Centrosomal protein TRIM43 restricts herpesvirus infection by regulating nuclear lamina integrity

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Supplementary Figures 1-11

Supplementary Figure Legends

Supplementary Table 1

Supplementary Information Content

The Supplementary information comprises the 11 Supplementary Figures, the Supplementary Figure Legends, and one Supplementary Table.

Supplemental Figures:

Supplementary Figure 1. Expression of TRIM43 in virus-infected cells and AIDS-associated Kaposi's sarcoma. The figure is related to Figure 1.

Supplementary Figure 2. Effect of TRIM43 knockdown or its ectopic expression on virus lytic replication or reactivation. The figure is related to Figure 1.

Supplementary Figure 3. TRIM43 and DUX4 expression in human tissues or cells. The figure is related to Figure 2.

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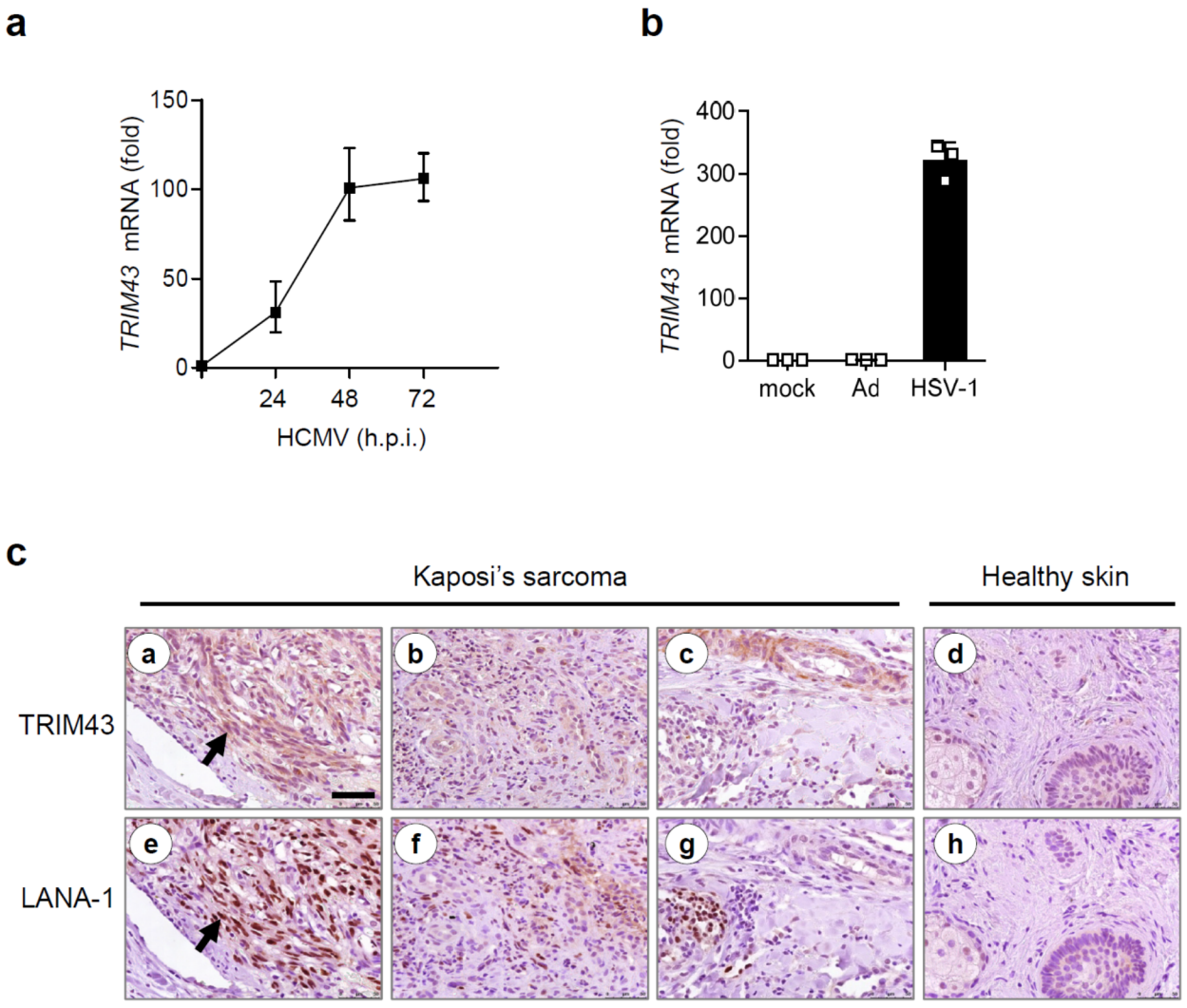
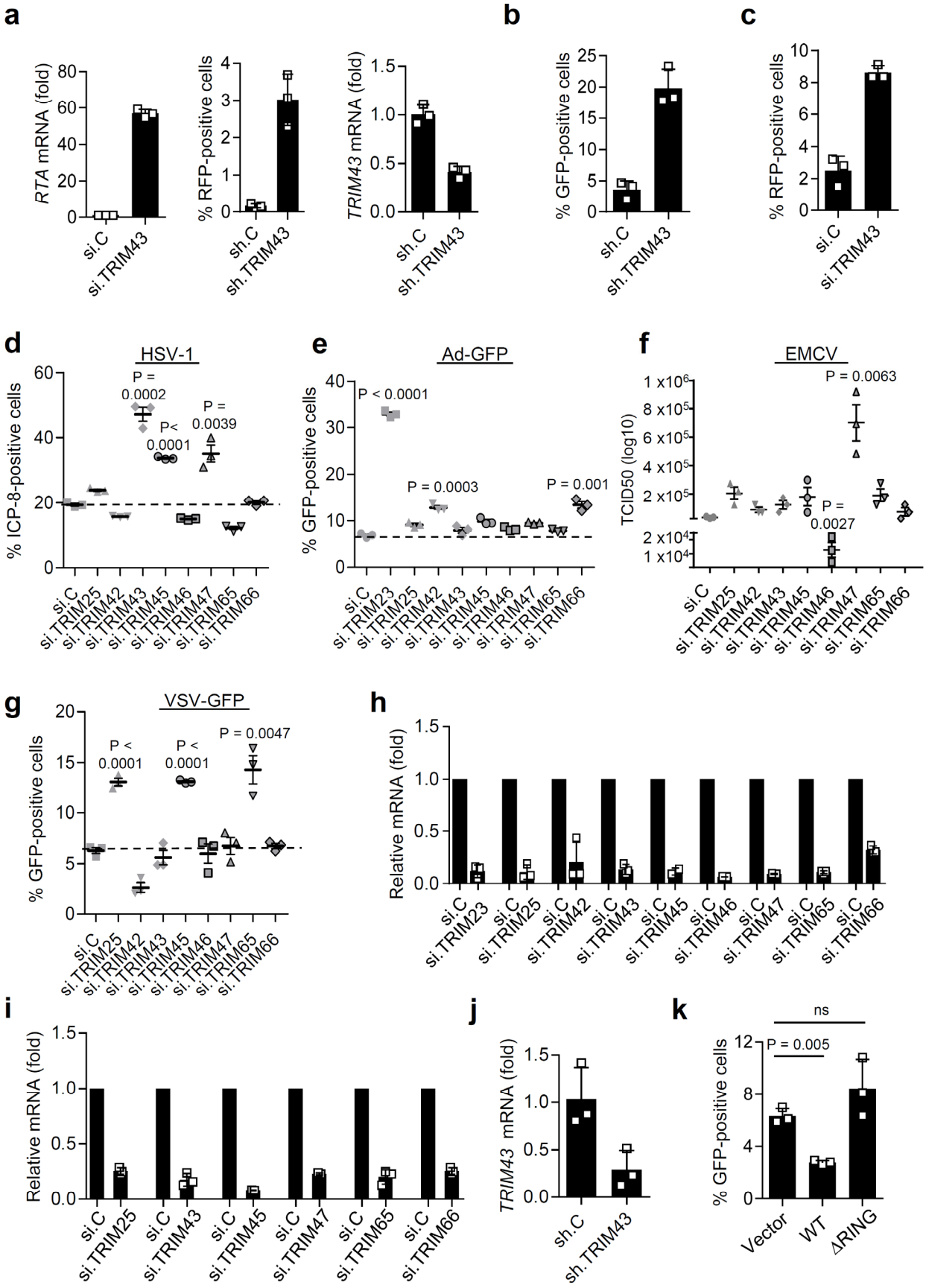
Supplementary Figure 11. The figure contains all raw Western Blot images of the study.

Supplementary Tables:

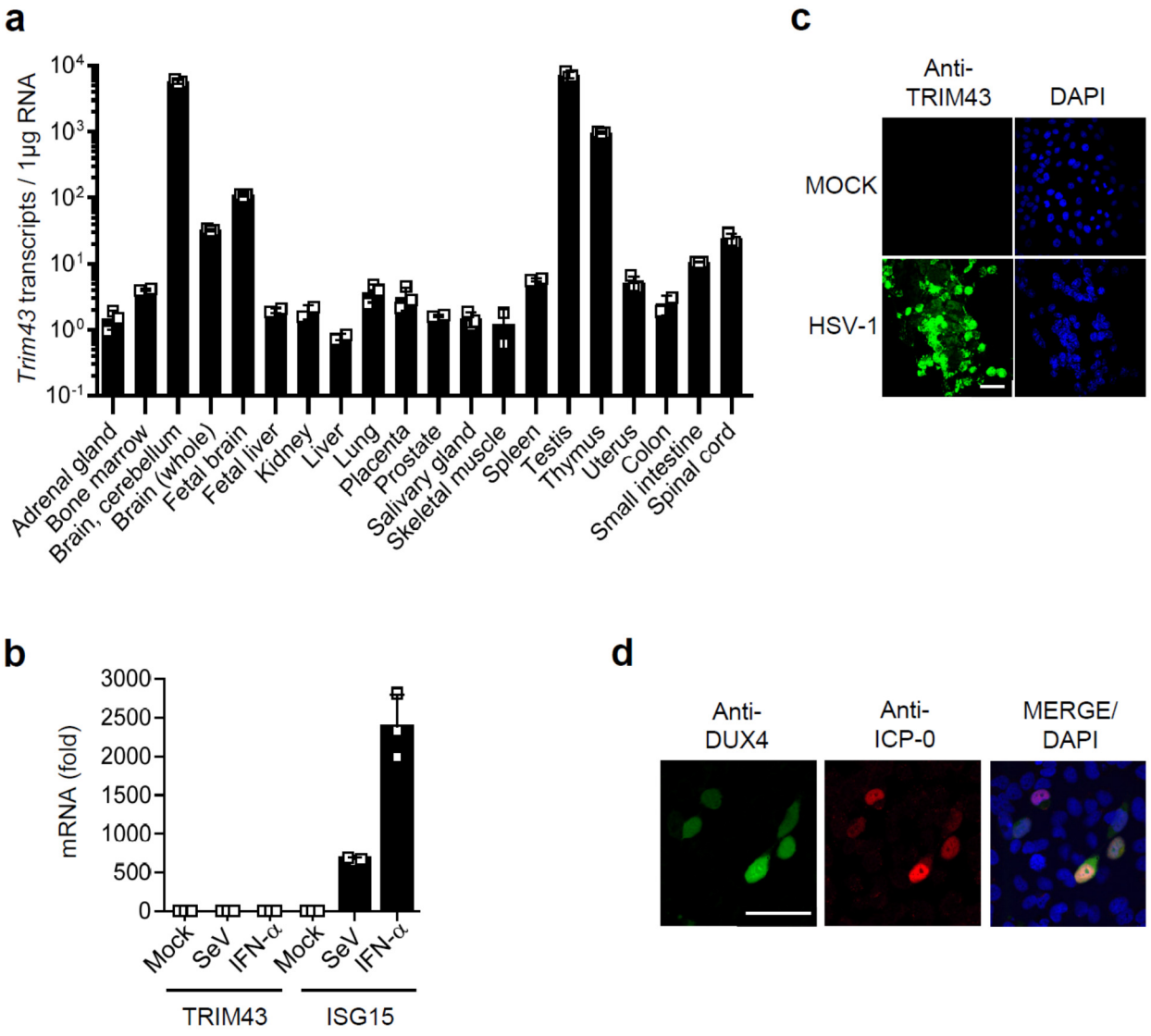
Supplementary Table 1. P-values of statistical tests (Student's *t*-test) from the data shown in Figure 1b.



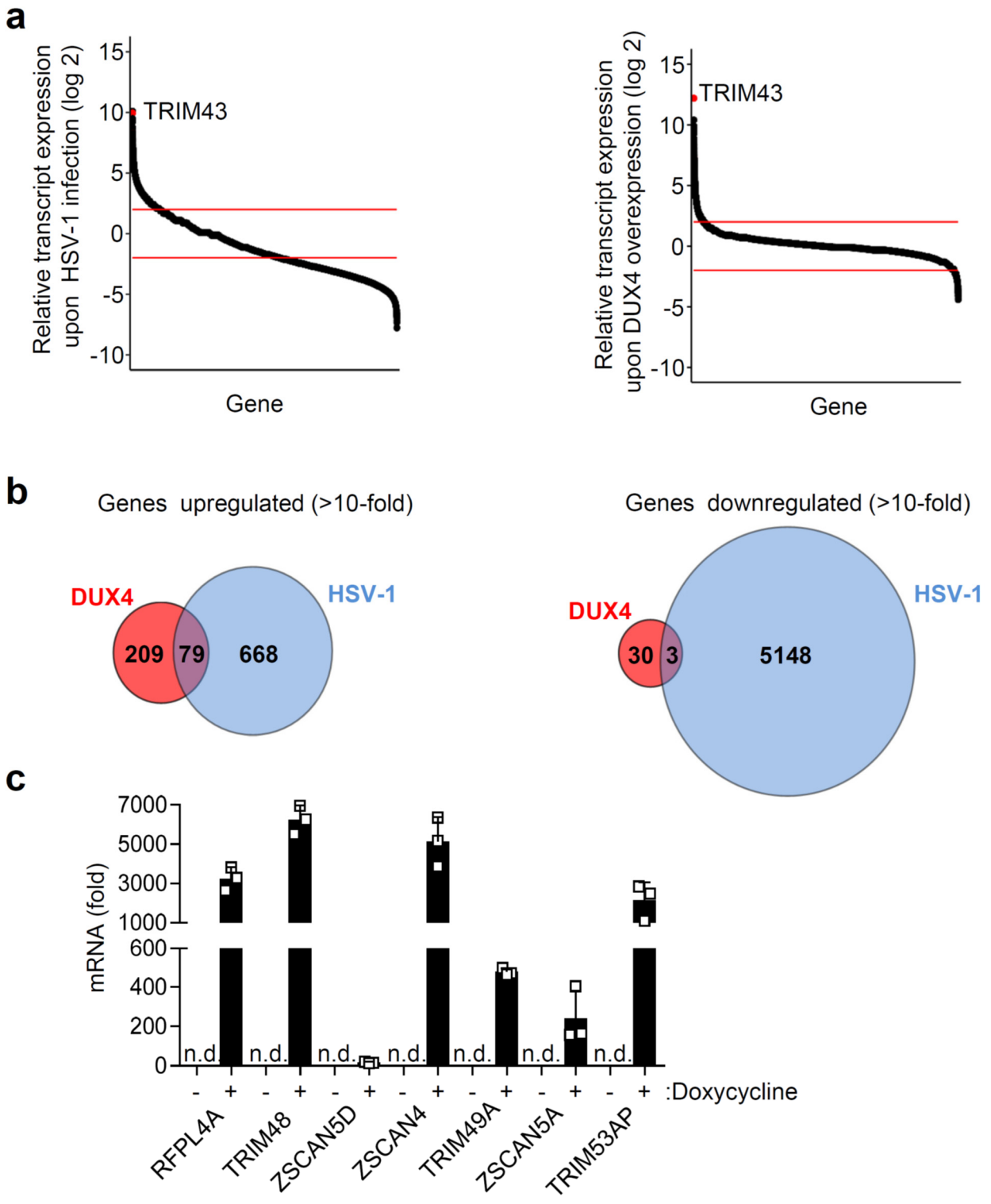
Supplementary Figure 1. Expression of TRIM43 in virus-infected cells and AIDS-associated Kaposi's sarcoma. **a**, *TRIM43* transcripts in primary human foreskin fibroblasts (HFFs) infected with HCMV (MOI 0.1) for the indicated times. Values were normalized to cellular *HPRT*, and are presented as fold induction relative to mock-infected control cells. **b**, *TRIM43* transcripts in HEK 293T cells infected with Ad or HSV-1 (MOI 0.2 for each) for 36 h. Values were normalized to cellular *HPRT*, and are presented as fold induction relative to mock-infected control cells. Data represent mean and s.d. of n = 3 (biological replicates). **c**, Immunohistochemical detection of TRIM43 protein in AIDS-associated Kaposi's sarcoma (AIDS-KS). TRIM43 protein was highly expressed in some AIDS-KS tissues, where TRIM43 was found to be expressed in LANA-1-positive KS spindle cells (a and e, arrows). In other AIDS-KS tissues TRIM43 was moderately expressed; in these tissues, TRIM43 expression was minimally (b and f), or not (c and g) localized to LANA-1-positive KS spindle cells. TRIM43 was minimally expressed in healthy skin (LANA-1-negative) of AIDS-KS patients (d and h). Scale bar, 50 μm. Results shown are representative of three independent experiments (**a**,**b**), or representative images of tissue samples from 17 individuals (**c**).


a
RTA mRNA (fold)
si.C
si.TRIM43
% RFP-positive cells
sh.C
sh.TRIM43
TRIM43 mRNA (fold)
b
% GFP-positive cells
c
% RFP-positive cells
d
HSV-1
% ICP-8-positive cells
P = 0.0002
P< 0.0001
P = 0.0039
si.C
si.TRIM25
si.TRIM42
si.TRIM43
si.TRIM45
si.TRIM46
si.TRIM47
si.TRIM65
si.TRIM66
e
Ad-GFP
% GFP-positive cells
P < 0.0001
P = 0.0003
P = 0.001
si.C
si.TRIM23
si.TRIM25
si.TRIM42
si.TRIM43
si.TRIM45
si.TRIM46
si.TRIM47
si.TRIM65
si.TRIM66
f
EMCV
TCID50 (log10)
P = 0.0063
P = 0.0027
si.C
si.TRIM25
si.TRIM42
si.TRIM43
si.TRIM45
si.TRIM46
si.TRIM47
si.TRIM65
si.TRIM66
g
VSV-GFP
% GFP-positive cells
P < 0.0001
P < 0.0001
P = 0.0047
si.C
si.TRIM25
si.TRIM42
si.TRIM43
si.TRIM45
si.TRIM46
si.TRIM47
si.TRIM65
si.TRIM66
h
Relative mRNA (fold)
si.C
si.TRIM23
si.TRIM25
si.TRIM42
si.TRIM43
si.TRIM45
si.TRIM46
si.TRIM47
si.TRIM65
si.TRIM66
i
Relative mRNA (fold)
si.C
si.TRIM25
si.TRIM43
si.TRIM45
si.TRIM47
si.TRIM65
si.TRIM66
j
TRIM43 mRNA (fold)
sh.C
sh.TRIM43
k
ns
P = 0.005
% GFP-positive cells
Vector
WT
ΔRING

Supplementary Figure 2. Effect of TRIM43 knockdown or its ectopic expression on virus lytic replication or reactivation. a*,* HEK 293T cells were transduced with non-targeting control shRNA (sh.C) or TRIM43-specific shRNA (sh.TRIM43) and selected with 1 µg/µL puromycin for 5 d. Cells were then infected with rKSHV.219 (MOI 2). Viral RTA/orf50 expression was determined by qRT-PCR at 48 h post-infection (left), or viral RFP expression analyzed by flow cytometry at 72 h post-infection (middle). TRIM43 knockdown efficiency was confirmed by qRT-PCR analysis (right). **b***,* Cell supernatants from (a) were collected at 72 h post-infection and used for *de novo* infection of HEK 293T cells. 24 h later, viral GFP expression, indicative of lytic replication, was analyzed by flow cytometry. **c***,* iSLK.219 cells were transfected with TRIM43-specific siRNA (si.TRIM43) or control non-targeting siRNA (si.C). 36 h later, cells were treated with 500 µM sodium butyrate to induce KSHV reactivation. 72 h later, viral RFP expression was analyzed by flow cytometry. **d–g**, Raw data for Figure 1h showing the effect of silencing of the indicated TRIM proteins on the replication of HSV-1 (d), Ad-GFP (e), EMCV (f) and VSV-GFP (g). HEK 293T (used for Ad-GFP and EMCV infection) or Huh7 cells (used for HSV-1 and VSV-GFP infection) were transfected either with si.C or the indicated TRIM-specific siRNAs. 36 h later, cells were infected with HSV-1 (MOI 0.1) for 36 h, Ad-GFP (MOI 0.1) for 72 h, EMCV (MOI 1) for 8 h, or VSV-GFP (MOI 0.01) for 24 h. Virus replication was determined by analyzing GFP-positive cells (Ad-GFP and VSV-GFP) or ICP-8-positive cells (HSV-1) using FACS, or TCID50 assay (EMCV). For the experiment with Ad-GFP, knockdown of TRIM23 served as additional control. **h***,***i***,* Knockdown efficiency of the indicated TRIM genes in HEK 293T (h) and Huh7 cells (i) for the experiments shown in (d–g), determined by qRT-PCR at 36 h after siRNA transfection. Relative knockdown efficiency is shown as compared to values for si.C-transfected control cells. *TRIM42* and *TRIM46* transcripts were not detectable in Huh7 cells. **j***,* Knockdown of endogenous *TRIM43* for the experiment shown in Figure 1k, determined by qRT-PCR at 48 h after siRNA transfection. **k***,* HEK 293T cells were transfected with empty vector or plasmids encoding wild-type (WT) TRIM43 or mutant TRIM43 ΔRING. 24 h later, cells were infected with HSV-1-GFP (MOI 0.01). 40 h later, virus-infected cells were analyzed by determining GFP using flow cytometry. Data represent mean and s.d. of n = 3 (biological replicates) (**a**–**k**), and statistical significance was calculated by unpaired two-tailed *t*-test. Data are representative of three (**a**–**c, h**–**k**) or two (**d**–**g**) independent experiments. ns, statistically not significant.

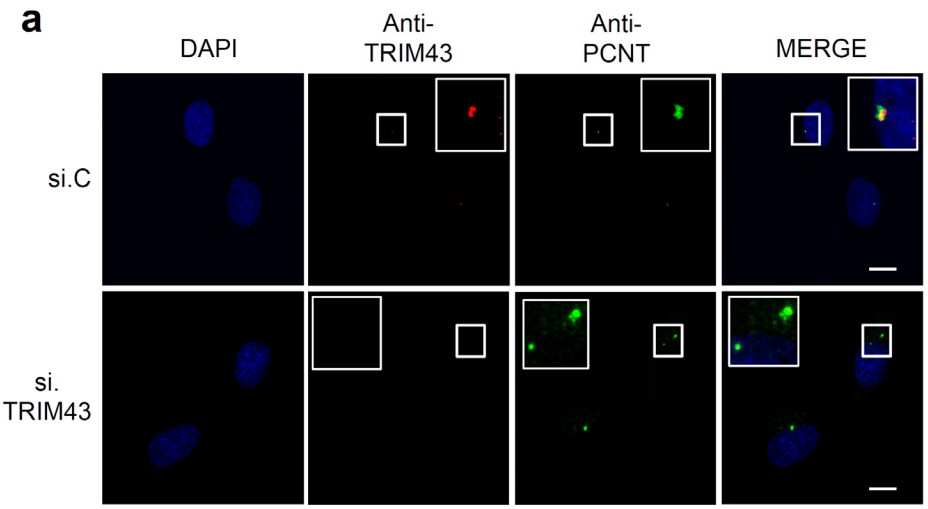


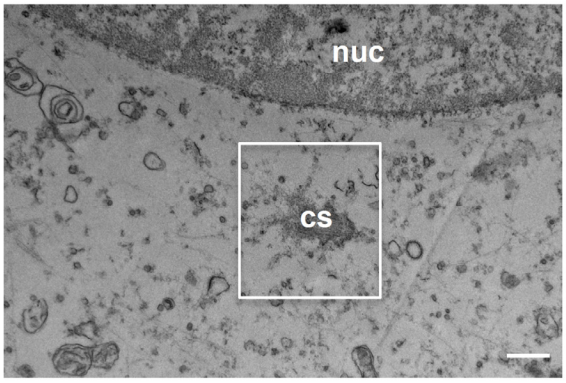
Supplementary Figure 3. TRIM43 and DUX4 expression in human tissues or cells. **a**, Absolute number of *TRIM43* transcripts in the indicated human tissues, determined by qRT-PCR analysis. **b**, Relative transcript amounts of *TRIM43* and *ISG15*, which served as positive control, in HEK 293T cells that were either mock-treated, treated with IFN-α2 (100 units/mL) or infected with Sendai virus (SeV) (50 HA units/mL) for 18 h. Values were normalized to *GAPDH* and are presented as fold induction relative to mock-treated cells. **c**, Endogenous TRIM43 protein expression in Vero cells that were infected with HSV-1 (MOI 5) for 20 h, or left uninfected (mock), determined by immunofluorescence (IF) analysis using an anti-TRIM43 antibody. Nuclei were stained with DAPI (blue). Scale bar, 100 µm. **d**, Expression of endogenous DUX4 protein in HeLa cells that were infected with HSV-1 (MOI 0.1) for 14 h, determined by IF using an anti-DUX4 antibody. HSV-1 ICP-0 was stained using an anti-ICP-0 antibody to visualize infected cells. Nuclei were stained with DAPI (blue). Scale bar, 100 µm. Data represent mean and s.d. of n = 3 (technical replicates) (**a**) or n = 3 (biological replicates) (**b**), and statistical significance was calculated by unpaired two-tailed *t*-test. Data are representative of three (**a**–**c**) or two (**d**) independent experiments.

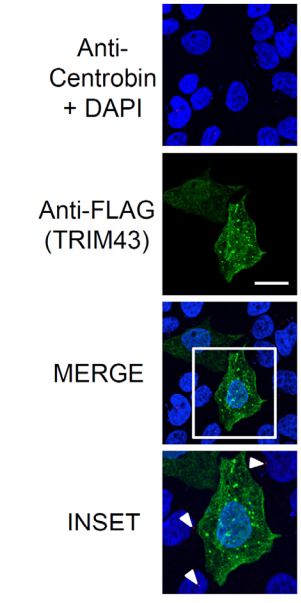


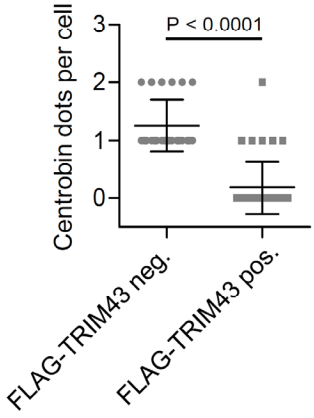
Supplementary Figure 4. Herpesvirus-induced transcriptional upregulation of DUX4 target genes. a, Global transcript expression in HEK 293T cells that were either infected with HSV-1 (MOI 5) for 18 h, or transfected with a plasmid encoding human DUX4 for 18 h, determined by RNAseq. Scatter blot shows relative gene expression upon HSV-1 infection (left) or DUX4 expression (right), relative to mock-treated control cells. Expression of *TRIM43* is shown in red

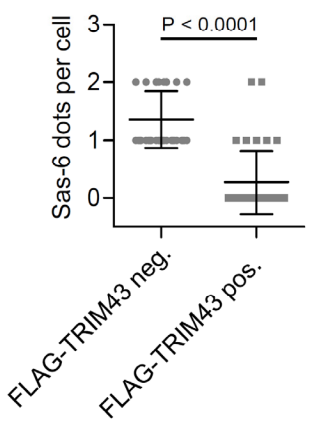
(dot). Red boundaries represent ± 4-fold change in gene expression. **b**, The overlap of genes from (a) that were upregulated (left panel) or downregulated (right panel) by > 10-fold relative to mock-treated control cells, shown by Venn diagrams. **c**, qRT-PCR analysis of the indicated transcripts, which are all known DUX4 target genes, in iSLK cells that were either left untreated, or were treated with doxycycline (1 μg/mL) for 96 h to induce KSHV reactivation. Values were normalized to cellular *HPRT*, and are presented as fold induction relative to mock-treated control cells. Ct values for transcripts that were undetectable (n.d.) in cells not treated with doxycycline were set to 45 (45 PCR cycles). Data represent mean and s.d. of n = 3 (biological replicates). Efficient KSHV reactivation upon doxycycline treatment was confirmed by monitoring viral RFP expression (not shown). Data are representative of two (**a**,**b**) or three (**c**) independent experiments.











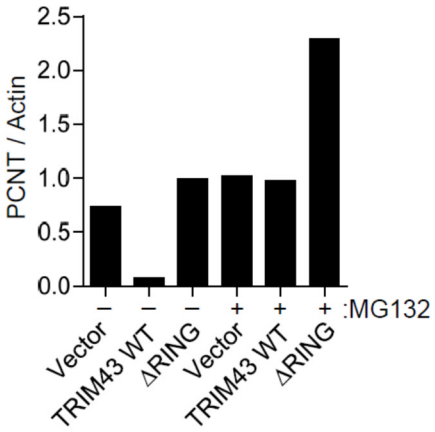
f

PCNT: 19 unique peptides

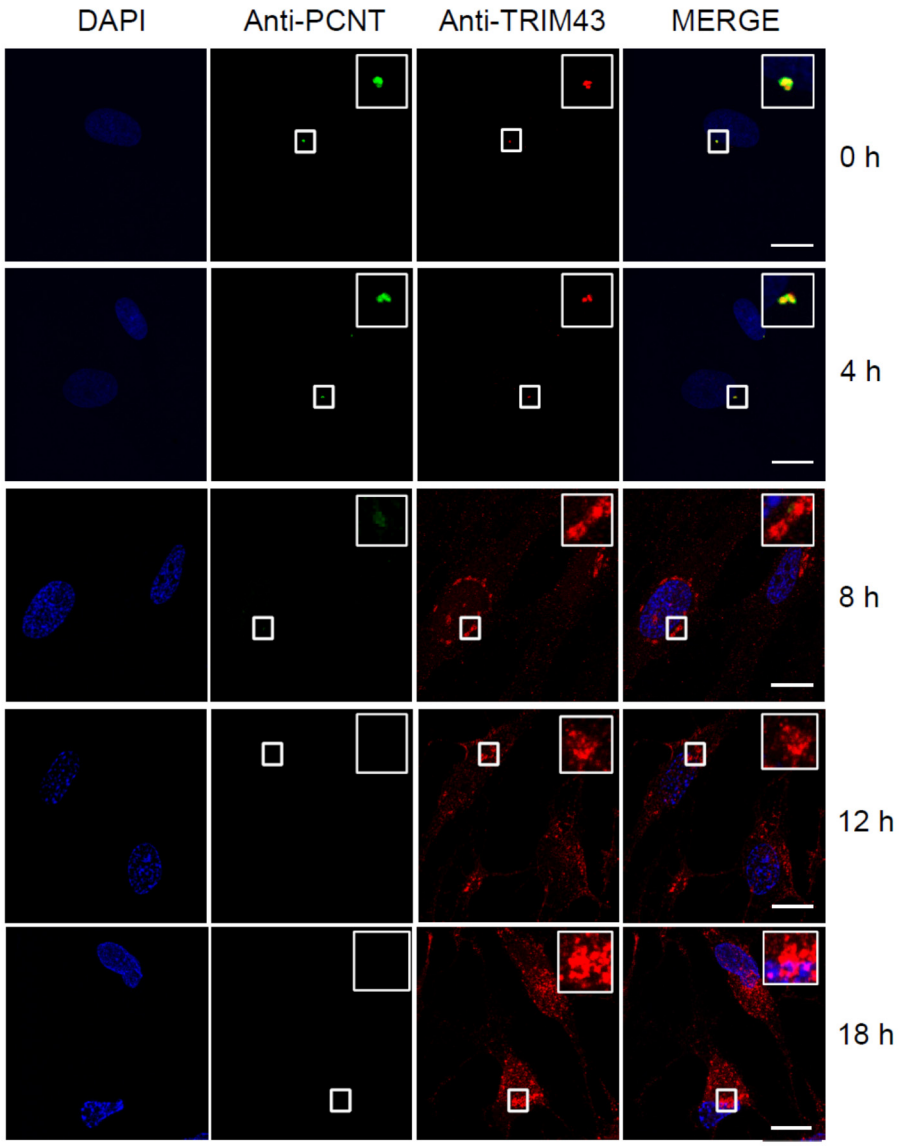
R.LLADQER.R
R.TVNDWTSSNEK.A
R.ELEEENTSLK.V
K.VAQLQEEVEKQK.N
R.FQAELEESHR.H
K.VAQLQEEVEK.Q
K.GSAVDASVQEESPVTK.E
K.QGALLAAR.V
R.LEEM*NINIR.K
R.GALQDALR.R
R.LLTEQLSQR.T
R.ALQSQLEEEQLR.H
K.LLAAEQTVVR.D
K.VDLVAQVK.Q
K.AELALELHK.T
R.LSPGSGGPEAQTAGPVTPASISGR.F
R.IQEFEAALK.A
R.TLELSEALR.H
R.VVDLQAMLEK.V

Supplementary Figure 5. TRIM43 is a centrosomal protein. a*,* HFF cells were transiently transfected with either non-targeting control siRNA (si.C) or TRIM43-specific siRNA (si.TRIM43). 48 h later, cells were immunostained with anti-TRIM43 (red) and anti-PCNT (green) antibodies and subjected to confocal microscopy analysis. Nuclei, DAPI (blue). Scale bars, 10 μm. **b***,* Lower-magnification image of Figure 3b showing primary HFF cells stained with anti-TRIM43 antibody and protein A coupled to 5 nm gold particles. nuc, nucleus; cs, centrosome. Scale bar, 500 nm. **c***,* HeLa cells were transiently transfected with FLAG-TRIM43. 48 h later, cells were immunostained with anti-FLAG (green) and anti-Centrobin (red) antibodies and subjected to confocal microscopy analysis. Nuclei, DAPI (blue). White arrows indicate Centrobin foci (red). Scale bar, 10 μm. **d***,* Quantification of Centrobin foci from the experiment in (c) for 40 cells each that expressed FLAG-TRIM43 or not. The graph shows mean and s.d., and statistical significance was calculated by unpaired two-tailed Student's *t*-test. **e***,* Quantification of Sas-6 foci in HeLa cells that were transfected as in (c) and immunostained for FLAG-TRIM43 as well as endogenous Sas-6 using an anti-Sas-6 antibody. 40 cells each that expressed FLAG-TRIM43, or not, were quantified. The graph shows mean and s.d., and statistical significance was calculated by unpaired two-tailed Student's *t*-test. **f***,* Unique peptides of PCNT identified by mass spectrometry analysis of affinity-purified FLAG-TRIM43ΔRING from transiently transfected HEK 293T cells. Data are representative of three (**a**), two (**b**–**e**), or one (**f**) independent experiments.

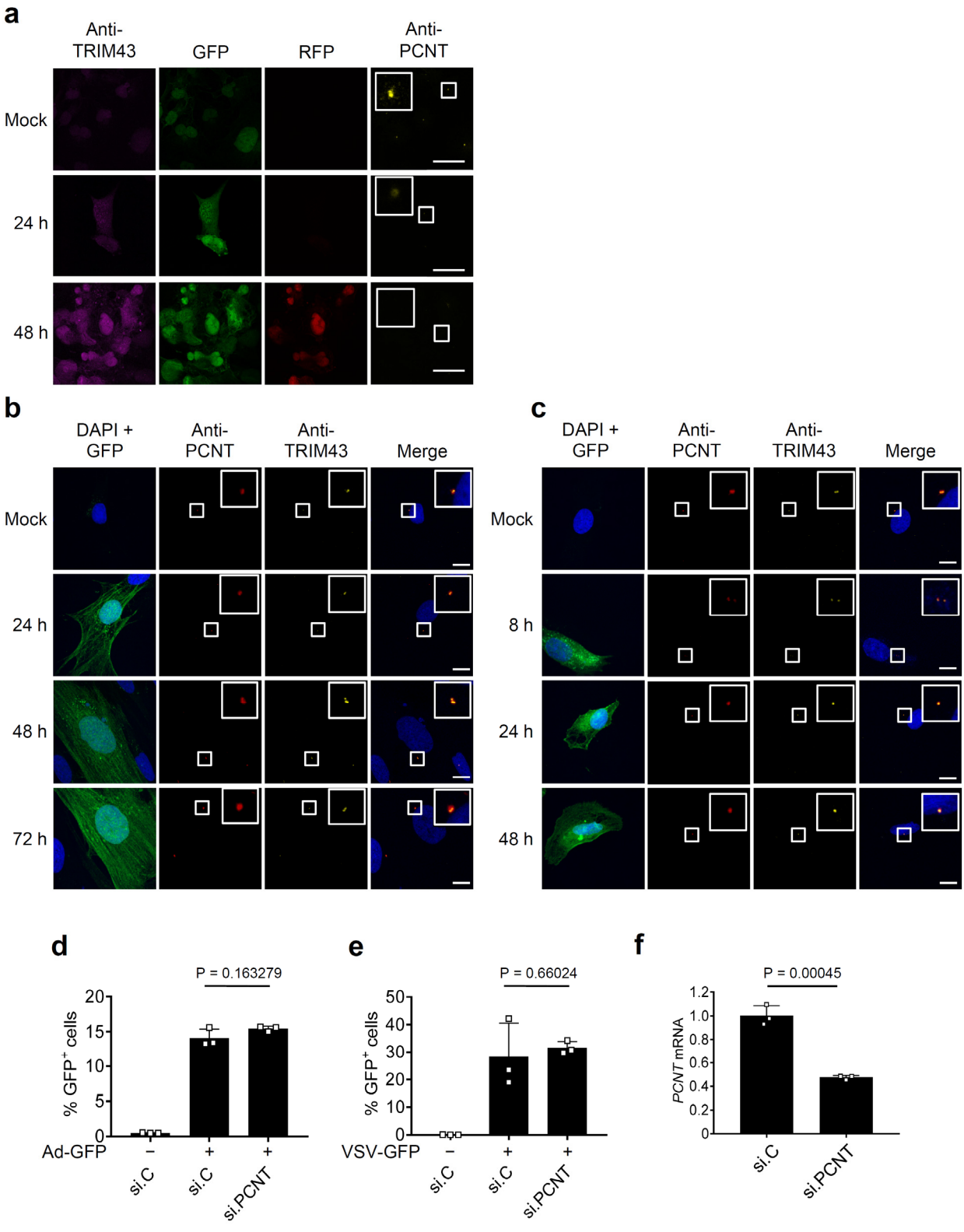
a


PCNT / Actin
2.5
2.0
1.5
1.0
0.5
0.0
– – – + + + :MG132
Vector
TRIM43 WT
ΔRING
Vector
TRIM43 WT
ΔRING

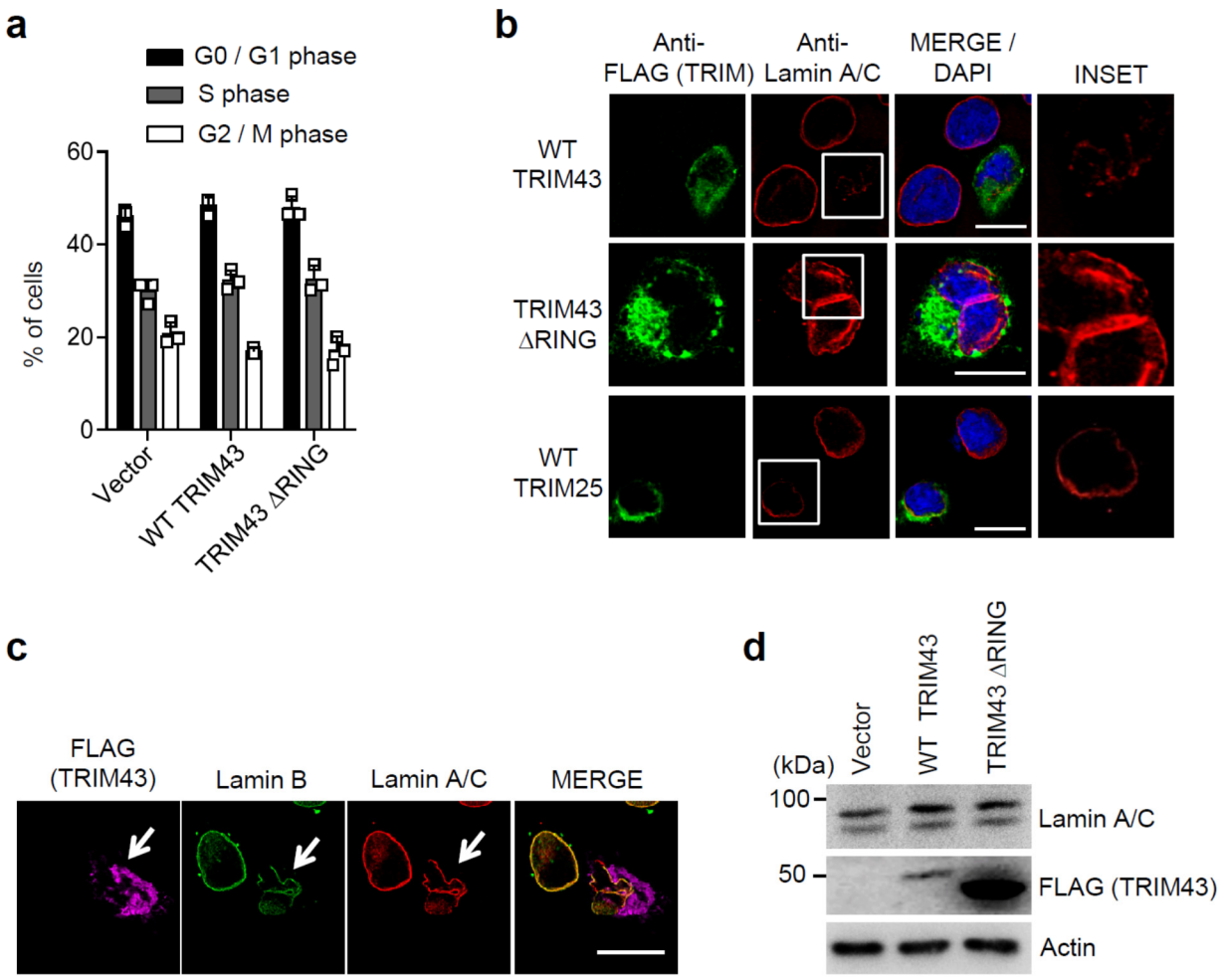
b


DAPI
Anti-PCNT
Anti-TRIM43
MERGE
0 h
4 h
8 h
12 h
18 h

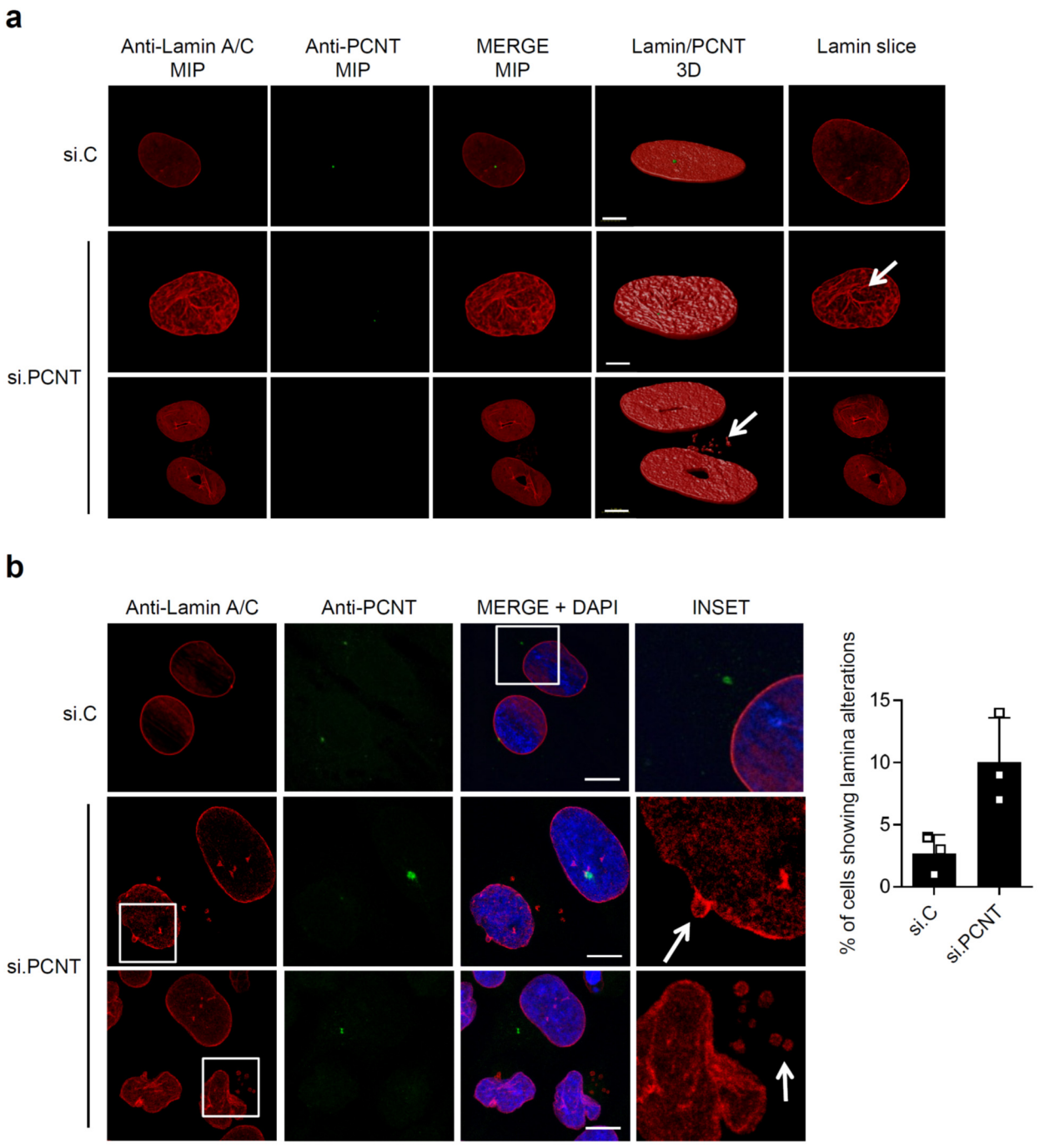
Supplementary Figure 6. PCNT degradation upon ectopic expression of TRIM43 or during HSV-1 infection. a*,* Densitometric analysis of endogenous PCNT protein abundance, normalized to cellular actin, from the Western blot shown in Figure 4a. **b***,* Endogenous TRIM43 and PCNT protein expression in primary HFF cells infected with HSV-1 (MOI 1) for the indicated times, determined by immunostaining using anti-TRIM43 (red) and anti-PCNT (green) antibodies, followed by confocal microscopy analysis. Nuclei, DAPI (blue). Scale bars, 20 μm. Data are representative of two (**a**) or three (**b**) independent experiments.


a
Anti-TRIM43
GFP
RFP
Anti-PCNT
Mock
24 h
48 h
b
DAPI + GFP
Anti-PCNT
Anti-TRIM43
Merge
Mock
24 h
48 h
72 h
c
DAPI + GFP
Anti-PCNT
Anti-TRIM43
Merge
Mock
8 h
24 h
48 h
d
P = 0.163279
% GFP+ cells
Ad-GFP
si.C
si.C
si.PCNT
e
P = 0.66024
% GFP+ cells
VSV-GFP
si.C
si.C
si.PCNT
f
P = 0.00045
PCNT mRNA
si.C
si.PCNT

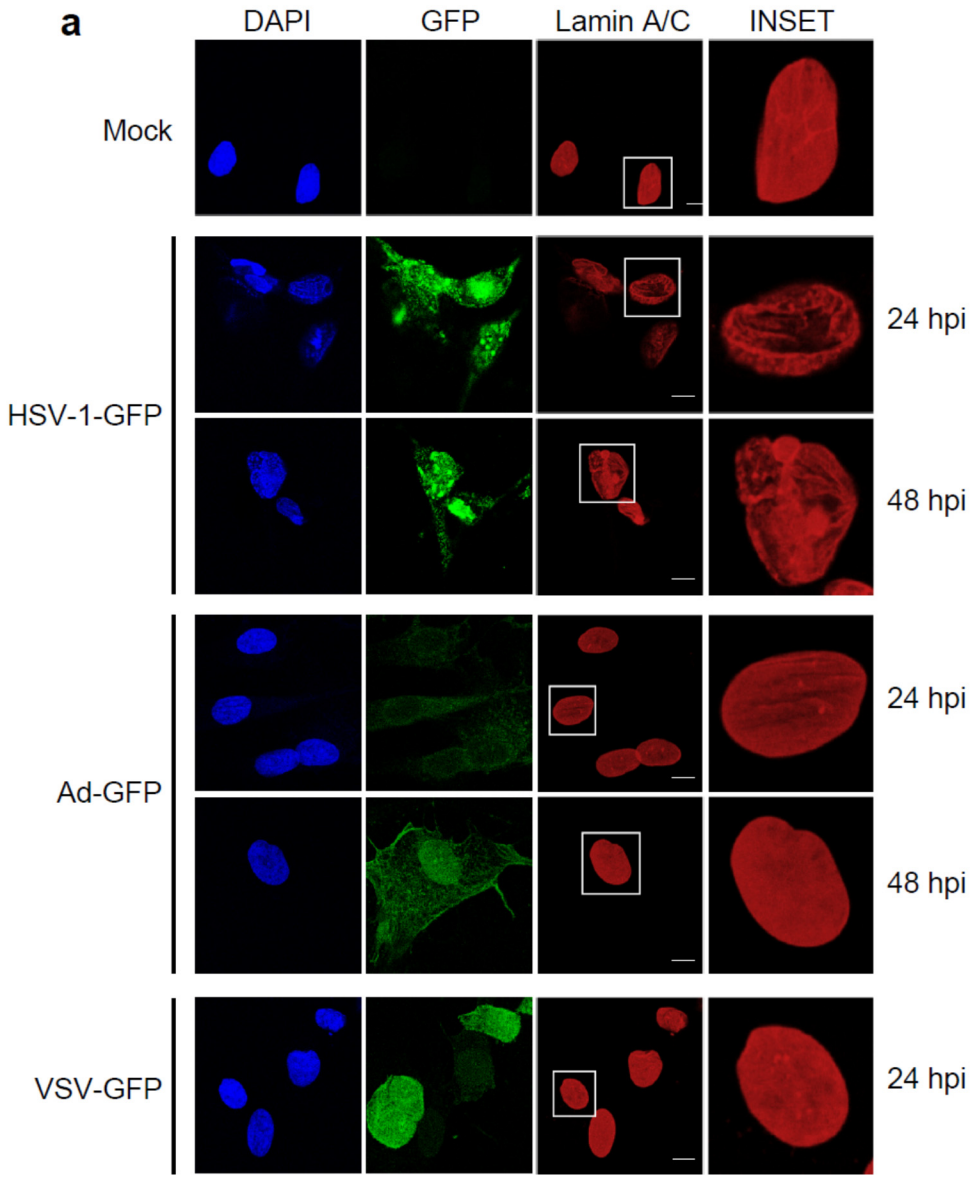
Supplementary Figure 7. TRIM43 and PCNT protein expression in virus-infected cells. **a**, Endogenous TRIM43 and PCNT protein expression in iSLK.219 cells that were either mock-treated or stimulated with 1 μg/mL of doxycycline for the indicated times, determined by immunostaining using anti-TRIM43 and anti-PCNT antibodies followed by confocal microscopy analysis. GFP- and RFP-positive cells represent KSHV.219-positive and reactivated cells, respectively. Nuclei, DAPI (blue). Scale bars, 20 μm. **b**,**c**, Endogenous TRIM43 and PCNT protein expression in HFF cells infected with Ad-GFP (MOI 30) (b) or VSV-GFP (MOI 5) (c) for the indicated times, determined as in (a). Nuclei, DAPI (blue). Scale bars, 10 μm. **d**,**e**, Replication of Ad-GFP (d) or VSV-GFP (e) in HFF cells that were transfected for 24 h with either non-targeting control siRNA (si.C) or PCNT-specific siRNA (si.PCNT) and then infected with Ad-GFP (MOI 30) or VSV-GFP (MOI 1) for 48 h. Virus-infected cells were determined by analyzing GFP-positive cells using FACS. **f**, Representative knockdown of PCNT for (d,e), determined by qRT-PCR analysis at 24 h after siRNA transfection. Data (**d**–**f**) represent mean and s.d. of n = 3 (biological replicates), and statistical significance was calculated by unpaired two-tailed *t*-test. Data are representative of three (**a, d**–**f**) or two (**b**,**c**) independent experiments.



Supplementary Figure 8. Enforced expression of TRIM43 induces nuclear lamina alterations. **a**, Cell cycle analysis of HEK 293 cells transfected with empty vector, FLAG-tagged TRIM43 WT or its ΔRING mutant. 36 h later, cells were stained with propidium iodide and analyzed by flow cytometry. Data represent mean and s.d. of n = 3 (biological replicates). **b**, Nuclear lamina morphology in HeLa cells that were transiently transfected with FLAG-tagged WT TRIM43 or its ΔRING mutant, or FLAG-tagged WT TRIM25 (control). 24 h after transfection, cells were immunostained with anti-Lamin A/C (red) and anti-FLAG (green) antibodies, followed by confocal microscopy analysis. Nuclei, DAPI (blue). Scale bar, 20 μm. **c**, HeLa cells were transfected with FLAG-tagged TRIM43. 36 h later, cells were immunostained for FLAG (magenta), endogenous Lamin A/C (red) and Lamin B (green) and subjected to confocal microscopy analysis. Nuclei, DAPI (blue). Scale bar, 20 μm. **d**, HEK 293T cells were transfected with empty vector or plasmids encoding FLAG-tagged TRIM43 WT or its ΔRING mutant. 36 h later, cells were lysed and whole cell lysates subjected to IB analysis with anti-Lamin A/C, anti-FLAG, and anti-Actin antibodies. Data are representative of three (**a**,**b**) or two (**c**,**d**) independent experiments.



Supplementary Figure 9. Nuclear lamina alterations upon PCNT knockdown. a,**b**, HFF (**a**) or HeLa (**b**) cells were transfected with non-targeting control siRNA (si.C) or siRNA targeting PCNT (si.PCNT). 48 h later, cells were immunostained with anti-Lamin A/C (red) and anti-PCNT (green) antibodies and subjected to confocal microscopy analysis. In (**a**), stacks of laser-scanning confocal images were subjected to 3D surface rendering. MIP: maximum intensity projection. Arrows indicate morphological lamin abnormalities or extranuclear lamin. Scale bars, 5 μm (**a**) or 10 μm (**b**). **c**, Quantification of nuclear lamina alterations from (b) (n = 300 cells). Data (**a**–**c**) are representative of three independent experiments.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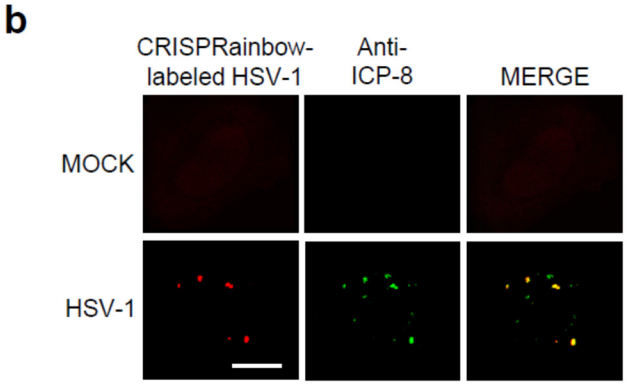
DAPI
GFP
Lamin A/C
INSET
Mock
HSV-1-GFP
24 hpi
48 hpi
Ad-GFP
24 hpi
48 hpi
VSV-GFP
24 hpi


b
CRISPRainbow-labeled HSV-1
Anti-ICP-8
MERGE
MOCK
HSV-1

Supplementary Figure 10. Nuclear lamina morphology in cells infected with HSV-1, Ad or VSV. a*,* Endogenous lamin A/C morphology in primary HFF cells that were either left uninfected (mock), or infected with HSV-1-GFP (MOI 0.1), Ad-GFP (MOI 30), or VSV-GFP (MOI 5) for the indicated times, determined by IF analysis using an anti-lamin A/C antibody. Nuclei, DAPI (blue). Scale bar, 10 µm. **b***,* Validation of CRISPRainbow labeling of HSV-1 genomes. Co-localization of CRISPRainbow-labeled HSV-1 genomes (red) with viral ICP-8 (green) in HeLa cells infected with HSV-1 (MOI 10) for 3 h, determined by confocal microscopy. Cells that were left uninfected (mock) served as control. Scale bar, 20 µm. Data are representative of two (**a***,***b**) independent experiments.

Figure 1j

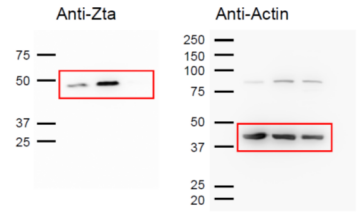


Figure 2c

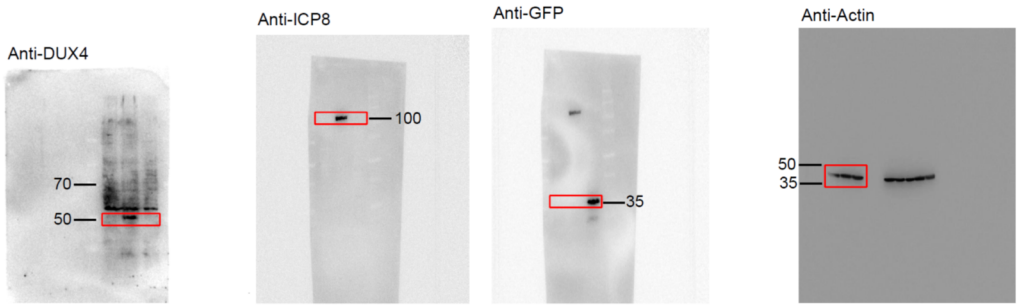


Figure 2f

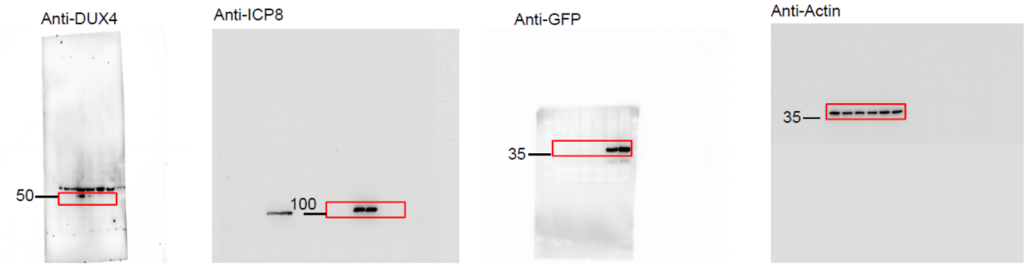


Figure 4a

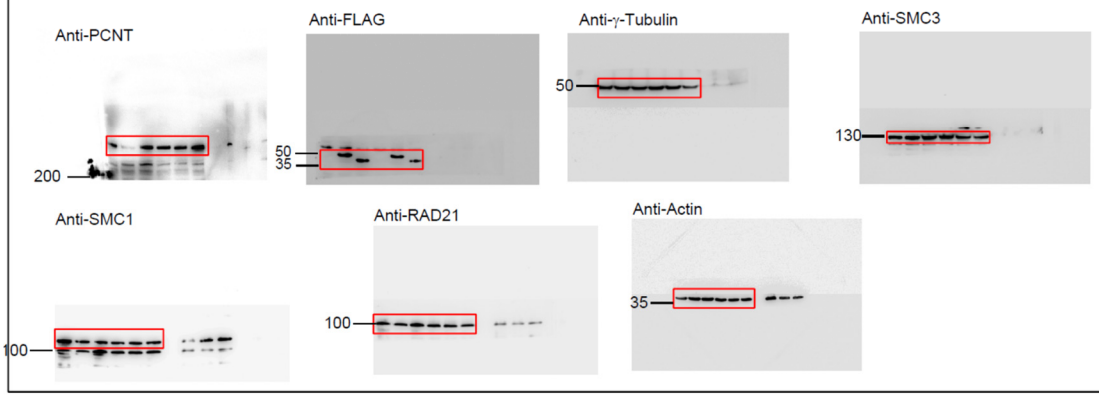


Figure 4c

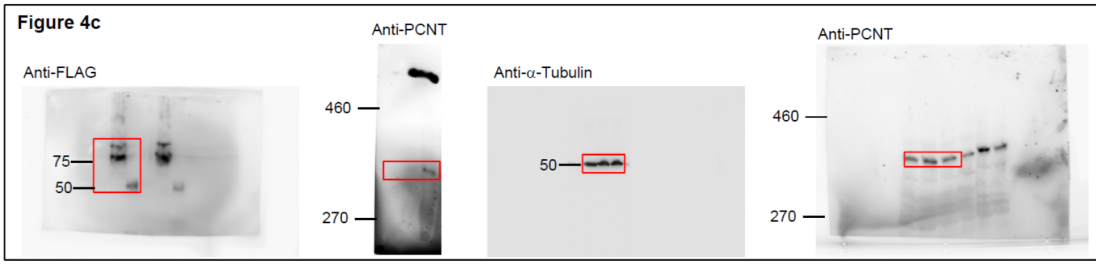
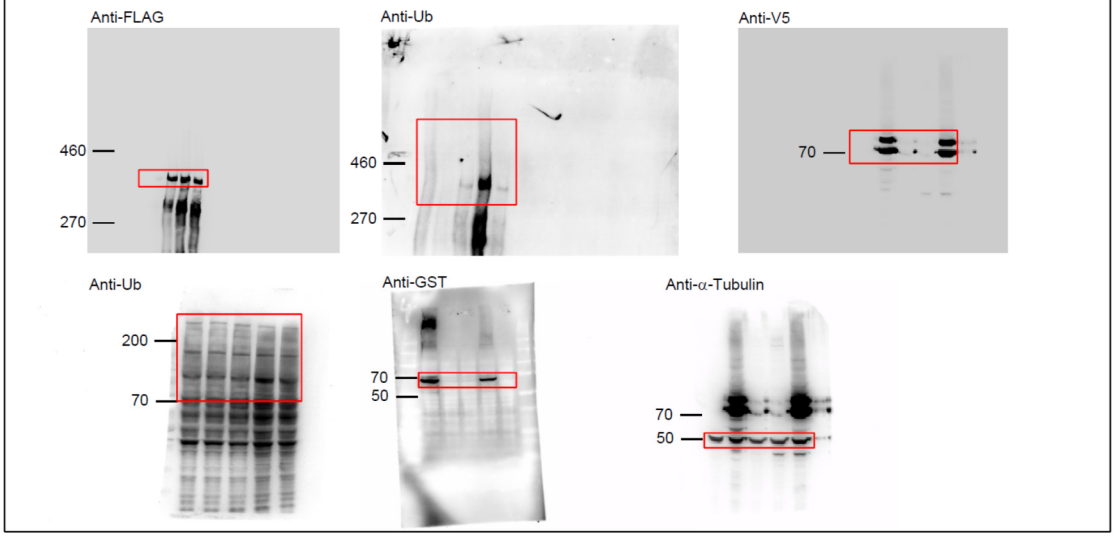
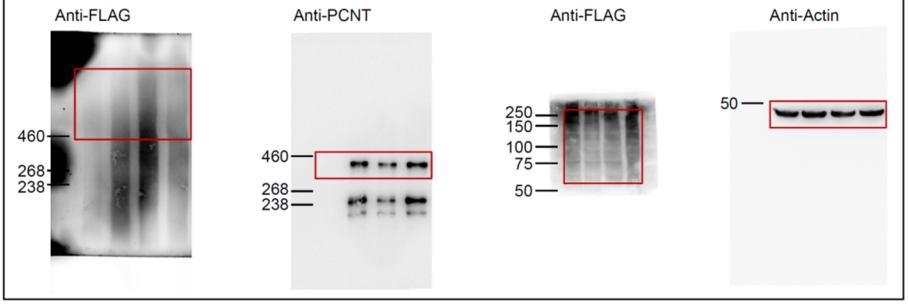


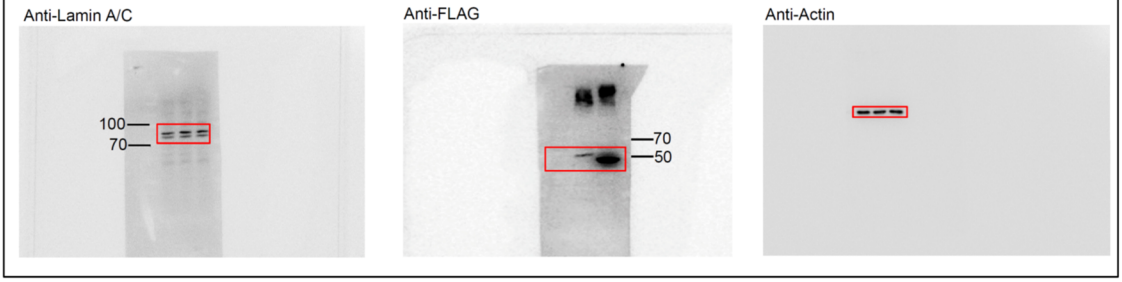
Figure 4e



Supplementary Figure 4f



Supplementary Figure 8c



Supplementary Figure 11. Complete western blot images of the indicated figures of the manuscript. Red boxes indicate the cropped areas shown in the indicated figures. Numbers indicate molecular weight markers (in kDa).

Supplementary Table 1. Exact P values for the data shown in Figure 1b.

Figure 1b		Student's *t*-test, two-sided	P-value
	shRNA mediated knockdown of TRIM proteins in 293.rKSHV219 cells; RFP by flow cytometry		
		si.C vs si.TRIM8	0.0017
		si.C vs si.TRIM15	0.0016
		si.C vs si.TRIM20	0.0411
		si.C vs si.TRIM33	0.0079
		si.C vs si.TRIM34	0.0013
		si.C vs si.TRIM36	0.0089
		si.C vs SI.TRIM41	0.0095
		si.C vs si.TRIM42	0.0024
		si.C vs si.TRIM43	>0.0001
		si.C vs si.TRIM45	0.0001
		si.C vs si.TRIM46	0.0015
		si.C vs si.TRIM47	0.0002
		si.C vs si.TRIM54	0.0059
		si.C vs si.TRIM65	0.0004
		si.C vs si.TRIM66	0.0003
		si.C vs si.TRIM69	0.013

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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	ABI 7500 software Leica LAS AF BD FACS DIVA Software

Data analysis	MS Excel GraphPad Prism 7.03 ImageJ TRIM Galore! Cutadapt FastQC Tophat2 HTSeq-count R ggplot2 ABI 7500 software Leica LAS AF Huygens Professional Flowjo FCS Express

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

No restrictions on availability of data or unique materials. The RNAseq data from this study are deposited in NCBI GEO under accession code GSE101435 and will be made available to the public before publication of our manuscript.

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The number of experiments and replicates was based on standard practices. In general, two or three independent experiments were conducted with three biological or technical replicates where applicable. Details for each experiment are included in the respective figure legend.
Data exclusions	No data were excluded from the analysis.
Replication	The experimental findings were reliably reproduced. Data from cell lines were generated using at least three independent biological replicates, and independently repeated as indicated in the figure legends. Data involving human tissues/samples were generated using multiple individual donors.
Randomization	Randomization is not relevant to our study because it does not contain randomized controlled in vivo research involving animals or human subjects. Patient tissues were assigned to different groups based on being negative or positive for viruses (KSHV and HSV-1 respectively) as stated in detail in the Methods section.
Blinding	No blinding was performed.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

Broncheoalveolar lavage (BAL) samples were selected from the sample repository of the diagnostics department of the Institute for Clinical and Molecular Virology at the University Hospital Erlangen (Erlangen, Germany). Exclusively samples that were no longer needed for diagnostic purposes and assigned for disposal were used. Samples were anonymized and no patient information was available.

Kaposi's sarcoma (KS) tissues were obtained from skin biopsies of 17 HIV-1-infected individuals with AIDS in the context of medically required diagnostic or therapeutic procedures. Uninvolved tissues of the skin of five of the patients as well as cerebellum tissue of an HIV-1-negative person were obtained as controls. The use of these post-diagnostic tissues was approved by the ethics committee of the University Hospital Erlangen, Germany. Samples were anonymized and no patient information was available.

Antibodies

Antibodies used

Information on all antibodies used in our study is provided in the Materials & Methods section of the manuscript, including manufacturer and clone numbers.

Western Blot:
anti-FLAG (M2; 1:2,000; Sigma), anti-HA (1:2,000; clone HA-7; Sigma), anti-ICP-8 (1:5,000, provided by David Knipe, Harvard), anti-Zta (1:500, BZ1, Santa Cruz), anti-V5 (1:5,000, R960-25, Novex), anti-GST (1:5,000, Sigma), anti-PCNT (1:1,000, clone 28144, Abcam), anti-Lamin A/C Antibody (clone 636, Santa Cruz Biotech), anti-γ-Tubulin (1:1,000, clone GTU-88, Abcam), anti-DUX4 (1:1,000, clone 9A12, Millipore), anti-SMC1a (1:500, rabbit polyclonal, Bethyl antibodies) anti-SMC3 (1:500, ab9263, Abcam), anti-RAD21 (1:500, 05-908, Millipore), anti-α-Tubulin (1:1,000, rabbit polyclonal, Genscript), anti-ubiquitin (1:500, clone P4D1, Santa Cruz Biotech), anti-β-Actin (1:10,000; Abcam or AC-15, Sigma).

Immunofluorescence:
anti-TRIM43 (1:400; Abcam 80460), anti-DUX4 (1:4,000; clone 9A12, Millipore), anti-ICP-8 (1:1,000; provided by David Knipe, Harvard), anti-PCNT (1:400; clone 28144, Abcam), anti-γ-Tubulin (1:400; clone GTU-88, Abcam), anti-FLAG (1:1,000; M2, Sigma), anti-Lamin A/C Antibody (clone 636, Santa Cruz Biotech), anti-Lamin B1 (clone B10, Santa Cruz Biotech), anti-Centrobin (1:500; Sigma), anti-SAS-6 (1:400; clone G1, Santa Cruz).

Validation

Commercially obtained antibodies were validated by manufacturers usually by Western blot analysis. We have validated the TRIM43 antibody by performing IF analysis in cells in which TRIM43 was silenced using specific siRNA, as compared to cells transfected with non-targeting control siRNA. The ICP-8 antibody was a gift from David Knipe (Harvard Medical School) and validated in Knipe DM, Senechek D, Rice SA, Smith JL. 1987. Stages in the nuclear association of the herpes simplex virus transcriptional activator protein ICP4. J. Virol. 61:276–284.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK 293 (ATCC), HEK 293T (ATCC), HeLa (ATCC), A549 (ATCC), Vero (ATCC). Huh7, iSLK, iSLK.219, AKBM, BJAB and AGS-EBV cells were obtained from other investigators, who have published these cell lines and confirmed their authentication.

Authentication

Cell lines from ATCC were authenticated by the vendor and were not validated further in our laboratory. Cell lines that were obtained and validated by other groups were not further authenticated.

Huh7:
Growth of human hepatoma cells lines with differentiated functions in chemically defined medium.
Nakabayashi H, Taketa K, Miyano K, Yamane T, Sato J. Cancer Res. 1982 Sep;42(9):3858-63.

iSLK and iSLK.219:
Generation of a doxycycline-inducible KSHV producer cell line of endothelial origin: maintenance of tight latency with efficient reactivation upon induction. Myoung J, Ganem D. J Virol Methods. 2011 Jun;174(1-2):12-21.

AGS-EBV:
Infection of Epstein–Barr virus in a gastric carcinoma cell line induces anchorage independence and global changes in gene expression. Marquitz et al. PNAS 2012 Jun;109(24)9593-58.

AKBM:
Impaired Transporter Associated with Antigen Processing-Dependent Peptide Transport during Productive EBV Infection. Ressing M. E. et al. J. Immunol. 2005 Jun;174(11):6829-38.

Mycoplasma contamination: Cell lines have been regularly tested for potential mycoplasma contamination by PCR and/or using the MycoAlert Kit (Lonza).

Commonly misidentified lines (See ICLAC register): SLK cells have been misidentified as Caki-1 cells in the ICLAC database; however, iSLK cells are a genetically engineered version of SLK cells for production of KSHV and widely used in the KSHV field, not necessarily as a model cell line for endothelial tissue.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation: Cells were harvested by detaching them using 0.1% EDTA (in PBS, pH 7.4), and then pelleted by centrifugation at 300 × g for 5 min. Cells were fixed with 2% (w/v) PFA (in PBS) for 15 min, followed by washing cells twice with PBS. For antibody staining, cells were incubated with blocking/permeabilization buffer (5% [v/v] FBS and 0.1% [w/v] Saponin in PBS), followed by staining with primary (anti-ICP-8; 1:1,000) and secondary antibody (anti-rabbit-Alexa 488 (1:400) on ice in blocking/ permeabilization buffer for 30 min each.

Instrument: LSRII flow cytometer (BD Biosciences)

Software: FACS Diva (BD Biosciences), FlowJo (Tree Star) and FCS Express 3 softwares (De Novo Software)

Cell population abundance: N.A.

Gating strategy: The main cell population was gated in the FSC/SSC blot and then analyzed in either the GFP/Alexa-488 or RFP channel. Positive cells were above cutoff value, which is set to 98% of measured events of the negative control.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.