SUPPLEMENTAL INFORMATION

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KEY RESOURCES TABLE

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Antibodies and reagents		
Anti-PIAS1 (for Western Blot, C-term)	Cell Signaling Tech	Cat# 3550
Anti-PIAS1 (for Western Blot, N-term)	Abcam	Cat# ab109388
Anti-cleaved PARP	Cell Signaling Tech	Cat# 5625
Cleaved caspase antibody kit	Cell Signaling Tech	Cat# 9929
Anti-cleaved caspase-8	Cell Signaling Tech	Cat# 9496
Anti-cleaved caspase substrate motif	Cell Signaling Tech	Cat# 8698
Anti-PIAS1 (for ChIP)	Abcam	Cat# 77231
Anti-SUMO2/3	MBL International	Cat# M114-3
Anti-V5-HRP	Invitrogen/Thermo Fisher	Cat# R961-25
Anti-HA-HRP	Cell Signaling Tech	Cat# 14031
Anti-HA (for ChIP)	Cell Signaling Tech	Cat# 3724
Anti-HA magnetic beads	Thermo Fisher	Cat# 88836
Anti-Flag M2 magnetic beads	Sigma	Cat# M8823
Anti-Flag M2 HRP	Cell Signaling Tech	Cat# 86861
Anti-C/EBPβ	Bethyl	Cat# A302-738A-T
Mouse anti-β-actin antibody	MP Biomedicals	Cat# 691001
Anti-human IgG (for IgG cross-linking)	MP Biomedicals	Cat# 55087
anti-human IgM (for IgM cross-linking)	Southern Biotech	Cat# 2020-01
Anti-ZTA	Argene	Cat# 11-007, Discontinued
Anti-ZTA(BZ1)	Santa Cruz	Cat# sc-53904
Anti-RTA	Argene	Cat# 11-008, Discontinued
EBV LMP-2A Antibody (15F9)	Santa Cruz	Cat# sc-101315
Anti-BGLF4	(Wang et al., 2005)	Clone #s 2616 and 2224
Anti-H3K4Me3	Cell Signaling Tech	Cat# 9751

Anti-H3K27Me3	Cell Signaling Tech	Cat# 9733
Anti-H3K9Me3	Cell Signaling Tech	Cat# 9754
Anti-H3K9Ac	Cell Signaling Tech	Cat# 9671
Histone H3 (D2B12) (ChIP Formulated)	Cell Signaling Tech	Cat# 4720
Normal Rabbit IgG	Cell Signaling Tech	Cat# 2729
SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads)	Cell Signaling Tech	Cat# 9003
MG132	Sigma	Cat# M7449
chloroquine	Sigma	Cat# C6628
pan-caspase inhibitor (Z-VAD-FMK)	ApexBio	Cat# A1902
Caspase-3/7 inhibitor (Z-DEVD-FMK)	ApexBio	Cat# A1920
Caspase-6 inhibitor (Z-VEID-FMK)	ApexBio	Cat# A1923
Caspase-8 inhibitor (Z-IETD-FMK)	ApexBio	Cat# B3232
Active human caspases group IV	ApexBio	Cat# K2060
Halo-tag protein purification kit	VWR/Promega	PAG6790
TPA	Fisher Scientific	Cat# NC9325685
Sodium Butyrate (NaBu)	Millipore	Cat# 19-137
Gemcitabine	Santa Cruz	Cat# SC-204763
Constructs		
pLX304-PIAS1	DNASU	Clone# HsCD00443565
pLX304-PIAS1 (with PAM mutated)	This study	pRL1
pLX304-PIAS1-D100/433A (with PAM mutated)	This study	pKZ89
pLX304	Addgene	#25890
lentiCRISPR v2 vector	Addgene	#52961
pMD2.G	Addgene	#12259
psPAX2	Addgene	#12260
pMSCV-N-LMP2A	Addgene	#37963
pMSCV-N-GFP	Addgene	#37855
pCMV-VSV-G	Addgene, gift via Yue Sun	#8454
pUMVC-gag-pol	Addgene, gift via Yue Sun	#8449
PIAS1-sg-1	This study	pKZ1
PIAS1-sg-2	This study	pKZ21
sg-NC (Non-targeting control)	This study	pKZ5c
Zp-Luc (-240 to +39)	Hayward Lab Collection	pGL516
Rp-Luc (-412 to +13)	Hayward Lab Collection	pGL263
HSV-TKp (-206 to -1)	Hayward Lab Collection	pGL535
BGLF2p-Luc (-619 to -1)	This study	pDL0092
C/EBPβ	Hayward Lab Collection	pGL210
ZTA	Hayward Lab Collection	NA
HA-RTA	Hayward Lab Collection	pGL196
V5-PIAS1	(Cox et al., 2017)	NA
V5-PIAS1	This study	pKZ6b
V5-PIAS1-C351S	This study	pKZ11
V5-PIAS1 (1-415)	This study	pKZ15
V5-PIAS1 (409-651)	This study	pKZ16
V5-PIAS1 (1-433)	This study	pKZ103
V5-PIAS1 (434-651)	This study	pKZ95
V5-PIAS1 (101-433)	This study	pKZ83
Halo-V5-PIAS1	This study	pKZ28
Halo-V5-PIAS1-D100A	This study	pKZ60
Halo-V5-PIAS1-D148A	This study	pKZ61
Halo-V5-PIAS1-D433A	This study	pKZ65

Halo-V5-PIAS1-D100/433A	This study	pKZ68
Cell lines		
Akata (EBV+)	Hayward Lab Collection	NA
Akata-4E3 (EBV-)	Hayward Lab Collection	NA
Akata-BX1 (EBV+)	(Molesworth et al., 2000)	NA
Akata (EBV+)-PIAS1-sg1	This study	NA
Akata (EBV+)-PIAS1-sg2	This study	NA
Akata-BX1 (EBV+)-PIAS1-sg1	This study	NA
Akata-BX1 (EBV+)-PIAS1-sg2	This study	NA
Akata (EBV+)-PIAS1-Knockout (KO)	This study	NA
Akata (EBV+)-PIAS1-sg1-pLX-PIAS1	This study	NA
Akata (EBV+)-PIAS1-KO-pLX-Vector	This study	NA
Akata (EBV+)-PIAS1-KO-pLX-PIAS1	This study	NA
Akata (EBV+)-PIAS1-KO-pLX-PIAS1-D100/433A	This study	NA
293T cells	Hayward Lab Collection	NA
LCL	Coriell Institute	GM19099
LCL	Coriell Institute	GM11830
LCL	Coriell Institute	GM11993
Kem-I	Sample Lab (Kosowicz et al., 2017)	NA
P3HR-1	ATCC	HTB-62
Primary human B cells	Precision Bioservices	Cat# 84400
SNU-719	Hayward Lab Collection	NA
BC3	Hayward Lab Collection	NA
BCBL1	Hayward Lab Collection	NA
Akata-LMP2A	This study	NA
Akata-GFP	This study	NA
Oligonuceotides		
Primers for qPCR and cloning	This study	See Table S3
Software and Algorithms		
COBALT Multiple Alignment Tool	NCBI	www.ncbi. nlm.nih.gov/tools/cobalt/
CaspDB	(Kumar et al., 2014)	caspdb.sanfordburnham.org
Cascleave	(Song et al., 2010)	sunflower.kuicr.kyoto-u.ac.jp/~sjn/Cascleave/
Cascleave 2.0	(Wang et al., 2014)	structbioinfor.org/cascleave2
I-TASSER	(Roy et al., 2010; Yang et al., 2015; Zhang, 2008)	https://zhanglab.ccmb.med.umich.edu/I-TASSER/
Chimera package	(Pettersen et al., 2004)	http://www.rbvi.ucsf.edu/chimera

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Renfeng Li (rli@vcu.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics Statement

CD19-positive primary B cells were purchased from the Precision for Medicine (Cat# 84400 Frederick, MD). As these were derived from anonymous blood donors, no ethical approval is required.

Cell Lines and Cultures

The Akata (EBV+), Akata-4E3 (EBV-) and Akata-BX1 (EBV+) cells were grown in RPMI 1640 media supplemented with 10% FBS (Cat# 26140079, Thermo Fisher Scientific) in 5% CO_2 at 37°C (Li et al., 2015; Li et al., 2012; Li et al., 2011). 293T cells were grown in DMEM media supplemented with 10% FBS in 5% CO_2 at 37°C.

The P3HR-1 cell line (ATCC, HTB-62) was purchased from ATCC. The EBV-transformed lymphoblast cell lines (LCLs, GM19099, GM11830 and GM11993) were purchased from the Coriell Institute for Medical Research (Camden, NJ). The P3HR-1 cell, Kem-I cell (a gift from Jeffery Sample) and SNU-719 (gift from Diane Hayward), a naturally derived EBV-infected gastric carcinoma cell line, were grown in RPMI 1640 media supplemented with 10% FBS. The LCL cells were cultured in RPMI 1640 media supplemented with 15% FBS. The KSHV-positive B-lymphoma cells (BC3 and BCBL1, gifts from Diane Hayward) were maintained in RPMI 1640 media with 10% FBS. Human CD19+ negative selected primary B cell was purchased from Precision Bioservices (Cat# 84400). The primary B cells were thawed and cultured following the protocol provided by the manufacturer. All cells were incubated at 37°C with 5% CO_2.

METHOD DETAILS

PIAS1 Depletion and Knockout by CRISPR/Cas9 Genome Editing

To knock out PIAS1, two different sgRNAs targeting human PIAS1 were designed and cloned into lentiCRISPR v2 vector (a gift from Feng Zhang; Addgene plasmid # 52961) (Sanjana et al., 2014). Packaging 293T cells were transfected with PIAS1 sgRNAs or negative controls (non-targeting sgRNA-NC) and helper vectors (pMD2.G and psPAX2; gifts from Didier Trono; Addgene plasmid #s 12259 and 12260) using Lipofectamine 2000 reagent (Cat# 11668019, Life Technologies). Medium containing lentiviral particles and 8 μg/mL polybrene (Sigma-Aldrich, St. Louis) was used to infect EBV-positive and KSHV-positive cells. Infected cells were selected in medium containing 2 μg/mL puromycin. The target guides sequences are listed in Table S3.

To obtain PIAS1-knockout single cell clones, the Akata (EBV+)-PIAS1-sg1 pool cells (with puromycin resistant gene) were plated in 96-well plates at a density of 1 cell per well. The parental Akata (EBV+) cells (without puromycin resistant gene) were pretreated by puromycin to kill the cells for two days and 10000 dying cells (as a structural, contact and nutrition support for single B cell growth) were be added in each well to support the growth of single Akata (EBV+) B cell. After 3 to 4 weeks, growing clones were identified and picked. The PIAS1 knockout status was first verified by western blot. Then genomic DNA was extracted and PIAS1 sequence spanning the gRNA targeting sites was amplified by primers (RL0371 and RL0372, Table S3) and the PIAS1 knockout status was confirmed by using Sanger sequencing of 12 clones.

Cloning, Gibson Assembly and Site-directed Mutagenesis

V5-PIAS1 (full length, 1-415, 409-651, 101-433, 434-651) and Halo-V5-PIAS1 plasmids were constructed using Gibson assembly (Gibson et al., 2009). Briefly, the DNA fragments were prepared by PCR amplification with 40 bp overlap to the adjacent sequence using specific primers (Table S3) and the Q5 High-Fidelity DNA polymerase (New England Biolabs, Cat# M0491S). The Gibson assembly reaction were carried out by using the 2x Gibson Assembly Master Mix (405 μl Isothermal Start Mix, 25 μl 1M DTT, 20 μl 25 mM dNTPs, 50 μl NAD+, 1 μl T5 exonuclease, 31.25 μl Phusion High Fidelity DNA Polymerase, 250 μl Taq Ligase, 467.75 μl H_2O). Aliquots of 10 μl were prepared and then used for a single Gibson reaction. Each assembly reaction contained approximately 100 ng of each insert and 50 ng of the linearized vector backbone and added to the 10 μl master mix in a 20 μl total volume reaction mixture. The reaction was incubated at 50°C for 60 min. After the assembly reactions, the reaction mixture was transformed into DH5α competent cells.

The V5-PIAS1 mutants (C351S, 1-433 stop) and Halo-V5-PIAS1 mutants (D100A, D148A, D433A and D100/433A) were generated by the QuikChange II site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions

The pGL2-BGLF2p619 luciferase reporter plasmid was constructed by inserting the sequence of −619 to −1 relative to the *BGLF2* ORF into pGL2-basic luciferase vector (Promega) using XhoI and HindIII restriction sites. The primer sets used are listed in Table S3.

Protein Expression and Purification

Halo-V5-PIAS1 and mutant proteins were expressed and purified as previously described (Li et al., 2015). Briefly, Halo-V5-PIAS1 or mutant plasmids were transfected into 293T cells. Two T175 flasks of transfected cells were harvested 48 hrs post-transfection at 100% confluence and lysed with 25 ml HaloTag Protein Purification Buffer (50 mM HEPES pH7.5, 150 mM NaCl, 1 mM DTT, 1 mM EDTA and 0.005% NP40/IGEPAL CA-630) with Protease Inhibitor Cocktail. Halo-tagged proteins were enriched using the Halo-tag resin and proteins were eluted from the resin by washing 3 times with 0.5 ml HaloTag Protein Purification Buffer containing 20 μl Halo-TEV protease.

Virus Titration
EBV titers were determined using a Raji cell infection assay (Li et al., 2011; Meng et al., 2010). Briefly, GFP-EBV recombinant virus was harvested from lytically induced Akata-BX1 cells carrying sgRNAs targeting PIAS1 or non-targeting control sgRNA. Raji cells (2×10^5 in 1 ml medium/well in 24-well plates) was infected with the GFP-virus and phorbol-12-myristate-13-acetate (TPA) (20 ng/ml) and sodium butyrate (3 mM) were added 24 hrs later. After a further 24 hrs, the GFP-positive Raji cells were scored using a fluorescence microscope. The number of green Raji cells was used to determine the concentration of infectious virus particles.

Cell Treatment
To induce the EBV lytic cycle, Akata (EBV+) cells were treated with anti-IgG antibody (1:200, Cat# 55087, MP Biomedicals) for 0 to 48 hrs. Akata-4E3 (EBV-) cells were treated similarly as controls. P3HR-1 cells were triggered by addition of TPA (20 ng/ml) and sodium butyrate (3 mM). The EBV lytic replication in SNU-719 cells and LCL cells was induced by addition of gemcitabine (1μg/mL). To induce the BCR activation, the LCL cells were treated with anti-IgM antibody (20μg/mL, Cat# 2020-01, Southern Biotech) for 0 to 48 hrs. For caspase inhibition assay, cells were untreated or pretreated with caspase inhibitors for 1 hrs and then treated with anti-IgG (1:200, Cat# 55087, MP Biomedicals) for additional 48 hrs.

CD19-positive primary human B cells (6×10^5 cells/ml) were pretreated with DMSO control or pan-caspase inhibitor Z-VAD-FMK (50 μM) for 1 hr and the treated with anti-IgM (20 μg/ml, Cat# 2020-01, Southern Biotech) to trigger BCR activation for 48 hrs.

Cell Lysis and Immunoblotting
Cells were harvested and lysed in 2x SDS-PAGE sample buffer and boiled for 5 minutes. The samples were separated on 4-20% TGX gels (Biorad), transferred to PVDF membranes, and probed with primary and horseradish peroxidase-conjugated secondary antibodies. The primary antibodies were diluted at 1:1000. For PIAS1 blotting, re-blot was normally required for detecting endogenous protein in Akata (EBV+) cells using the anti-PIAS1 antibody (Cell Signaling Tech, Cat# 3550).

Lentiviral Transduction of PIAS1
The pLX304-PIAS1 was purchased from DNASU Plasmid Repository. The CRISPR-resistant PIAS1 (nucleotide G888A, silent mutation) was generated by site-directed mutagenesis using the QuikChange II site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. Subsequently, the pLX304-PIAS1 (D100/D433A) mutant was generated sequentially using the CRISPR-resistant PIAS1 as a template (primers are listed in Table S3). To prepare lentiviruses, 293T cells were transfected with lentiviral vector pLX304 containing the gene of wild-type PIAS1 or cleavage-resistant mutant (D100/433A) and the help vectors (pMD2.G and psPAX2) using Lipofectamine 2000 reagent. The supernatants were collected 48 hrs after transfection and used for infection of PIAS-1 knockout cell lines. Infected cells were selected in medium containing 10 μg/mL blasticidin. Expression of PIAS1 was examined by western blot analysis.

Chromatin Immuno-precipitation (ChIP) Assay
ChIP assay was performed using a SimpleChIP Enzymatic Chromatin IP Kit (Cell Signaling Technology) according to the manufacturer's protocol. Briefly, the control (NC) and PIAS1-depleted (PIAS1-sg-1) Akata (EBV+) cells were cross-linked with 1% formaldehyde and digested with micrococcal nuclease to achieve DNA fragments of 150-900 bp. 1% of the chromatin was reserved as input sample. DNA-protein complexes were immunoprecipitated with Rabbit anti-PIAS1 antibody or Rabbit IgG control. After being reverse crosslinked, DNA samples were purified and quantified by qPCR with the primers listed in Table S3.

The SNU-719 cells plated at the 10 cm^2 dishes were transfected with indicated plasmids using Lipofectamine 2000 reagent. The following day, cells were washed twice with PBS and transferred to 15 cm^2 dishes. At 48 h post-transfection, the cells were cross-linked with 1% formaldehyde. DNA-protein complexes were immunoprecipitated with anti-PIAS1, anti-ZTA, anti-C/EBPβ or anti-HA antibody. The anti-IgG or anti-H3 antibody served as negative or positive control. After being reverse crosslinked, DNA samples were purified and quantified by qPCR with the primers listed in Table S3. The cells were also harvested using cell lysis buffer to check the transfection efficiency.

Immunoprecipitation Assay

293T cells were transfected with Lipofectamine 2000. The cells were harvested 48 hrs post-transfection using RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP40, 1% deoxycholate, 0.1% SDS and 1 mM EDTA) containing protease inhibitors and phosphatase cocktail I and II. The immunoprecipitation was carried out as previously described (Li et al., 2011). For immunoprecipitation from Akata (EBV+) cells, the cells were treated with anti-IgG antibody for 24 hrs and then harvested using the cell lysis buffer (Cell Signaling Tech, Cat# 9803). The cell pellets were removed by centrifugation at 14000 g for 10 min. The lysates were incubated with the indicated antibodies overnight, followed by incubation with 25 μL Protein A/G Plus-Agarose (Santa Cruz, SC-2003) for 3 hrs. After 4 times wash using cell lysis buffer, the samples were eluted in 2x SDS-PAGE buffer for SDS-PAGE and immunoblot.

Production of Retrovirus and Transduction of LMP2A

For retrovirus generation, 293T cells were transfected with pMSCV-N-LMP2A or pMSCV-N-GFP (gifts from Karl Munger, purchased from Addgene, #37963 and 37855) (Rozenblatt-Rosen et al., 2012) and the help vectors (pCMV-VSV-G and pUMVC-gag-pol, gifts from Bob Weinberg via Yue Sun, Addgene plasmids # 8454 and 8449) (Stewart et al., 2003) using Lipofectamine 2000 reagent. The supernatants were collected 48 and 72 hrs after transfection and filtered through a 0.45 μM filter. The Akata (EBV+) cells were infected twice with retroviral particles in the presence of 8 μg/mL polybrene. Infected cells were selected in medium containing 2 μg/mL puromycin.

Luciferase Assay

Transfection of 293T or SNU-719 cells was performed with lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Briefly, 293T or SNU-719 cells seeded in 12-well plates 1 day prior to transfection. The cells were co-transfected with the firefly luciferase reporter vectors along with C/EBPβ, ZTA, or RTA and *renilla* expression plasmids using Lipofectamine 2000 reagent (Cat# 11668019, Life Technologies) (Huang et al., 2006). Thirty-six hours after transfection, cell extracts were prepared and assayed with the dual-luciferase assay kit from Promega (Cat # E1960, Madison, WI, USA). Each condition was performed in triplicate.

***In vitro* Caspase Cleavage Assay**

Purified PIAS protein and caspases (active human caspases group IV; ApexBio, Cat# K2060) were incubated in caspase assay buffer (50 mM HEPES, pH7.2, 50 mM NaCl, 0.1% Chaps, 10 mM EDTA, 5% Glycerol and 10mM DTT) at 37°C for 2 hrs. Reactions were stopped by boiling in 2× SDS sample buffer and samples were analyzed by western blot.

Reverse Transcription and Quantitative PCR (RT-qPCR)

Total RNA was extracted from cells following induction using Isolate II RNA Mini Kit (Bioline). RNA was reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Invitrogen). qPCR was performed using Brilliant SYBR Green qPCR master mix (Agilent Technology) with specific primers. The relative expression of mRNA was normalized to *β-actin* expression using the comparative Ct method. Primers are listed in Table S3.

EBV DNA Detection

To measure cell associated viral DNA, total genomic DNA was extracted using the Genomic DNA Purification Kit (Cat# A1120, Promega). The relative viral genome copy numbers were determined by quantitative PCR using primers to *BALF5* and *BHLF1* genes with and *β-actin* as a control. The values were normalized by *β-actin*. Primers are listed in Table S3.

Structure Analysis for PIAS1

Full length PIAS1 sequence (1-651 aa) was uploaded to the I-TASSER Protein Structure and Function Prediction tool (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) (Roy et al., 2010; Yang et al., 2015; Zhang, 2008) using default settings. Five models of PIAS1 3-dimentional (3D) structure were generated. One PIAS1 3D model (model-4) with surface-exposed cleavage sites (D100 and D433) was selected for visualization. Molecular graphics and analyses were performed with the UCSF Chimera package (Pettersen et al., 2004) (http://www.rbvi.ucsf.edu/chimera). Chimera is developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIGMS P41-GM103311).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses employed a two-tailed Student's t test. A p value of ≤ 0.05 was considered statistically significant. Values are given as the mean of replicate experiments. Error bars represent the standard deviation (SD) from triplicate samples.

SUPPLEMENTAL FIGURES

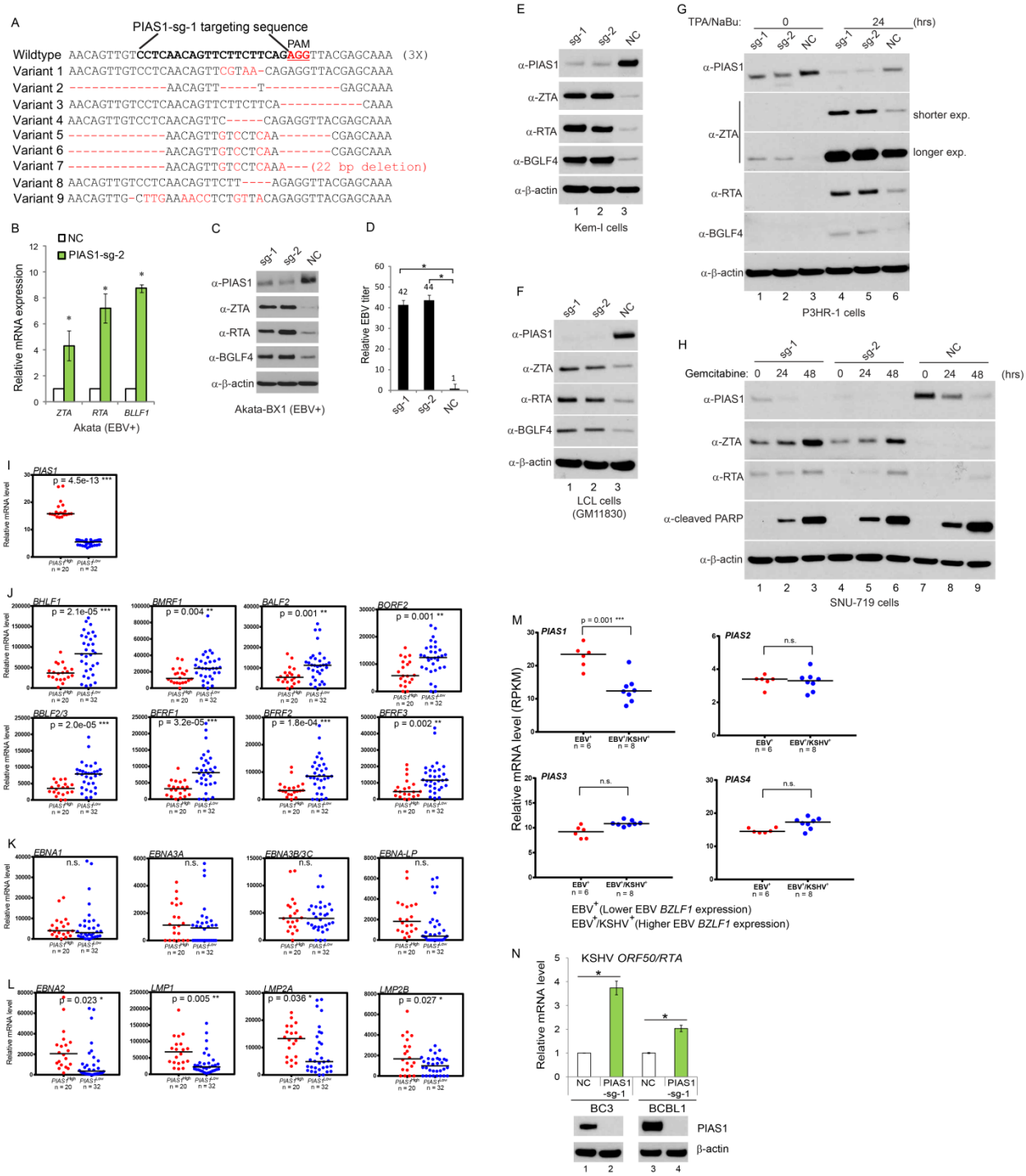

Figure S1, Related to Figure 1. PIAS1 depletion facilitates EBV and KSHV reactivation.
(A) The sequencing of PIAS1-sg-1 cell line showing that 9 out of 12 clones contain PIAS1 frame shift mutations in Akata (EBV+) cells.
(B) PIAS1-depletion leads to enhanced lytic gene expression. RNAs from the PIAS1-depleted (sg-2) and control (NC) Akata (EBV+) cells was extracted and analyzed by RT-qPCR using primers as indicated.
(C and D) PIAS1-depletion leads to EBV reactivation of Akata-BX1 (EBV+) cells. Akata-BX1 (EBV+) cells were used to establish stable cell lines using guide RNA constructs sg-1, sg-2 and a non-targeting control (NC). Western blot analyses showing PIAS1 depletion and EBV lytic gene expression in the two cell lines using antibodies as

indicated (C). The relative EBV titer upon PIAS1 depletion was measured by a Raji cell infection assay (D). The EBV titer in the control (NC) cells was set as 1. Results from three biological replicates are presented. Error bars indicate the standard deviation. *, $p < 0.01$.
(E) PIAS1 depletion facilitates EBV reactivation in Kem-I cells. EBV-positive Kem-I cells were used to establish stable cell lines using guide RNA constructs sg-1,sg-2 and a non-targeting control (NC). PIAS1 and EBV lytic protein expression was monitored by WB using antibodies as indicated. β-actin blot served as loading controls.
(F) PIAS1 depletion facilitates EBV reactivation in LCL cells. EBV-immortalized LCL cells were used to establish stable cell lines using guide RNA constructs sg-1,sg-2 and a non-targeting control (NC). PIAS1 and viral protein expression was monitored by WB using antibodies as indicated. β-actin blot served as loading controls.
(G) PIAS1 depletion leads to enhanced EBV protein expression in P3HR-1 cells. P3HR-1 cells were used to establish stable cell lines using guide RNA constructs sg-1,sg-2 and a non-targeting control (NC). The PIAS1-depleted and control cells were either untreated or treated with TPA and sodium butyrate (NaBu) to induce lytic reactivation. PIAS1 and viral protein expression was monitored by WB using antibodies as indicated. β-actin blot served as loading controls.
(H) PIAS1 depletion leads to enhanced EBV protein expression in EBV-positive gastric cancer cells. SUN-719 cells were used to establish stable cell lines using guide RNA constructs sg-1, sg-2 and a non-targeting control (NC). The PIAS1-depleted and control cells were either untreated or treated with Gemcitabine to induce lytic reactivation. PIAS1 and viral protein expression was monitored by WB using antibodies as indicated. Cleaved-PARP served as the marker for caspase activation and β-actin blot served as loading controls.

(I-L) PIAS1 expression level inversely correlates with EBV lytic gene expression in LCLs. See also Table S1.
(I) LCLs were stratified into $PIAS1^{High}$ and $PIAS1^{Low}$ groups based on *PIAS1* expression level extracted from 201 RNA-seq analyses of 146 LCLs (Arvey et al., 2012).
(J) The transcriptional levels of EBV lytic genes *BHLF1, BMRF1, BALF2, BORF2, BBLF2/3*, *BFRF1, BFRF2* and *BFRF3* were significantly higher in $PIAS1^{Low}$ group than those in $PIAS1^{High}$ group.
(K) The transcriptional levels of EBV latent genes *EBNA1, EBNA3A, EBNA3B/3C* and *EBNA-LP* were not affected by *PIAS1* expression.
(L) The transcriptional levels of EBV latent genes *EBNA2, LMP1, LMP2A* and *LMP2B* were lower in $PIAS1^{Low}$ group than those in $PIAS1^{High}$ group.
Statistical significance was evaluated by Student *t-test* (two-tailed distribution with unequal variance). *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$; n.s., not significant.

(M) EBV lytic gene expression inversely correlates the expression level of PIAS1. The *PIAS1, PIAS2, PIAS3* and *PIAS4* mRNA levels were extracted from RNA-seq analysis of 2 EBV-positive (3 replicates/cell line) and 3 EBV/KSHV dual-positive (2 or 3 replicates/cell line) tumor cell lines derived from infected huNSG mice (McHugh et al., 2017). The dually-infected cells have higher EBV *BZLF1* expression. Values represent mean ± standard deviation. ***, $p = 0.001$; n.s., not significant. Detailed statistical analysis was described in Mchugh et al (McHugh et al., 2017). RPKM, Reads Per Kilobase of exon per Million mapped reads.

(N) PIAS1 depletion facilitates KSHV lytic gene expression. KSHV-positive BC3 and BCBL1 cells were used to establish stable cell lines using guide RNA constructs sg-1 and a non-targeting control (NC). RNAs from PIAS1-depleted (sg-1) and control (NC) cells were extracted and analyzed by RT-qPCR using primers to KSHV *ORF50/RTA*. The values of NC control were set as 1. Western blot analysis showing PIAS1 depletion in the two cell lines as indicated. Representative results from three biological replicates are presented. Values represent mean ± standard deviation. *, $p < 0.01$.

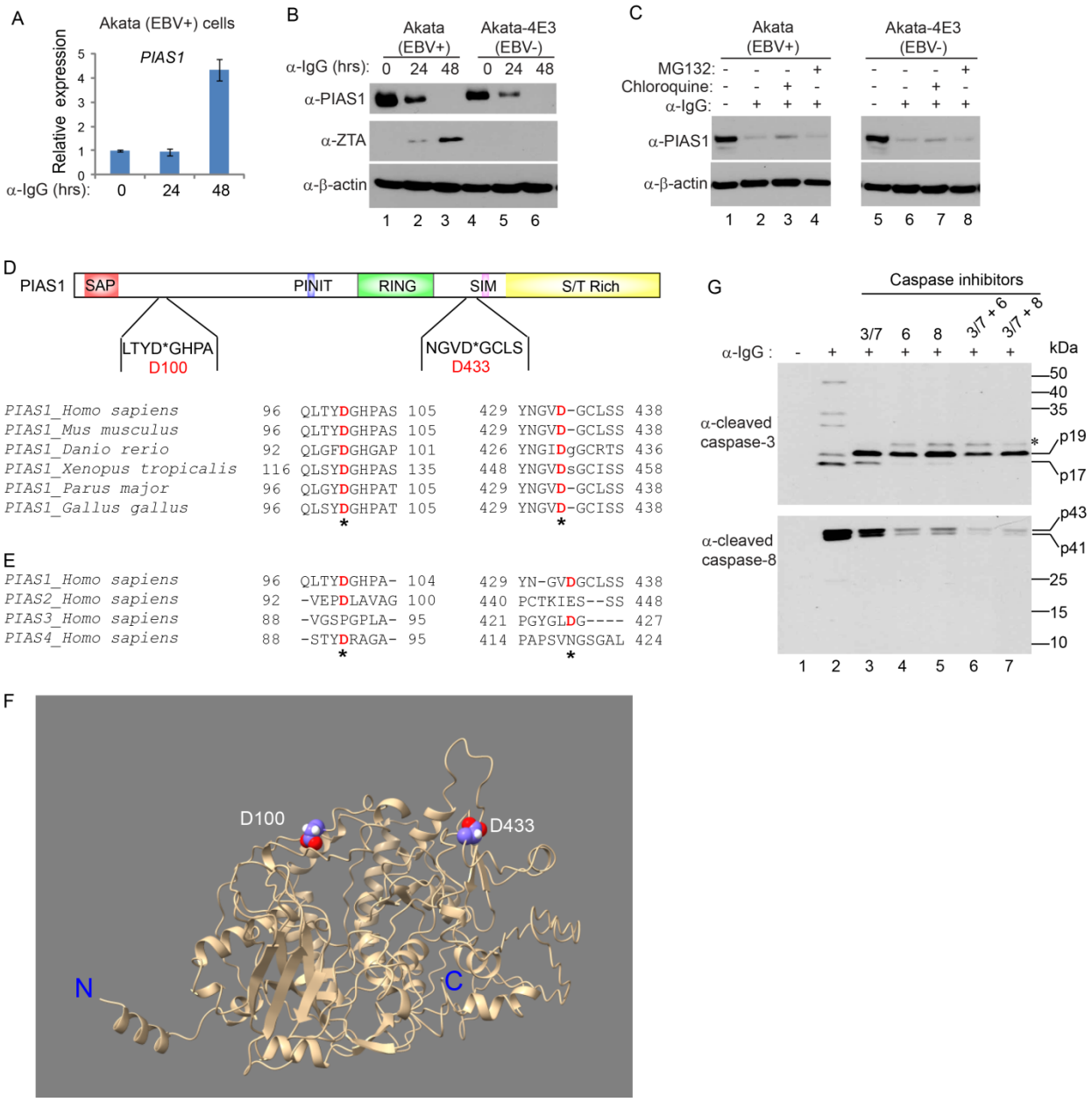

Figure S2, related to Figures 3-6. PIAS1 is downregulated upon BCR activation.
(A) RT-qPCR showing the relative PIAS1 mRNA level upon IgG cross-linking. Results from three biological replicates are presented. Error bars indicate the standard deviation.
(B) Immunoblot showing PIAS1 downregulation by IgG cross-linking induced BCR activation. Akata (EBV+) and Akata-4E3 (EBV-) cells were treated with anti-IgG antibody as indicated.
(C) Western blot analyses showing that proteasome and lysosome inhibition could not block PIAS1 degradation in both cell lines. The cells were either untreated or treated with anti-IgG (48 hrs), chloroquine (50 μM, 48 hrs) or MG132 (50 μM, added 6 hrs before cell harvest).
(D) The cleavage sites on PIAS1 are conserved across different species. Sequence alignment showing that D100 and D433 are highly conserved from *Homo sapiens* to *Gallus gallus*. PIAS1 sequences from different species were aligned by NCBI COBALT. The amino acids surrounding PIAS1 D100 and D433 are presented.
(E) Sequence alignment showing that D100 and D433 are less conserved among human PIAS1 to PIAS4. PIAS1 to PIAS4 sequences were aligned by NCBI COBALT. The amino acids surrounding PIAS1 D100 and D433 are presented.

(F) Cleavage sites-guided structure prediction for PIAS1. The 3-dimentional (3D) structure of PIAS1 was generated by using the I-TASSER Protein Structure and Function Prediction tool (https://zhanglab.ccmb.med.umich.edu/I-TASSER/). One model with surface-exposed cleavage sites (D100 and D433) was presented. The locations of N- and C-terminal PIAS1 were labeled as indicated. Molecular graphics and analyses were performed with the UCSF Chimera package (http://www.rbvi.ucsf.edu/chimera).
(G) Caspase inhibition suppresses the activation of caspase-3 and caspase-8. Immunoblot showing the effects of caspase inhibitors on the generation of active caspase-3 and caspase-8. Same samples from Figure 6B were analyzed. *; not fully processed caspase-3 fragment.

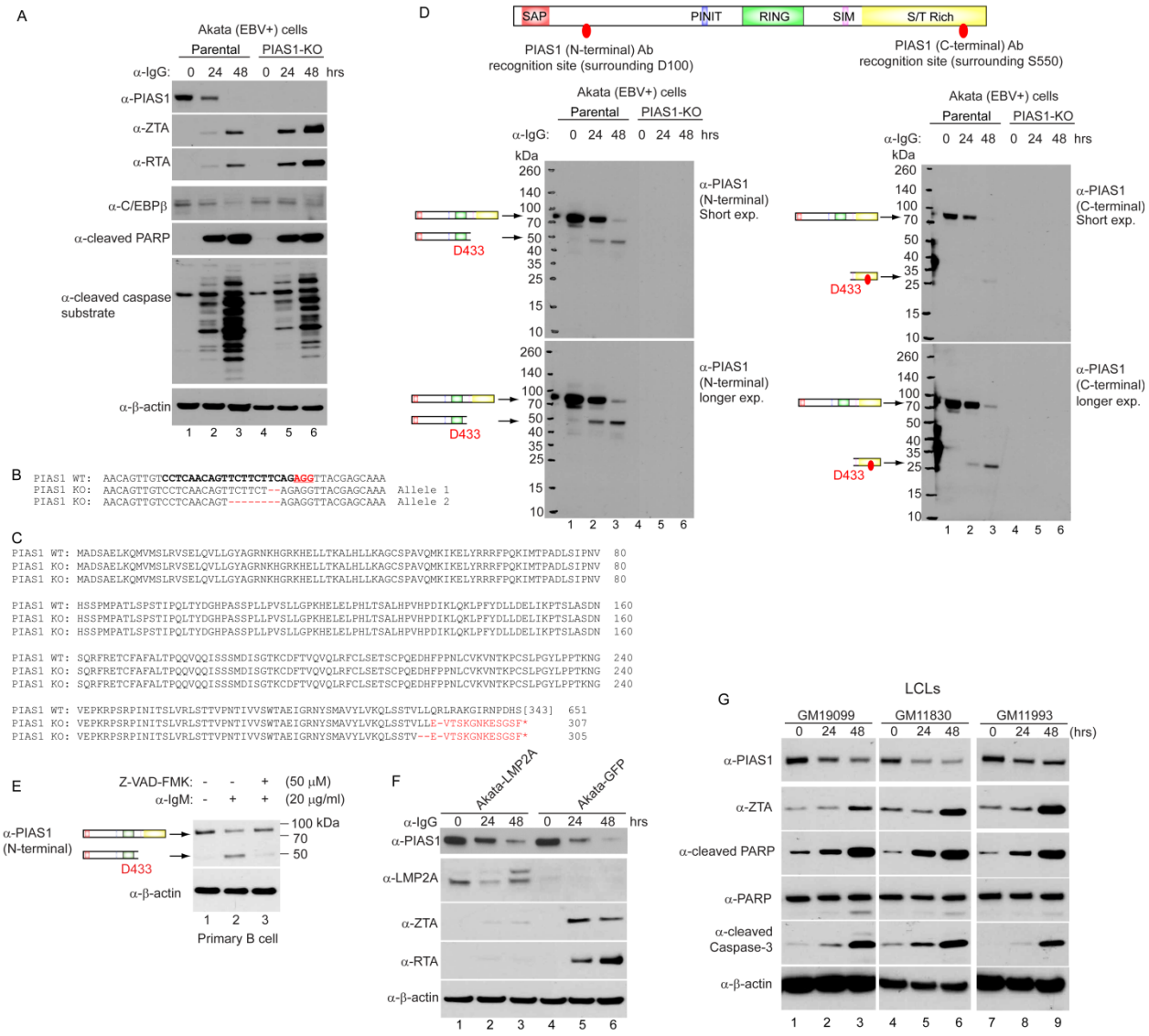

Figure S3, related to Figures 3 and 6.

(A-D) Establishment and characterization of a PIAS1-knockout single cell clone.

(A) PIAS1 knockout facilitates EBV lytic replication. Akata (EBV+) sg-1 cells were used to establish a PIAS1-knockout (PIAS1-KO) single cell clone. The parental and PIAS1-KO cells were then stimulated with anti-IgG cross-linking. Western blot analyses showing the expression levels of PIAS1, ZTA, RTA and C/EBPβ, and the generation of cleaved-PARP and other cleaved-caspase substrates using antibodies as indicated.

(B) Sequencing results depicting the deletion of nucleotides within PIAS1 at the respective loci.

(C) Sequence alignment showing the frame shifts-induced truncations of PIAS1 due to the generation of stop codons. PIAS1 sequences were aligned by NCBI COBALT. Frame shifts-induced mutations of PIAS1 are highlighted with red color.

(D) Western blot analysis showing the PIAS1 cleavage pattern in the parental cells and the PIAS1 knockout status in the PIAS1-KO cells. The cell extract from panel (A) was analyzed using two anti-PIAS1 antibodies that recognize N-terminal and C-terminal PIAS1. PIAS1 cleavage fragments were labeled as indicated. The absence of N-terminal truncated PIAS1 in the PIAS1-KO cells was revealed by an antibody recognizing the N-terminal of PIAS1.

(E) BCR activation triggers PIAS1 cleavage in primary human B cells. CD19-positive primary B cells were pretreated with DMSO control or pan-caspase inhibitor Z-VAD-FMK (50 μM) for 1 hr and then treated with anti-IgM (20 μg/ml) as indicated to trigger BCR activation for 48 hrs. Western blot analysis showing that PIAS1 is cleaved in anti-IgM treated B cells and the cleavage is reversed by pretreatment with Z-VAD-FMK.

(F) LMP2A suppresses PIAS1 degradation and the accumulation of EBV ZTA and RTA protein. Akata (EBV+) cells were transduced with retroviral constructs expressing LMP2A or GFP control. Lytic induction was trigger by adding anti-IgG for 0, 24 and 48 hrs as indicated. Immunoblot showing the suppression of PIAS1 degradation and EBV ZTA and RTA expression in the LMP2A-expressing cells.
(G) BCR activation by anti-IgM-mediated crosslinking promotes caspase activation, PIAS1 degradation and EBV lytic gene expression**.** Immunoblot showing PIAS1 degradation, the generation of cleaved PARP and caspase-3, and the accumulation of EBV ZTA. Three different LCLs were treated with anti-IgM for 0, 24 and 48 hrs.

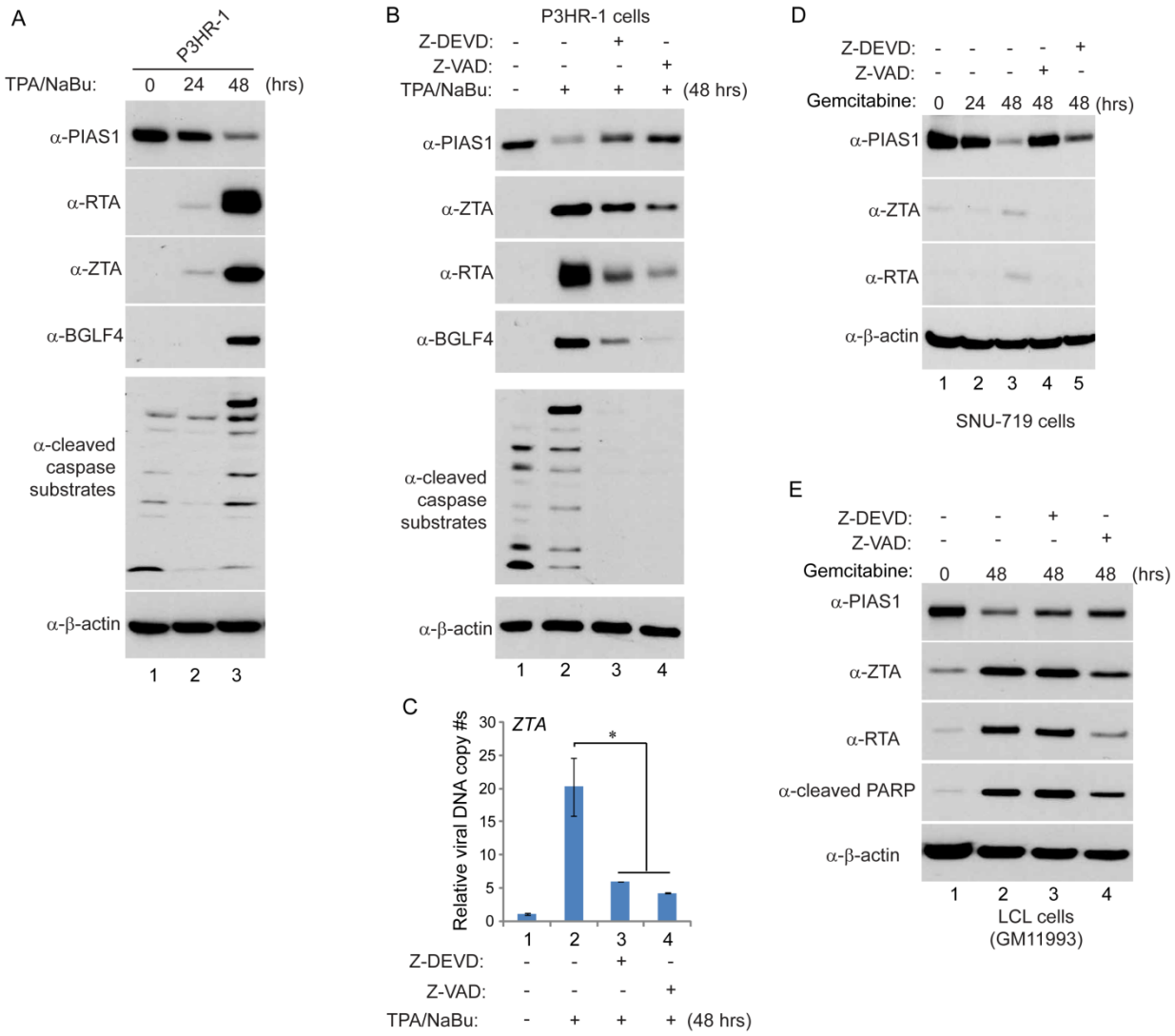

Figure S4, related to Figures 3 and 6. PIAS1 is downregulated upon lytic induction and caspase inhibition suppresses EBV lytic replication through PIAS1 stabilization.

(A) Immunoblot showing PIAS1 degradation, the generation of cleaved caspase substrates, and the accumulation of EBV RTA, ZTA and BGLF4. P3HR-1 (EBV+) cells were treated with TPA and sodium butyrate (NaBu) for 0 to 48 hrs as indicated

(B-C) Caspase inhibition blocks EBV lytic replication. P3HR-1 (EBV+) cells were either untreated or pretreated with caspase inhibitors (50 μM) for 1 hr and then TPA and sodium butyrate (NaBu) were added for 48 hrs as indicated. EBV ZTA, RTA and BGLF4 expression, and the generation of cleaved caspase substrates were monitored by western blot (B). Relative EBV DNA copy numbers were measured by qPCR using primers specific for *ZTA* (C). The value of cells without treatment (lane 1) was set as 1. Representative results from three biological replicates are presented. Error bars indicate the standard deviation. *, $p < 0.01$.

(D-E) Caspase inhibition suppresses EBV lytic protein expression through PIAS1 stabilization.

(D) SUN-719 cells were either untreated or pretreated with caspase inhibitors (50 μM) for 1 hr and then TPA and sodium butyrate (NaBu) were added for 48 hrs as indicated. PIAS1, EBV ZTA and RTA expression was monitored by western blot.

(E) EBV-immortalized LCL cells were either untreated or pretreated with caspase inhibitors (50 μM) for 1 hr and then gemcitabine was added for 48 hrs as indicated. PIAS1, EBV ZTA and RTA expression and the generation of cleaved PARP were monitored by western blot.

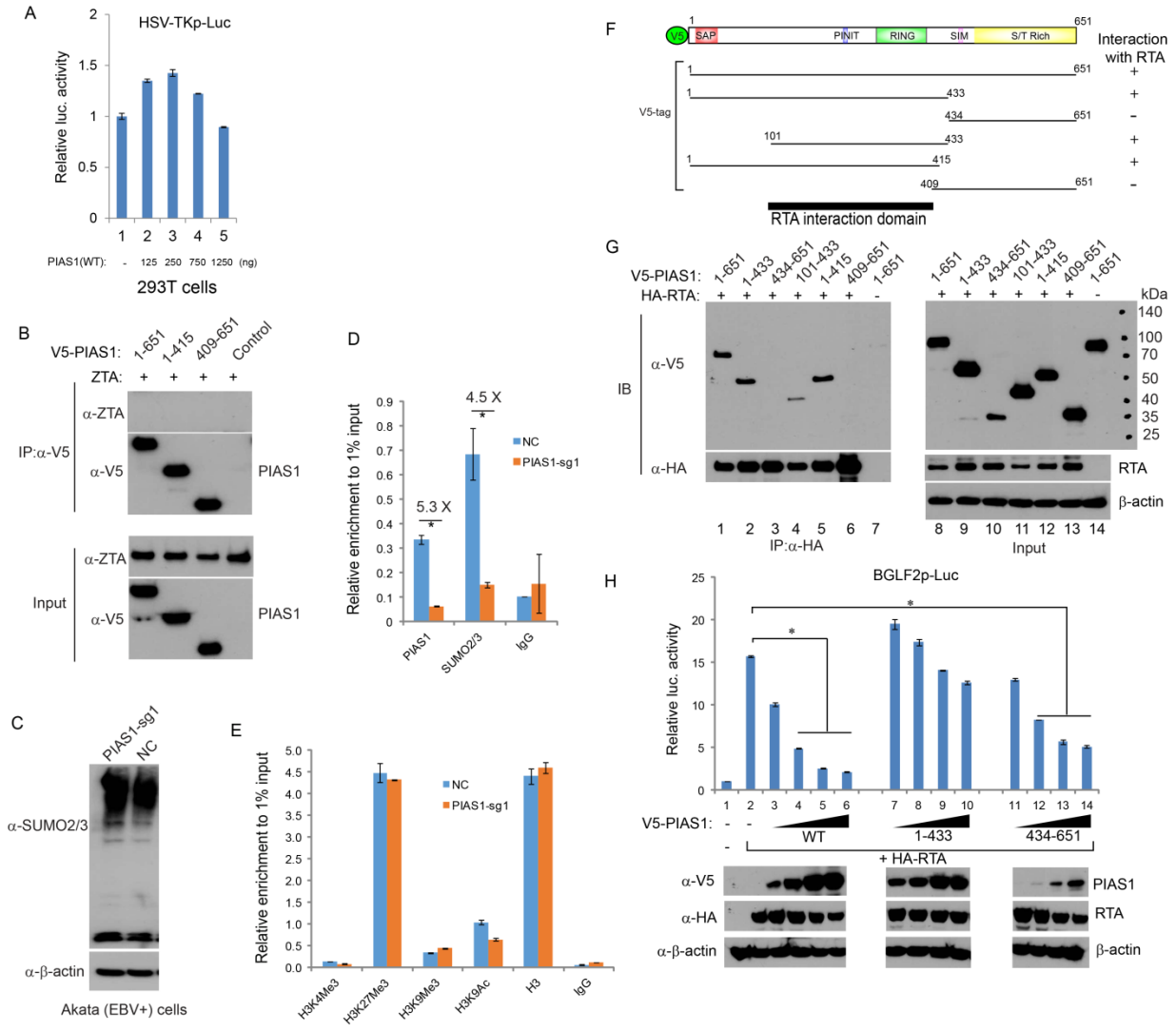

Figure S5, related to Figure 7.

(A) PIAS1 does not affect Luciferase activity driven by a HSV-TK promoter. 293T cells were transfected with 250 ng plasmid DNA encoding HSV-TKp-Luc and increasing amount of PIAS1 as indicated. The total amount of plasmid DNA used in each transfection was normalized by adding vector DNA. Representative results from three biological replicates are presented. Error bars indicate the standard deviation.

(B) PIAS1 does not interact with ZTA. 293T cells were co-transfected with ZTA, full length PIAS1 (aa 1-651) or PIAS1 truncation mutants (aa 1-415 and aa 409 to 651). Western blot analysis showing that no ZTA is co-immunoprecipitated (Co-IPed) with PIAS1. IP, immunoprecipitation.

(C) PIAS1 depletion does not affect the overall SUMOylation level of SUMO2/3. Western blot analysis of Akata (EBV+) cells carrying control (NC) or PIAS1-depleting (PIAS1-sg-1) constructs showing that global protein SUMOylation by SUMO2/3 was not affected by PIAS1 depletion.

(D and E) PIAS1 depletion leads to reduced SUMOylation of Zp-associated proteins. ChIP-qPCR analysis performed on Akata (EBV+) cells showing that the enrichment of PIAS1 and SUMO2/3 (D) but not histone H3 and various H3 markers (E) on Zp was reduced by PIAS1 depletion (PIAS1-sg1). ChIP was performed using anti-PIAS1, anti-SUMO2/3, anti-H3 antibodies and a nonspecific IgG as indicated. qPCR was performed using Zp specific primers. Representative results from three biological replicates are presented. Error bars indicate the standard deviation. *, $p < 0.01$.

(F-H) PIAS1 domains responsible for RTA interaction and for the suppression of RTA-mediated lytic gene promoter activation.

(F) The schematic representation of full length PIAS1 (aa 1-651) or PIAS1 truncation mutants. The interaction of PIAS1 with RTA was summarized in the right part.
(G) The N-terminal and central part of PIAS1 interacts with RTA. 293T cells were co-transfected with HA-RTA and full length PIAS1 (aa 1-651) or PIAS1 truncation mutants as indicated. Western blot analysis showing that RTA is co-immunoprecipitated (Co-IPed) with the N-terminal and central part of PIAS1. IP, immunoprecipitation; IB; immunoblot. β-actin blot was included as loading controls.
(H) The C-terminal PIAS1 suppresses RTA-mediated BGLF2 promoter activation. 293T cells were transfected with 250 ng of plasmid DNA encoding BGLF2p-Luc, and effector plasmid DNA expressing RTA and increasing amount (125, 250, 750 and 1250 ng) of wild-type (WT) or cleavage-mimicking PIAS1 (aa 1-433 and aa 434-651) as indicated. The relative luciferase activity was measured using the Dual-luciferase assay kit as described in the method. The expression levels of PIAS1 and RTA were monitored by Western blot. β-actin blot was included as loading controls. Representative results from three biological replicates are presented. Error bars indicate the standard deviation. *, $p < 0.01$.

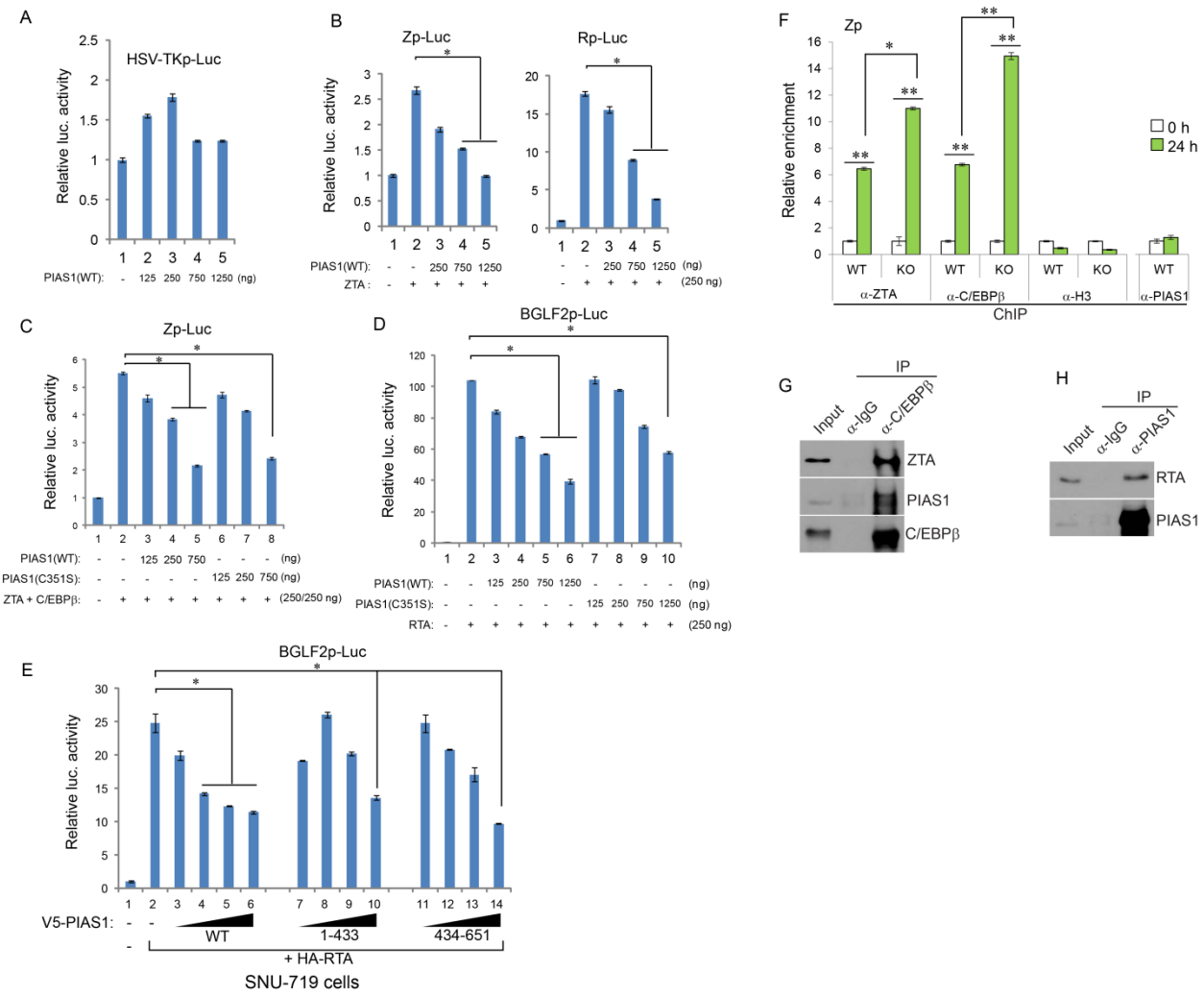

Figure S6, related to Figure 7.

(A-E) PIAS1 blocks C/EBPβ, ZTA and RTA-mediated lytic gene activation in SNU-719 cells.

(A) PIAS1 does not affect Luciferase activity driven by a HSV-TK promoter. SNU-719 cells were transfected with 250 ng plasmid DNA encoding HSV-TKp-Luc and increasing amount of PIAS1 as indicated. The total amount of plasmid DNA used in each transfection was normalized by adding vector DNA.

(B) Suppression of Zp- and Rp-Luc reporters by PIAS1. SNU-719 cells were transfected with 250 ng of plasmid DNA encoding Zp or Rp-Luc, and effector plasmid DNA expressing ZTA and increasing amount of PIAS1 as indicated. The total amount of effector plasmid DNA used in each transfection was normalized by adding vector DNA.

(C) PIAS1 suppresses ZTA-C/EBPβ-mediated Zp promoter activation. SNU-719 cells were transfected with 250 ng of plasmid DNA encoding Zp-Luc, and effector plasmid DNA expressing ZTA plus C/EBPβ and increasing amount of wild-type or SUMO ligase-deficient PIAS1 as indicated.

(D) PIAS1 suppresses RTA-mediated BGLF2 promoter activation. SNU-719 cells were transfected with 250 ng of plasmid DNA encoding Rp-Luc, and effector plasmid DNA expressing RTA and increasing amount of wild-type or SUMO ligase-deficient PIAS1 as indicated.

(E) The C-terminal PIAS1 suppresses RTA-mediated BGLF2 promoter activation. SNU-719 cells were transfected with 250 ng of plasmid DNA encoding BGLF2p-Luc, and effector plasmid DNA expressing RTA and increasing amount (125, 250, 750 and 1250 ng) of wild-type (WT) or cleavage-mimicking PIAS1 (aa 1-433 and aa 434-651) as indicated. The relative luciferase activity was measured using the Dual-luciferase assay kit as described in the method.

Representative results from three biological replicates are presented. Error bars indicate the standard deviation. *, $p < 0.01$.

(F-H) PIAS1 knockout facilitates the recruitment of ZTA and C/EBPβ to EBV *ZTA* promoter (Zp) upon lytic induction.
(F) The parental wild-type (WT) and PIAS1-knockout (KO) Akata (EBV+) cells were untreated (0 h) or treated with anti-IgG cross-linking of BCR (24 h). ChIP-qPCR analysis performed on these cells showing the increased enrichment of ZTA and C/EBPβ but not histone H3 on Zp in the absence of PIAS1 upon BCR stimulation. The values of untreated group (0 h) were set as 1. Representative results from three biological replicates are presented. Error bars indicate the standard deviation. *, $p < 0.01$; **, $p< 0.001$
(G-H) The parental Akata (EBV+) cells were treated with anti-IgG 24 h. Co-immunoprecipitation (Co-IP) showing that C/EBPβ interacts with ZTA and PIAS1 (G) and PIAS1 interactes with RTA (H) *in vivo*. 1% input was loaded as control.

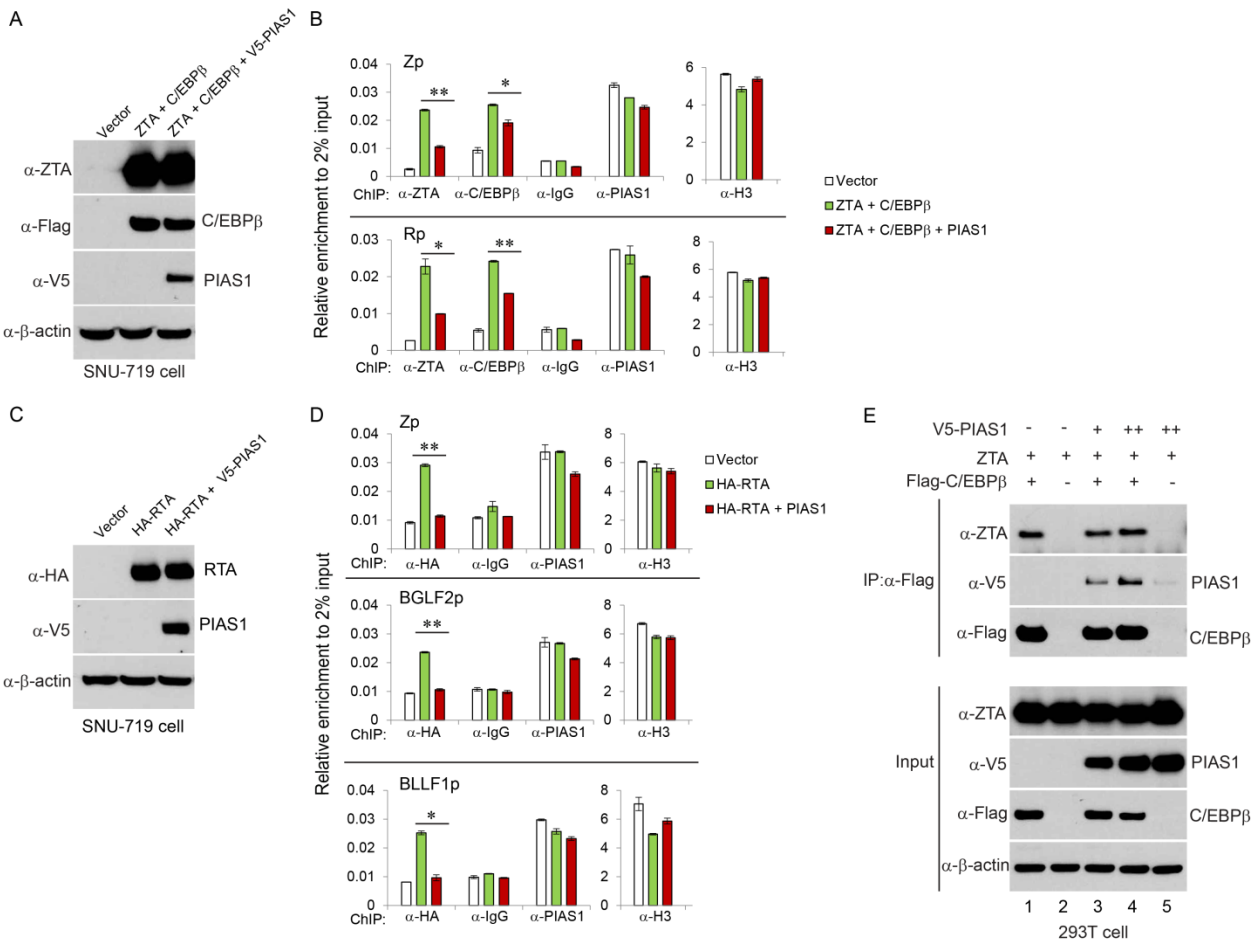

Figure S7, related to Figure 7. PIAS1 blocks the recruitment of ZTA, C/EBPβ and RTA to EBV lytic gene promoters.

(A-B) PIAS1 blocks the recruitment of ZTA and C/EBPβ to EBV Zp and Rp. SNU-719 cells were transfected with Vector control, ZTA and C/EBPβ or ZTA and C/EBPβ plus PIAS1. Immunoblot analysis showing the expression of ZTA, C/EBPβ and PIAS1 as indicated in (A). ChIP-qPCR analysis performed on these cells showing that the enrichment of ZTA and C/EBPβ but not IgG control or histone H3 on Zp and Rp was suppressed by PIAS1 (B).

(C-D) PIAS1 expression blocks the recruitment of RTA to EBV lytic gene promoters. SNU-719 cells were transfected with Vector control, RTA or RTA plus PIAS1. Immunoblot analysis showing the expression of RTA and PIAS1 as indicated in (C). ChIP-qPCR analysis performed on these cells showing that the enrichment of RTA but not IgG control or histone H3 on Zp, BGLF2p and BLLF1p was suppressed by PIAS1 (D).

(E) C/EBPβ interacts with ZTA and PIAS1. 293T cells were co-transfected with V5-PIAS1, ZTA, Flag-C/EBPβ as indicated. Western blot analysis showing that ZTA and PIAS1 are co-immunoprecipitated (Co-IPed) with C/EBPβ. IP, immunoprecipitation; β-actin blot was included as loading controls.

Representative results from three biological replicates are presented. Error bars indicate the standard deviation. *, $p < 0.01$. **, $p < 0.001$.

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