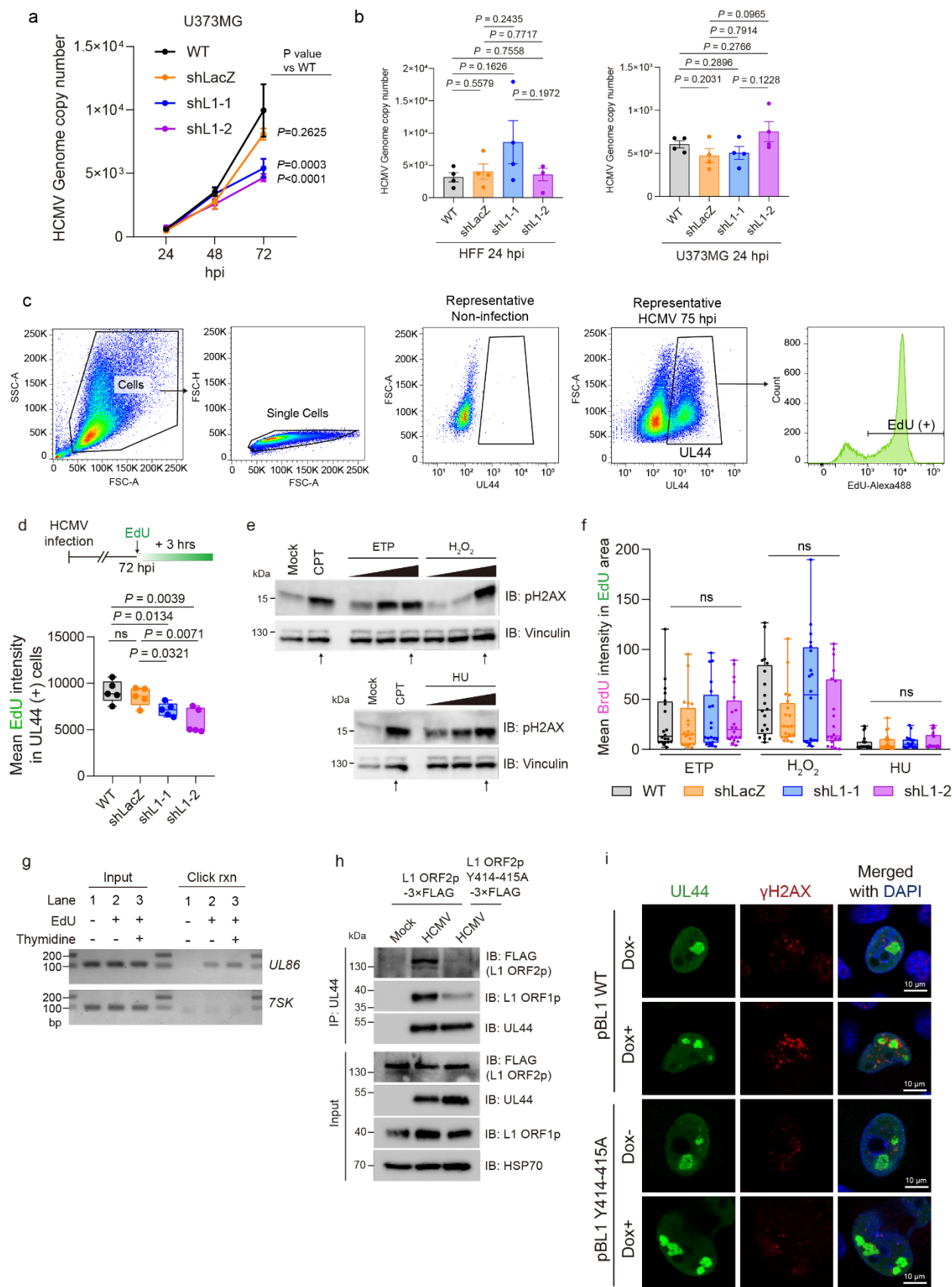
b

Sample Name	≥ 30X	Mean Cov.	Median	% Aligned	M Aligned	% Dups
shL1-1_rep1	97.0%	16 114.9X	16 474.0X	100.0%	27.5	11.0%
shL1-1_rep2	97.0%	15 996.5X	16 484.0X	100.0%	27.9	12.7%
shL1-2_rep1	97.0%	16 056.6X	16 482.0X	100.0%	30.5	19.9%
shL1-2_rep2	97.0%	16 051.2X	16 466.0X	100.0%	28.2	13.2%
shLacZ_rep1	97.0%	16 151.0X	16 470.0X	100.0%	31.6	22.3%
shLacZ_rep2	97.0%	16 180.4X	16 465.0X	100.0%	29.7	17.0%
Toledo WT BAC	97.0%	16 013.8X	16 486.0X	100.0%	30.2	19.5%
WT_rep1	97.0%	16 203.9X	16 485.0X	100.0%	32.0	23.1%
WT_rep2	97.0%	16 055.7X	16 478.0X	100.0%	30.6	20.5%

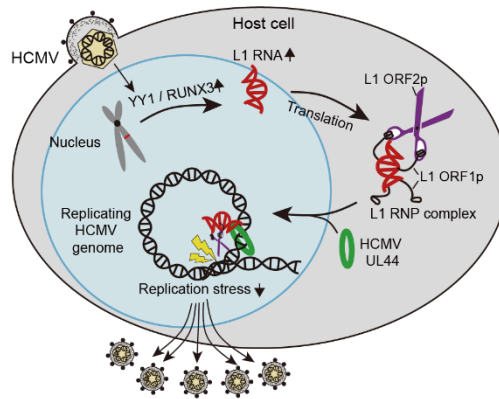
Sample Name	≥ 30X	Mean Cov.	Median	% Aligned	M Aligned	% Dups
shL1-1_rep1	97.0%	24 978.0X	25 842.0X	100.0%	47.0	18.8%
shL1-1_rep2	97.0%	24 966.9X	26 243.0X	100.0%	49.8	23.4%
shL1-2_rep1	97.0%	25 038.3X	25 800.0X	100.0%	47.4	19.4%
shL1-2_rep2	97.0%	25 037.3X	25 897.0X	100.0%	47.2	19.2%
shLacZ_rep1	97.0%	24 986.5X	25 825.0X	100.0%	50.8	24.9%
shLacZ_rep2	97.0%	24 935.8X	25 975.0X	100.0%	60.0	23.8%
WT_rep1	97.0%	25 149.2X	26 131.0X	100.0%	49.8	23.1%
WT_rep2	97.0%	24 958.7X	25 910.0X	100.0%	51.6	25.9%



Supplementary Figure 9. Detection of single-nucleotide polymorphisms and indels in HCMV genome (related to Figure 5). (a) Computational pipeline for calling SNPs and indels using Mutect2 and Varscan2. Both variant callers were used in paired mode with Toledo BAC serving as a control. (b) Sequencing coverage and duplication rates across WGS datasets after removing possible contaminating reads from hg38 and performing downsampling to the lowest median coverage within each batch. (c) Distribution of substitution types among SNP calls. (d) Distribution of variant allele frequencies of SNP calls. (e-f) Circos plots of combined Batch 1 and Batch 2 SNPs detected from Varscan2 (e) and Mutect2 (f). Inner rings show genomic coordinates in kb (brown) and major sequence features of HCMV (green). Middle tracks show SNP calls in WT (purple), shLacZ (blue), shL1-1 (red inner), and shL1-2 (red outer) samples. The outer ring shows GenBank HCMV Toledo sequence annotations.



Supplementary Figure 10. L1 accelerates HCMV DNA replication (related to Figure 6). (a) Measurement of HCMV DNA copy number. U373MG cells were infected with indicated HCMV (1 MOI). Total DNA was quantified via qPCR (UL86/7SK). Data are presented as mean mean $\pm$ SEM of $n = 4$ independent samples. Two-way ANOVA and Dunnett's multiple comparisons test. (b) Entered HCMV DNA level at 24 hpi of HFF (left panel; Fig. 6a) and U373MG (right panel; Fig. S10a). Two-sided student's t -test ($n = 4$ independent replicates). (c) Gating strategy used for EdU incorporation assay. Cells were stained with HCMV UL44 antibody to determine HCMV-infected cells at 75 hpi. The intensity of EdU was analyzed in HCMV-infected and EdU-positive portion. (d) Mean EdU intensity of HCMV-infected virus at 75 hpi. Two-sided student's t -test ($n = 5$ independent replicates). (e) Immunoblot of pH2AX upon drug treatment. Primary HFFs were treated with 1 μ M camptothecin (CPT) for 1 h; 1, 5, or 10 μ M Etoposide (ETP) for 1 h; 20, 50, or 100 μ M hydrogen peroxide (H_2O_2) for 1 h; or 5, 20, or 50 mM hydroxyurea (HU) for 6 h. Arrows indicate the selected concentration of each drug for (f). Representative blot of $n=2$ independent replicates. (f) EdU-BrdU dual labeling assay using 10 μ M ETP, 100 μ M H_2O_2 , and 50 mM HU. The intensity of BrdU were measured in the EdU area. Dots in box plot shows the values from $n = 3$ independent replicates. Two-sided Kolmogorov–Smirnov test. (g) aniPOND-PCR assay of HCMV DNA (UL86) and host DNA (7SK). Representative gel image from $n = 2$ independent replicates. (h) IP assay for endogenous UL44 co-IP assay. U373MG cells expressing L1 ORF2p-3 $\times$ FLAG WT or Y414-415A mutant were infected with HCMV, treated with 50 ng/mL dox, and harvested at 4 dpi. Representative blot of $n = 2$ independent replicates. (i) Immunofluorescence assay using dox-inducible L1 expressing U373MG (pBL1 U373MG) at 48 hpi. Representative images ($n = 2$ independent replicates) for UL44 (green), pH2AX (red), and DAPI (blue) are indicated with the scale bar (10 μ m).



Supplementary Figure 11. A proposed model for HCMV-hijacking of L1 retrotransposon for efficient replication.

HCMV infection switches on L1 retrotransposon expression through the upregulation of L1 transcription factors, YY1 and RUNX3. L1 ORF1p and L1 ORF2p form the L1 RNP complex. HCMV DNA processivity factor UL44 then interacts with L1 RNP and is recruited to HCMV replication compartment where the viral genomic DNA is actively replicated. Access of L1 RNP to HCMV replication compartments enables HCMV DNA to replicate seamlessly by resolution replication stress.

Supplementary Tables

Table S1. SCRAMble putative L1 insertions

Table S2. Coordinates of mutations in HCMV DNA

Table S3. Primer sequences for qPCR or RT-qPCR

Table S4. Primer sequences for BAC recombineering

Table S1. SCRAMble putative L1 insertions

Library	Insertion	MEI Family	Insertion Direction	Clipped Reads in Cluster	Alignment Score	Alignment Percent Length	Alignment Percent Identity	Clipped Sequence	Clipped Side	Start In MEI	Stop In MEI	polyA Position	polyA Seq	polyA Supporting Reads	TSD	TSD length	Manual inspection note
WT	AC146905.1: 147408	alu	Plus	1	101.3012848	94.17475728	85.56701031	GCTCATGCCTGTAATGC CAGCACTTTGGGAGGCT GAGGCAGGCAGATCAC TTGAGGTGAGGAGTTTG AAACCAGCCTGGCTAAC ATGGTGAAACCCCAACT CT	right	15	109	147326	AAGAA TAAAA A	1	TGCGTCTTCT CTTTCCGAGG CCGTGGATAA CGCGTCTAGT CGCGCAACT TAGTGTGCGT GAGTTCGGTG AGTGGCGTGG TG	82	Mis-pairing PCR molecule. An AluSq fragment from human gDNA.
shL1-1	AC146905.1: 77273	alu	Plus	2	103.1755295	94.62365591	86.36363636	AGGAGAATTGCTTGAAC CCAGGAGGCCGAGGTT GCAGTGAGCCGAGATA GTGCCACTGCACTCCAG CCTGGGGGATAGAGTG ATACTCTATCT	left	188	275	77248	AAACA AAAAT	1	CACATTACAC ACACGGGGG CGCAAA	25	Homopolymer region
shL1-1	AC146905.1: 77268	alu	Plus	2	70.88728333	97.70992366	78.90625	TGCCTGTAGTTGCAGCT ACTCAGGAGGCTGAGG TGGGAGGATCACTTGAG ACTGGGAGGTTGAGGC TACAGTGAGCTGTGATC GCGCTACTGCACTCCAG CCTGGATGACAGAGTGA GACCCTGTCTCAAA	left	155	282	77248	AAACA AAAAT	1	CACATTACAC ACACGGGGG C	20	Homopolymer region
shL1-1	AC146905.1: 77291	l1	Plus	2	225.2827911	100	94.30894309	TCCTGATGAACATTGA TGCAAAATCCTCAATA AAATACTGGCAACCGA ATCCAGCAGCACATCAA AAAGCTTATCCACAATG ATCAAGTTGGCTTCATT CCTAGAATGCAAGGCTG GTTT	left	3590	3712	77248	AAACA AAAAT	1	CACATTACAC ACACGGGGG CGCAAAACAAA AACAAAAAAA AAAT	43	Homopolymer region
shL1-1	AC146905.1: 17650	l1	Minus	2	165.8527374	100	94.44444444	ATGAACCTCATCCTTTTT ATGGCTGCATAGTATTC CATGGGTATATATGCC ACATTTTCTTAATCCAGT ATATCATTTATGGACATT TG	left	5623	5712	17749	TTCTT TTTGT T	1	GGTTGGTTCC CCGTCTTTTT GAACACCAAA GTCAACATTT GCGGCTACCT GGCGCCCATC GTGCTGATGG CGTACACGTA CATCCGTATG GTGCGGTTT	99	Mis-pairing PCR molecule. An L1PA5 fragment from human gDNA.
shL1-2	AC146905.1: 120515	l1	Minus	1	116.5564423	90.27777778	93.84615385	TGATGATAGTTTCTTTT CTGTGCAGGAGCTCTTT AGTTTAAATTAGATCCCA CTTATCAATTTTGGGGG AGG	left	5008	5072	120564	TTTTG TTTTT GTC	1	TTTATTTAGG GCTACGCGAC GGCGTCTCGG GTGTGGCGCT ACACACCGT	49	Mis-pairing PCR molecule. An L1PA7 fragment from human gDNA.
shLacZ_P6	AC146905.1: 58552	l1	Plus	1	136.1681366	93.38842975	87.61061947	CTCCAGTCTACAGCTCC CAGGGTGAGTGACGCA GAAGACGGGTGATTCT GCATTTCCAACTAAGGT ACTGGGTTTATCTCACT GGGCTTGTGCGGACAG TGGGTGCAGCCCATGG AGCAT	left	35	146	58532	ATACA AAAAA	1	ACTACGCCGG ACAGCCGCAC	20	Mis-pairing PCR molecule. An L1PA5 fragment from human gDNA.

Table S2. Coordinates of mutations in HCMV DNA

Callset	Filtering	Coords	Group-filtered_Coords	Coords_count	Group-filtered_Coords_count
Mutect2	raw	['3717', '4023', '4031', '6899', '12547', '17263', '74444', '78264', '92687', '95538', '100095', '103121', '117465', '117575', '141577', '165428', '170718', '173936', '179420', '179477', '201593', '209210']	[3717, 4023, 4031, 6899, 12547, 17263, 74444, 78264, 92687, 95538, 100095, 103121, 117465, 117575, 141577, 165428, 170718, 173936, 179420, 179477, 201593, 209210]	22	22
Mutect2	raw	['11226', '13869', '16906', '22526', '24708', '48787', '70354', '83864', '119125', '135508', '137180', '144885', '147877', '150706', '160756', '172318', '178750', '187945', '188017', '214597']	[11226, 13869, 16906, 22526, 24708, 48787, 70354, 83864, 119125, 135508, 137180, 144885, 147877, 150706, 160756, 172318, 178750, 187945, 188017, 214597]	20	20
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Mutect2	batch-filtering	['13869', '16906', '24708', '48787', '119125', '160756', '188017']	[13869, 16906, 24708, 48787, 119125, 160756, 188017]	7	7
Mutect2	batch-filtering	['33205', '37241', '92940', '96827', '101867', '169879', '218538', '226711']	[33205, 37241, 92940, 96827, 101867, 169879, 218538, 226711]	8	8
Mutect2	batch-filtering	['11725', '12475', '35690', '55332', '56418', '57298', '82032', '88961', '94691', '99951', '110716', '120162', '121498', '129149', '134186', '140611', '146054', '161853', '172549', '182152', '187599', '188254', '214098', '217343', '221798']	[11725, 12475, 35690, 55332, 56418, 57298, 82032, 88961, 94691, 99951, 110716, 120162, 121498, 129149, 134186, 140611, 146054, 161853, 172549, 182152, 187599, 188254, 214098, 217343, 221798]	25	25
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High confidence	raw	[4023, '12547', '17263', '74444', '78264', '92687', '95538', '103121', '117465', '117575', '141577', '165428', '170718', '173936', '179420', '179477', '201593', '209210']	[4023, 12547, 17263, 74444, 78264, 92687, 95538, 103121, 117465, 117575, 141577, 165428, 170718, 173936, 179420, 179477, 201593, 209210]	18	18
High confidence	raw	['11226', '16906', '22526', '24708', '48787', '70354', '83864', '135508', '137180', '144885', '147877', '150706', '172318', '178750', '187945', '188017', '214597']	[11226, 16906, 22526, 24708, 48787, 70354, 83864, 135508, 137180, 144885, 147877, 150706, 172318, 178750, 187945, 188017, 214597]	17	17
High confidence	raw	['33205', '37241', '92940', '96827', '101867', '169879', '218538']	[33205, 37241, 92940, 96827, 101867, 169879, 218538]	7	7
High confidence	raw	['55332', '94691', '110716', '182152', '187599', '217343']	[55332, 94691, 110716, 182152, 187599, 217343]	6	6
High confidence	batch-filtering	[4023, '12547', '17263', '74444', '78264', '92687', '95538', '103121', '117465', '117575', '141577', '165428', '170718', '173936', '179420', '179477', '201593', '209210']	[4023, 12547, 17263, 74444, 78264, 92687, 95538, 103121, 117465, 117575, 141577, 165428, 170718, 173936, 179420, 179477, 201593, 209210]	18	18
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High confidence	batch-filtering	['33205', '37241', '92940', '96827', '101867', '169879', '218538']	[33205, 37241, 92940, 96827, 101867, 169879, 218538]	7	7
High confidence	batch-filtering	['55332', '94691', '110716', '182152', '187599', '217343']	[55332, 94691, 110716, 182152, 187599, 217343]	6	6

Table S3. Primer sequences for qPCR or RT-qPCR

L1 5'UTR	
F	AGC CTA ACT GGG AGG CA
R	TTC CCC ATC TTT GTG GT

L1 ORF1	
F	GCA AGG CAG GCC AAC GTT CA
R	CCT TTC TCT CTG GCT GCC CT

L1 ORF2	
F	CAG GGC AAT CAG GCA GGA GA
R	TTG GGC TGA GAC GAT GGG GT

L1Hs Taqman	
F	ACA GCT TTG AAG AGA GCA GTG GTT
R	AGT CTG CCC GTT CTC AGA TCT

GAPDH	
F	GCA AAT TCC ATG GCA CCG T
R	TCG CCC CAC TTG ATT TTG G

IFIT1	
F	GCA GCC AAG TTT TAC CGA AG
R	GCC CTA TCT GGT GAT GCA GT

MxA	
F	AGG TCA GTT ACC AGG ACT AC
R	ATG GCA TTC TGG GCT TTA TT

OAS1	
F	TGG AGA CCC AAA GGG TTG GA
R	AGG AAG CAG GAG GTC TCA CC

IFITM1	
F	CCC CCA GCA CCA TCC TTC
R	ACC CCG TTT TTC CTG TAT TAT CTG T

7SK	
F	TGA CTA CCC TAC GTT CTC CTA C
R	GTC AAG GGT ATA CGA GTA GCT G

UL86	
F	TGC GTA AAG TCG AAG AAG GG
R	CGC ACG GTG AAC GAA ATA AAG

L1Hs Taqman Probe	
	/56-FAM/TCC CAG CAC GCA GC/36TAMSp/

7SK Probe	
	/56-FAM/CCCTGCTAG/ZEN/AACCTCCAAACAAGCT/3IABkFQ/

UL86 Probe	
	/56-FAM/CAAGGTGGG/ZEN/CAACATCACGCTCTA/3IABkFQ/

Table S4. Primer sequences for BAC recombineering

Primers for BAC recombineering	
US1-2 rpsL	
F	CGAGAGCACTGGCAGGGGTGGCAAAACGCAGTTGAGCGGGGTATGCGTG <u>GGCCTGGTGATGATGGCGGGATCG</u>
R	TCTTCCTCTCCGTCGCTGTCATCAAGCAGCCAGGTCACCTCCTCCGCTC <u>TCAGAAGAACTCGTCAAGAAGGCG</u>
Usage	50 nt homology arm of US1-2 region + <u>rpsL-neo binding sequence</u>
US1-2 shRNA	
F	CGAGAGCACTGGCAGGGGTGGCAAAACGCAGTTGAGCGGGGTATGCGTG <u>CGGATCTCGACGGTATCGATC</u>
R	TCTTCCTCTCCGTCGCTGTCATCAAGCAGCCAGGTCACCTCCTCCGCTC <u>GGATGAATACTGCCATTGTCTC</u>
Usage	50 nt homology arm of US1-2 region + <u>shRNA construct binding sequence</u>

Sequence information of shRNA	
shRNA	
shLacZ	GCGATCGTAATCACCCGAGTGCTCGAGCACTCGGGTGATTACGATCGC <u>TTTTT</u>
shL1-1	AAGAAGGCTTCAGACGATCAACTCGAGTTGATCGTCTGAAGCCTTCTT <u>TTTTT</u>
shL1-2	AAATGAAGCGAGAAGGGAAGTCTCGAGACTTCCCTTCTCGCTTCATT <u>TTTTT</u>
	specific shRNA sequence + <u>termination sequence</u>

Supplementary Notes

Supplementary Note 1. Increased L1-induced mutagenesis is not observed in HCMV for genetic adaptation.

We asked whether L1 retrotransposition facilitates the adaptation of human cytomegalovirus (HCMV) over cell culture passages. We hypothesized that if certain L1 insertions provided a selective advantage and increased the fitness of the carrier HCMV, these insertions could be inherited by progeny virus with sequential clonal expansion in the virus population. To test this, we aimed to identify and compare the number of clonal L1 insertions in four different HCMV strains at high passage numbers (see **Methods**). For this purpose, we deployed the Human Active Transposon-seq (HAT-seq), a targeted capture approach for sensitive enrichment and specific identification of L1Hs insertions from bulk genomic DNA, with minor modifications to profile clonal L1 insertions in HCMV genomes (see **Methods**). According to the benchmark performance from spike-in controls with known 1%, 0.1%, and 0.01% mosaicism, the sensitivities of HAT-seq for clonal low-level L1 insertions were 76.6%, 28%, and 30.1%, respectively, with an average of 60 million read pairs (2×150 bp) per library in previous report⁵.

First, we performed quality control on HAT-seq libraries constructed with HCMV gDNA samples. All of libraries were sequenced with higher throughput than previous report, with an average of 87.9 million read pairs per sample and 75.8 million as the minimum of throughput (**Supplementary Data 4**). Data pre-processing revealed that 95.6% reads of HAT-seq libraries bearing the 3' sequence of L1 capture primer (P7_Ns_L1Hs), indicating that the majority of libraries were constructed properly. This allowed us to reliably call clonal L1 insertions from HCMV genomes.

Second, we extracted L1Hs-derived reads and determined the putative L1 integration sites based on the alignment of the 3' genomic flanking sequences. It is worth noting that all of HCMV HAT-seq libraries showed an extremely low percentage of L1Hs-derived reads (**Supplementary Data 4**, average of 'Read2_w_L1_3end_' = 1.33%), indicating that there were limited L1-genome templates in the input gDNA of L1 enrichment PCR. As a result, most of the PCR products were generated from non-specific amplification. Furthermore, the alignments of L1-genome junctions against human and HCMV genomes showed that most of L1-genome junctions were amplified from human gDNA (**Supplementary Data 4**, 'Unique_mapping_human_' and 'Trimmed_ctg_unique_mapping_human_'), while a tiny proportion of them could be aligned to the HCMV genome (**Supplementary Data 4**, 'Trimmed_ctg_unique_mapping_HCMV_'). As described in the HCMV gDNA extraction section, the centrifugation-based HCMV gDNA enrichment from the cell culture could inevitably recover low-level human gDNA carryover.

Third, the human gDNA carryover could be served as positive controls (*e.g.*, <5% "spike-ins" of known L1 insertion in human gDNA) within the HCMV gDNA samples themselves. HAT-seq successfully identified a decent number of reference (avg. = 656 insertions/library) and non-reference L1Hs (avg. = 119/library) insertions in the human gDNA carryover, confirming the exceptional sensitivity of our method and analytical pipeline. As an example, in the WT_Toledo library, 80.18% of raw reads (containing non-specific amplification products) could be uniquely mapped to the HCMV genome (**Supplementary Data 4**, 'Fastq_Screen_HCMV_'), while 9.42% of them uniquely aligned to the human reference genome (**Supplementary Data 4**, 'Fastq_Screen_human_'). Nevertheless, HAT-seq was capable of capturing both reference L1 insertions (**Supplementary Fig. 7a**) and known non-reference L1 insertions (**Fig. 5b** and **Supplementary Fig. 7b**) with a number of supporting reads from low-level human gDNA

carryover among all of the HCMV HAT-seq libraries.

Fourth, we applied the same analytical pipeline as we used to call non-reference L1 insertions in the human reference genome to identify putative novel L1 insertions in the HCMV genome. However, the tiny amount of L1-derived contigs that aligned to the HCMV genome yield only a few of putative integration peaks with limited number of supporting reads (**Supplementary Data 4**). Most of these events were backed with L1-genome junction sequence from one template with PCR duplicates (see examples in **Fig. 5c** and **Supplementary Fig. 7c**). Further manual inspection on the Integrative Genomics Viewer (IGV) rejected false positive peaks that 1) having jagged boundary of L1 and flanking genome, suggesting mis-pairing of chimeric reads at poly-A tail during PCR amplification; 2) showing incorrect peak patterns (split or truncated); 3) were systematic noisy signal presented in all of libraries. Remaining putative insertions were subject to experimental validation.

Experimental validation on putative low-level events was challenging. To increase the likelihood of re-sampling for the insertion signal, we tested three different templates as PCR input, including 1) the original HCMV gDNA, 2) adaptor-ligated HCMV fragments, and 3) the HAT seq library. First, we performed 3' junction nested PCR for seven candidate sites and examined the amplicons with expected sizes on 2% agarose gels (**Supplementary Data 5** and **Supplementary Fig. 8a–c**). Because of the presence of poly-A tail in the 3' L1-genome junction and the multi-copy nature of L1 element, it is possible that a chimeric amplicon could be formed during PCR amplification via mis-pairing of two unrelated molecules within a micro-homologues region; thus, it was vital to perform rigorous experimental validation to validate and resolve both ends of a predicted insertion. Next, two out of seven putative insertions validated by 3' junction PCR were then subject to step-wise 5' junction nested PCR to probe the location of the 5' end of L1 insertion. Amplicons were visualized on 2% gel and bands of the expected size were cut for amplicon sequencing to resolve internal sequences of 5' genome-L1 junctions (**Supplementary Data 5** and **Supplementary Fig. 8d–g**). Raw Amplicon-EZ data from Azenta, Inc. were pre-processed to trim adaptors and bases with low quality. Clean reads were mapped to the HCMV genome to verify the predicted integration sites using IGV. Unfortunately, none of putative L1 insertions were fully validated at both 3' and 5' junctions.

In conclusion, we successfully constructed HAT-seq libraries using bulk HCMV gDNA samples and conducted deep sequencing to obtain sufficient throughput for L1 profiling in HCMV genomes. Nonetheless, we were unable to identify high-confidence clonal L1 insertions through bioinformatic analysis of the sequencing dataset. In experimental validation, we were unable to fully confirm any predicted insertion at both ends. These findings suggest that L1 retrotransposition may not be the major mechanism responsible for HCMV adaptation.

Supplementary Note 2. Lack of Evidence for High-Confidence Clonal Transposon Insertions in Bulk HCMV gDNA Samples.

Except for the autonomous L1Hs retrotransposon, other active retrotransposon families, such as Alu and SVA, can also exploit the L1 machinery to facilitate their retrotransposition. We asked whether these subfamilies enhanced the fitness of HCMV via retrotransposition. To test this hypothesis, we performed ultra-deep Illumina whole genome sequencing (WGS) on HCMV gDNA samples to capture and identify low-level clonal transposon insertions (L1, Alu, and SVA) in HCMV genomes.

All of HCMV WGS libraries were sequenced to ultra-deep depth of coverage with an average of 27,637X and a minimum depth of 16,114X (**Supplementary Fig. 9b**). We applied SCRAMble⁶, a transposon insertion detection tool, to aligned BAM files to call putative L1, Alu, and SVA insertions. The tool identifies clusters of soft-clipped reads in a BAM file, builds consensus sequences, aligns to representative L1, Alu, and SVA consensus sequences, and outputs putative transposon insertion calls. We adjusted parameters to increase sensitivity for reporting low-level clonal events bearing putative insertional hallmarks (see **Methods**). Only 7 putative insertions (4 L1s and 3 Alus) with one or two supporting reads (i.e. <0.1% mosaicism) were called out from deep HCMV WGS data (**Table S1**). Manual inspection on predicted calls did not nominate any high-confidence events.

In conclusion, we successfully performed ultra-deep whole genome sequencing on HCMV genomes, but found no evidence of putative clonal insertions with greater than 0.1% mosaicism in the bulk gDNA samples. These observations suggest that L1, Alu, and SVA retrotranspositions may not be the major mechanism responsible for HCMV adaptation.

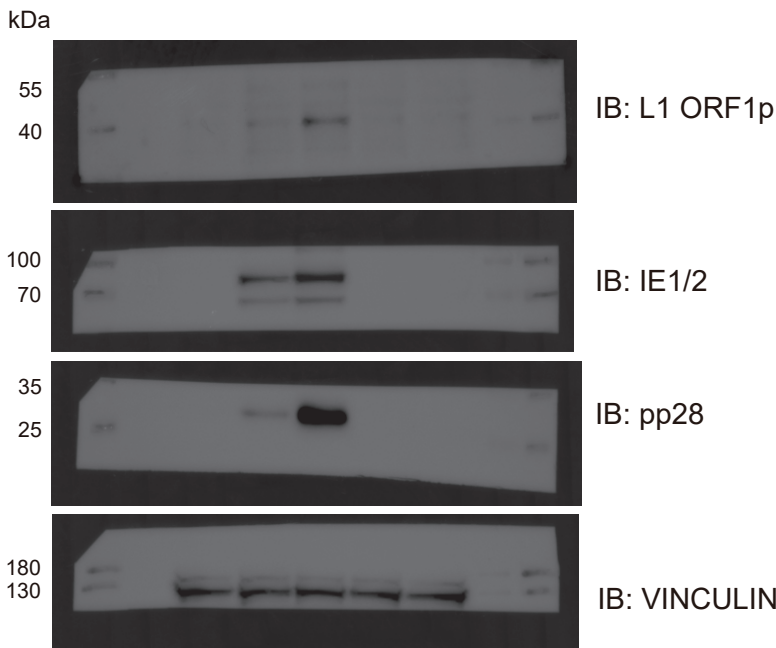
Supplementary References

- 1 Nogalski, M. T. *et al.* A tumor-specific endogenous repetitive element is induced by herpesviruses. *Nat Commun* **10**, 90 (2019). <https://doi.org:10.1038/s41467-018-07944-x>
- 2 Gulbis, J. M., Kelman, Z., Hurwitz, J., O'Donnell, M. & Kuriyan, J. Structure of the C-Terminal Region of p21WAF1/CIP1 Complexed with Human PCNA. *Cell* **87**, 297-306 (1996). [https://doi.org:https://doi.org/10.1016/S0092-8674\(00\)81347-1](https://doi.org:https://doi.org/10.1016/S0092-8674(00)81347-1)
- 3 Appleton, B. A., Loregian, A., Filman, D. J., Coen, D. M. & Hogle, J. M. The cytomegalovirus DNA polymerase subunit UL44 forms a C clamp-shaped dimer. *Mol Cell* **15**, 233-244 (2004). <https://doi.org:10.1016/j.molcel.2004.06.018>
- 4 Appleton, B. A. *et al.* Crystal Structure of the Cytomegalovirus DNA Polymerase Subunit UL44 in Complex with the C Terminus from the Catalytic Subunit: DIFFERENCES IN STRUCTURE AND FUNCTION RELATIVE TO UNLIGANDED UL44*. *Journal of Biological Chemistry* **281**, 5224-5232 (2006). <https://doi.org:https://doi.org/10.1074/jbc.M506900200>
- 5 Zhao, B. *et al.* Somatic LINE-1 retrotransposition in cortical neurons and non-brain tissues of Rett patients and healthy individuals. *PLoS Genet* **15**, e1008043 (2019). <https://doi.org:10.1371/journal.pgen.1008043>
- 6 Torene, R. I. *et al.* Mobile element insertion detection in 89,874 clinical exomes. *Genet. Med.* (2020). <https://doi.org:10.1038/s41436-020-0749-x>

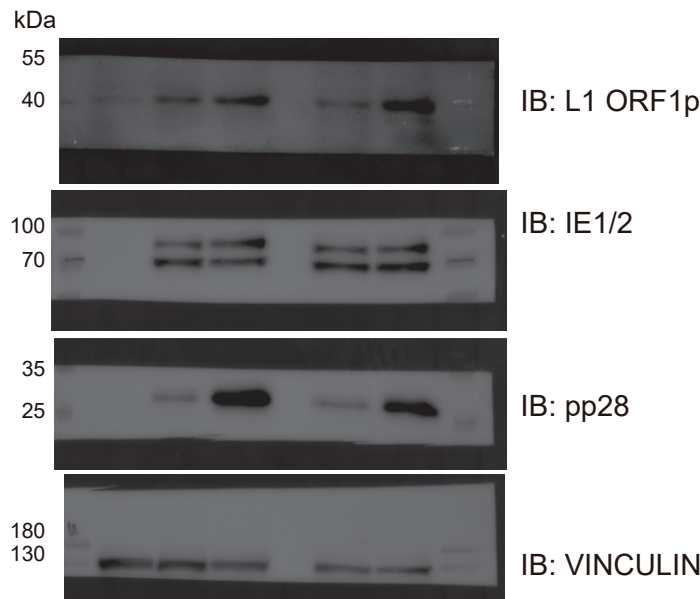
Source data

Supplementary Figure 1

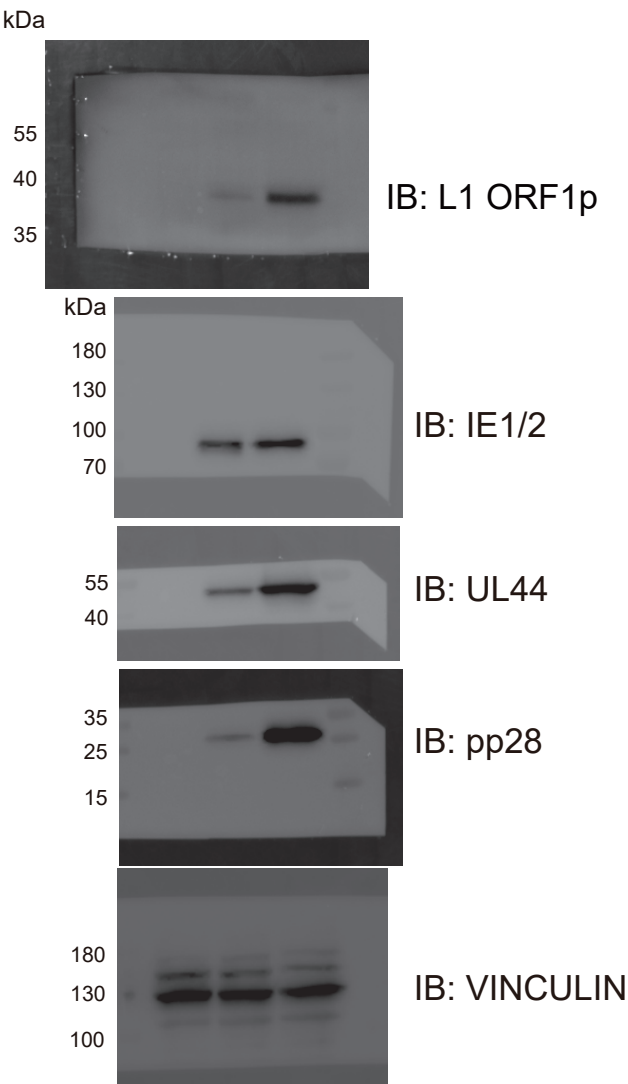
Supplementary Fig. 1c



Supplementary Fig. 1d



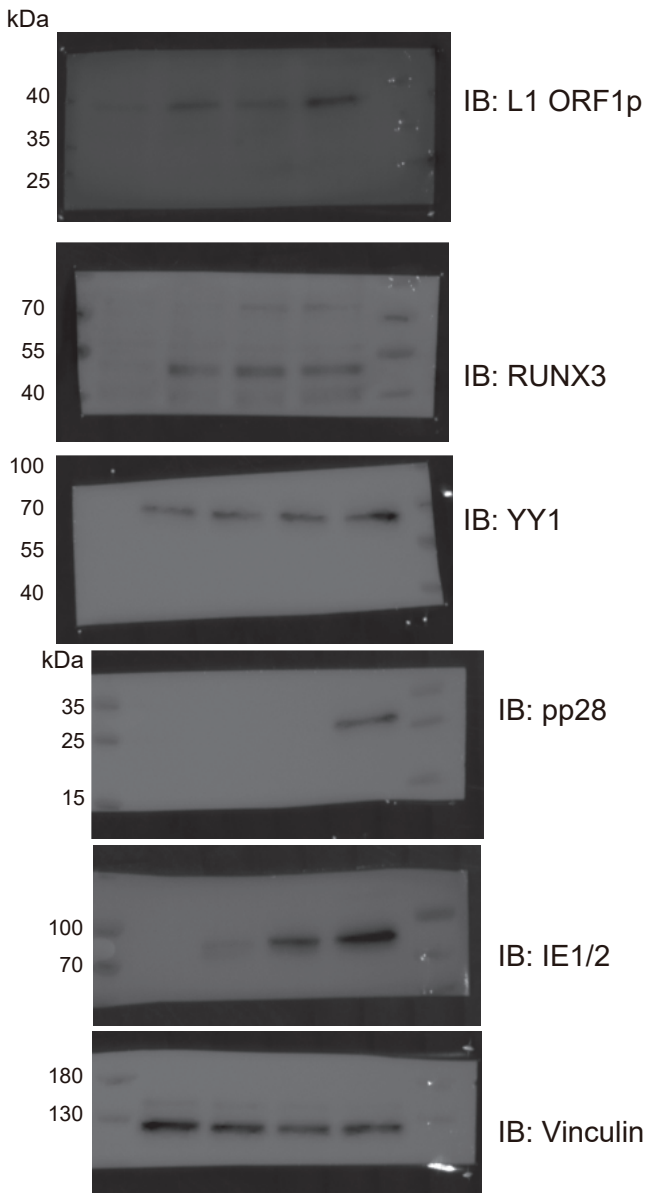
Supplementary Fig. 1e



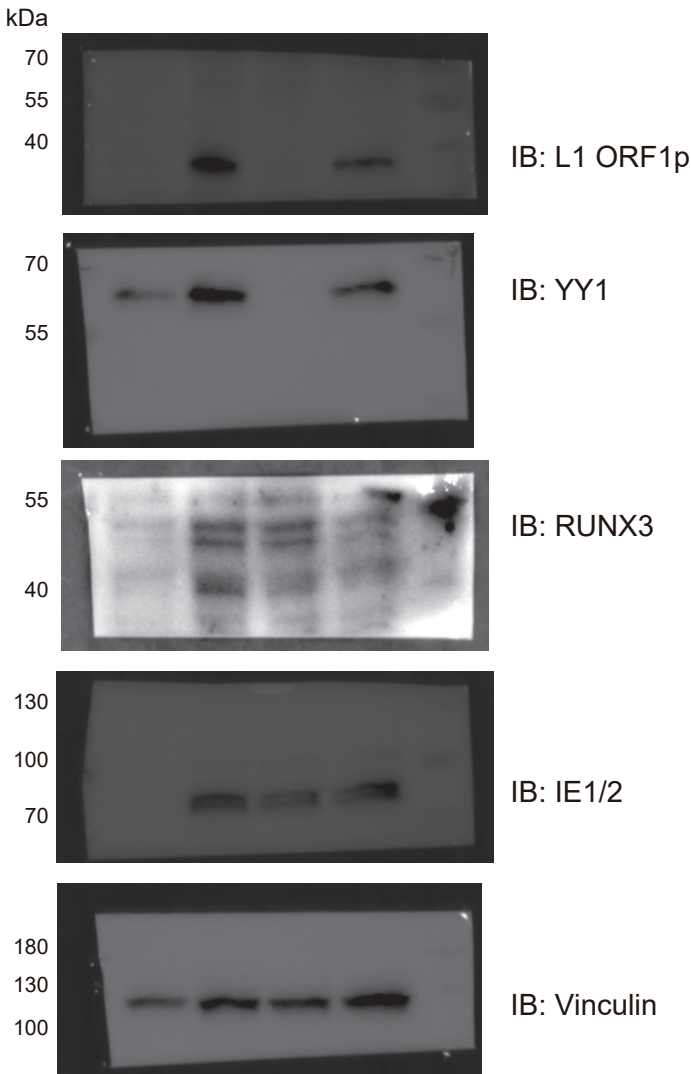
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Supplementary Figure 2

Supplementary Fig. 2e



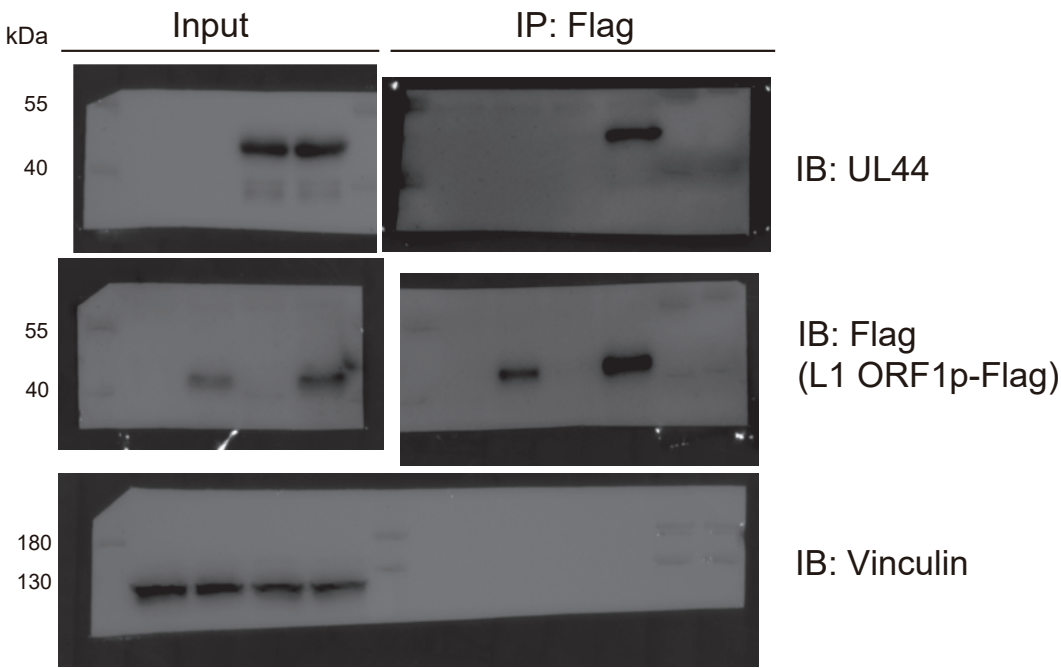
Supplementary Fig. 2f



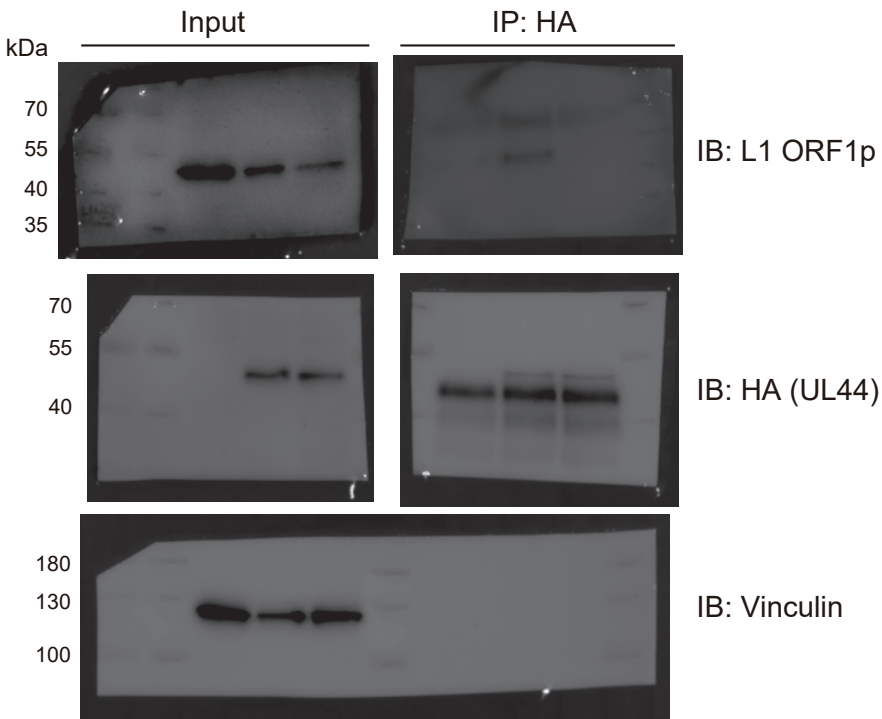
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Supplementary Figure 3

Supplementary Fig. 3b



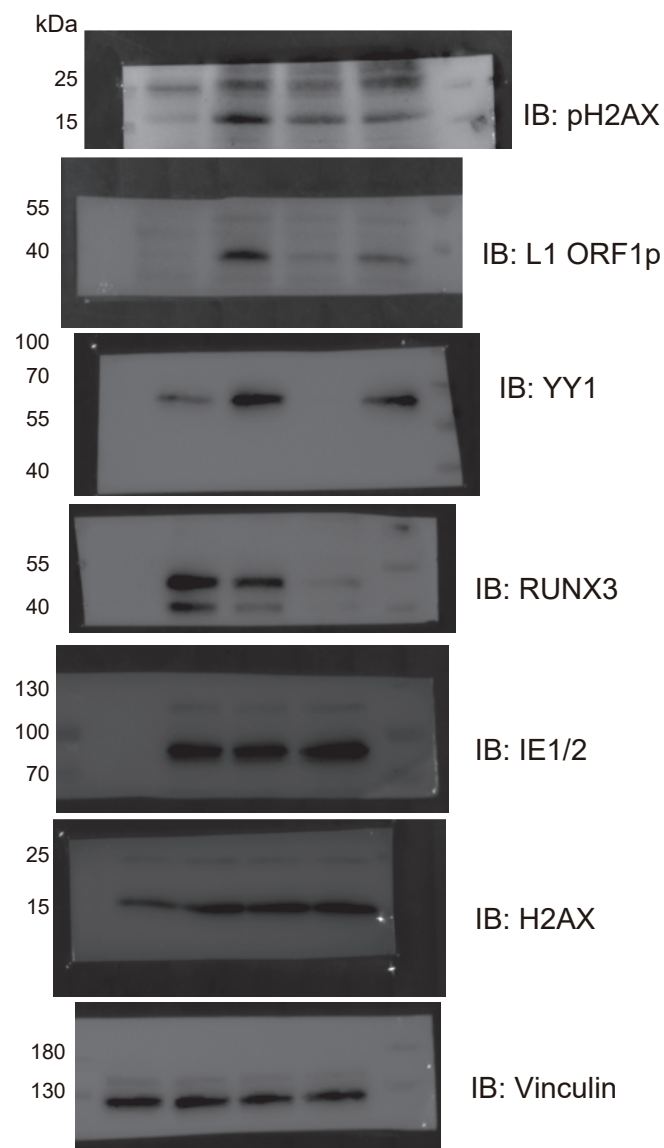
Supplementary Fig. 3c



Source data

Supplementary Figure 5

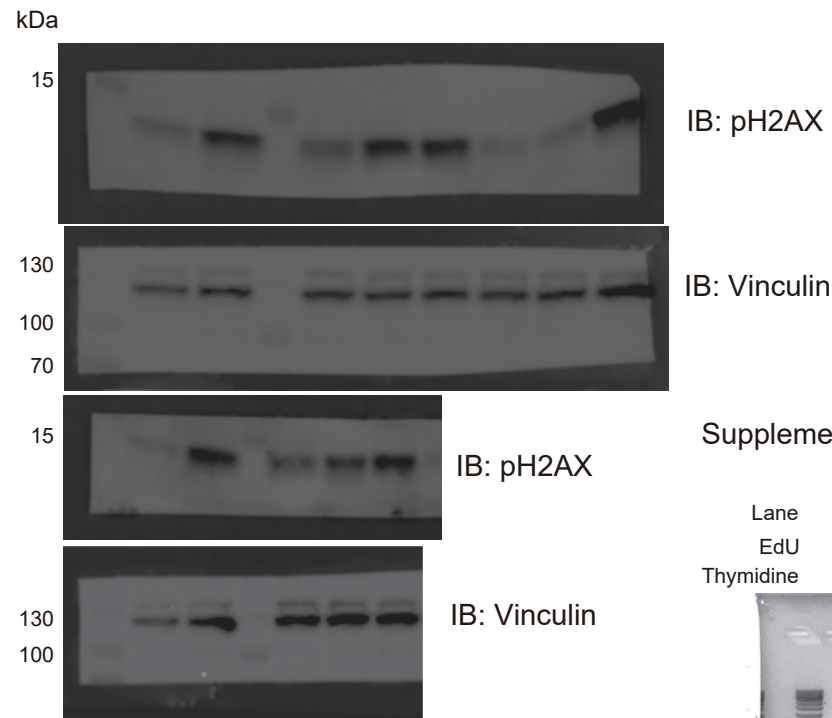
Supplementary Fig. 5b



Source data

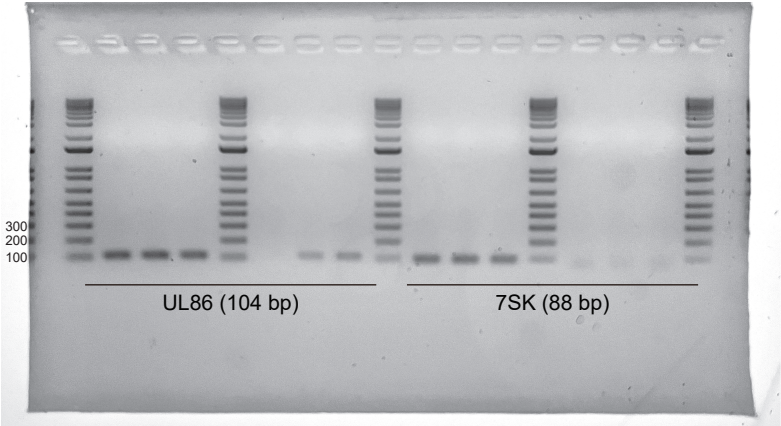
Supplementary Figure 10

Supplementary Fig. 10e



Supplementary Fig. 10g

Lane	Input			Click rxn			Input			Click rxn		
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EdU	-	+	+	-	+	+	-	+	+	-	+	+
Thymidine	-	-	+	-	-	+	-	-	+	-	-	+



Supplementary Fig. 10h

