Supplemental Information

MYC CONTROLS THE EPSTEIN-BARR VIRUS LYTIC SWITCH

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Figure S1 (related to Figure 1)

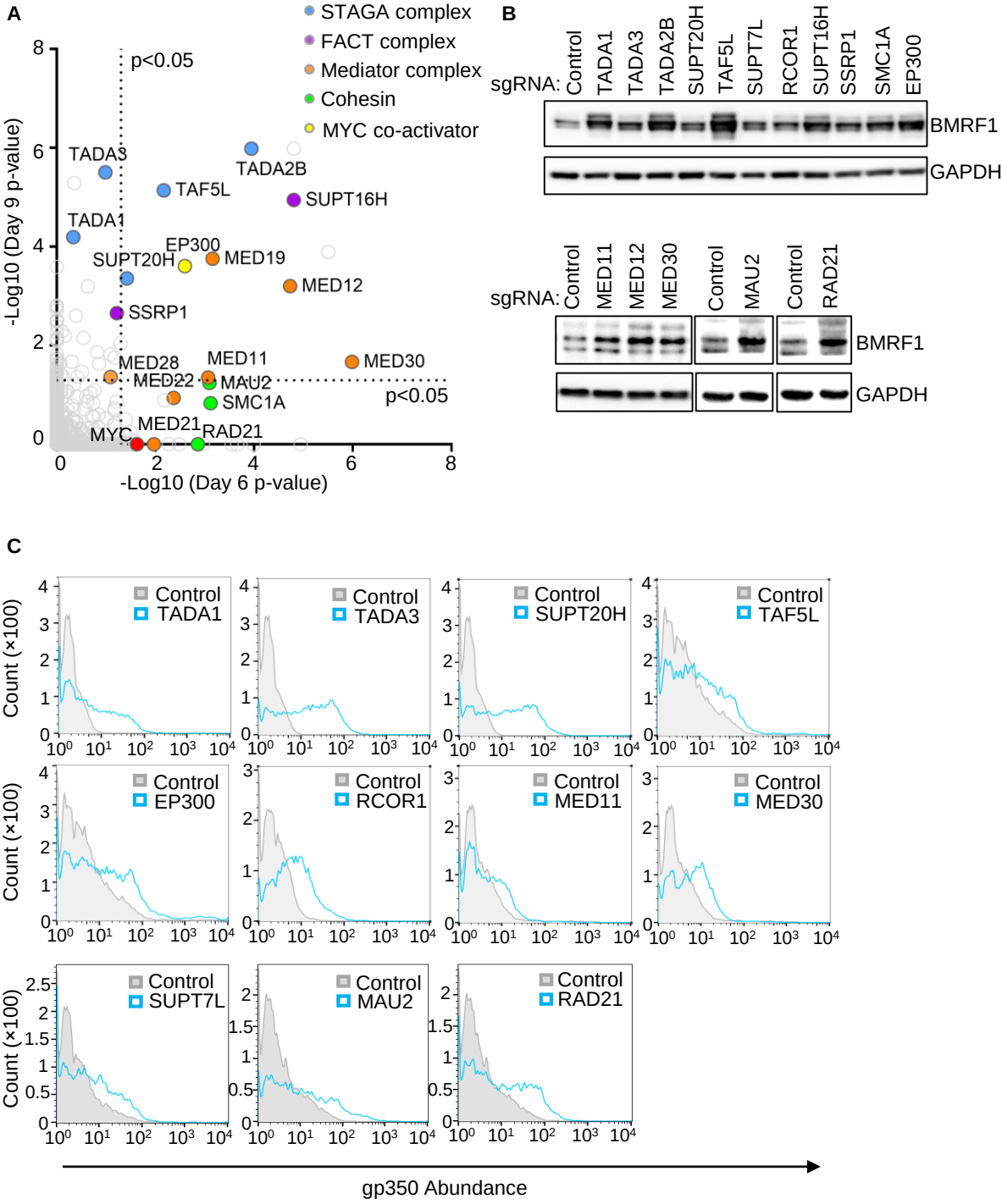


Figure S1 Initial Validation of Selected CRISPR Screen Hit, Related to Figure 1.

(A) Scatter plot showing the -Log10(p-value) screen hit STARS statistical significance on Day 9 (y-axis) versus Day 6 (x-axis). Selected screen hits are colored by biological function, as indicated.

(B) Immunoblot analysis of BMRF1 or GAPDH load-control abundances in whole cell lysates (WCL) from Cas9+ P3HR-1 cells expressing control sgRNA or sgRNA targeting the indicated CRISPR screen hits. Blots are representative of at least n=3 independent replicates.

(C) FACS analysis of PM gp350 expression in Cas9+ P3HR-1 cells expressing control sgRNA (gray) or sgRNA targeting the indicated screen hit (blue). Plots are representative of n=3 replicates.

Figure S2 (related to Figure 2)

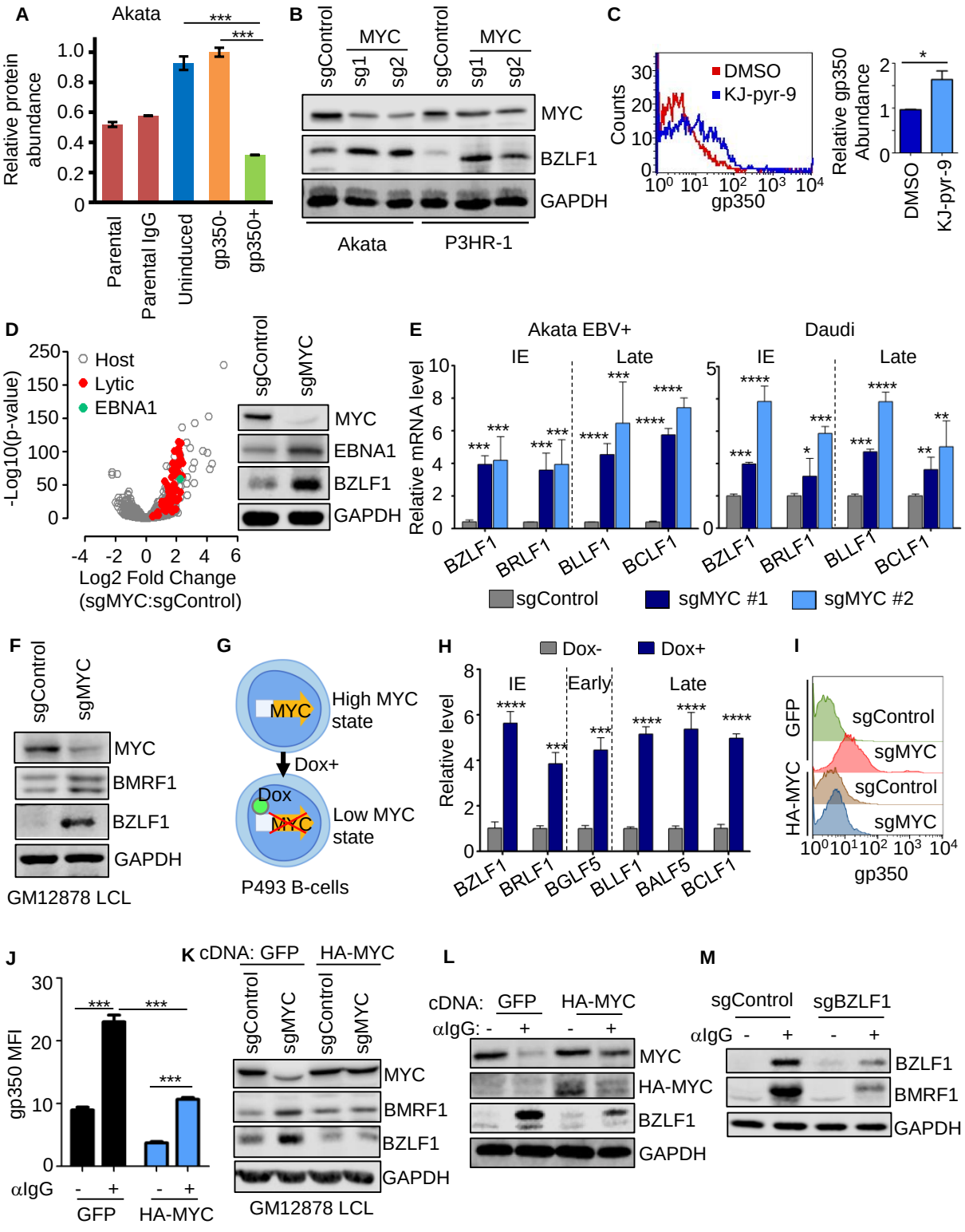


Figure S2, MYC Represses EBV Lytic Reactivation in BL, related to Figure 2.

(A) Relative MYC protein abundances in EBV-negative Akata that were either mock-induced by treatment with anti-IgG crosslinking for 48 hours (red), or in EBV+ Akata that were mock-induced or induced for lytic activation by IgG crosslinking for 48 hours. MYC protein levels in FACSorted gp350- (orange) vs gp350+ (green) Akata cells are shown. ***p < 0.001, calculated from biological triplicate replicates. Data were obtained from (Ersing et al., 2017).

(B) Immunoblot analysis of WCL from Akata or P3HR-1 BL cells expressing control or either of two MYC sgRNAs.

(C) FACS analysis of PM gp350 expression in P3HR-1 cells treated with DMSO vehicle control or 10 μM of KJ-Pyr-9 for 24 hours. Mean + SEM values of normalized PM gp350 abundance for n=3 replicates are show at right.

(D) Volcano plot comparing RNAseq values from Akata cells expressing control or MYC sgRNAs. -Log10 (p-value) (y-axis) and Log2 fold-change in mRNA abundance (x-axis) from n=3 replicates are shown. Significantly changed EBV lytic gene values are shown in red, and EBNA1 values are indicated by the green circle. At right, immunoblot of WCL from Akata expressing control or MYC sgRNAs.

(E) qRT-PCR analysis of EBV lytic gene mRNAs from Akata or Daudi BL expressing control or MYC sgRNAs. Mean + SD values from n=3 replicates are shown; ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05.

(F) Immunoblot analysis of WCL from GM12878 LCLs expressing control or MYC sgRNAs.

(G) Schematic diagram indicating repression of the conditional MYC allele in p493-6 B-cells by doxycycline (dox) addition.

(H) qRT-PCR analysis of EBV lytic gene mRNA abundances in p493-6 B cells that were grown in the absence or presence of doxycycline (1ug/ml) for 24 hours. Mean + SD values from n=3 replicates are shown; ****p < 0.0001, ***p < 0.001.

(I) FACS plots of PM gp350 abundances in Akata cells that stably express cDNA rescue constructs encoding control GFP or PAM site point mutant HA-MYC, together with control or MYC sgRNAs, as indicated.

(J) PM gp350 values in Akata BL stably expressing GFP or HA-tagged MYC and that were either mock- or αIgG-induced (10 μg/ml). At 48 hours post induction, PM gp350 mean fluorescence intensity (MFI) was quantified by FACS. Mean + SD MFI values from n=3 replicates are shown. ***p < 0.001.

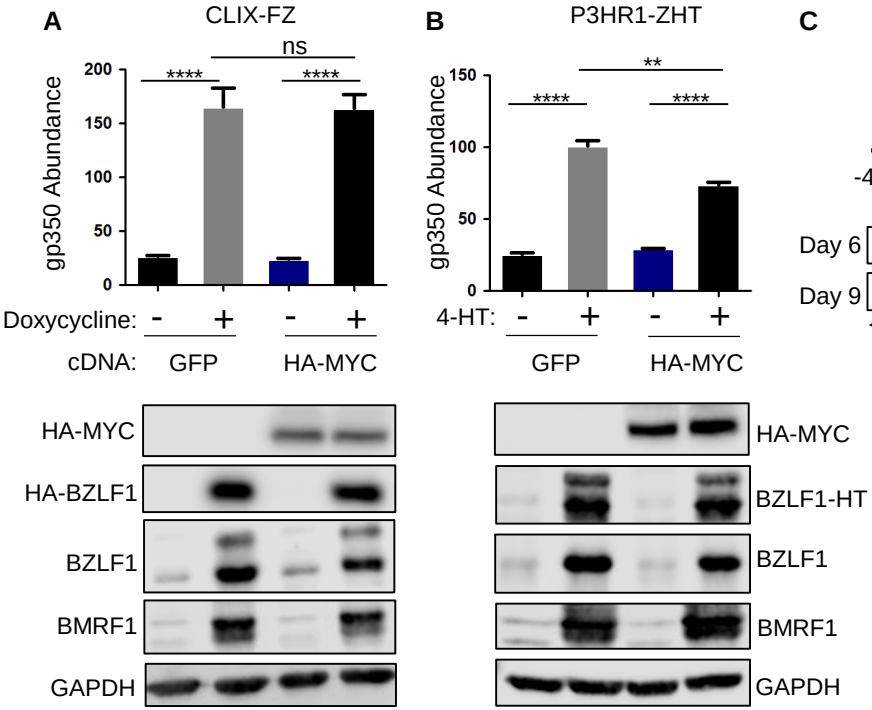
(K) Immunoblot analysis of WCL from Cas9+ GM12878 LCLs stably expressing GFP or sgRNA-resistant HA-MYC rescue cDNA and either control or MYC sgRNAs, as indicated.

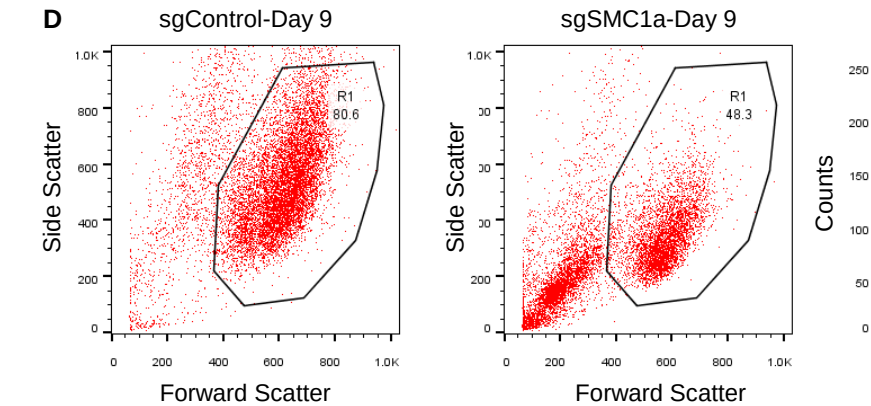
(L) Akata cells with stable GFP or HA-tagged MYC expression were mock-induced or induced by αIgG cross-linking (10 μg/ml) for 48 hours. Shown are immunoblots of WCL for the indicated proteins.

(M) Akata cells with control or BZLF1 sgRNA expression were mock or αIgG induced for 48 hours. Shown are immunoblots obtained from the indicated conditions.

B, D, F K-M blots are representative of at least n=3 replicates.

Figure S3 (related to Figure 2 and 3)





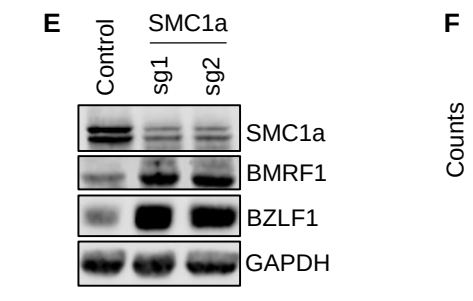


Figure S3, Related to Figures 2 and 3. Conditional BZLF1 Expression Bypasses MYC Depletion, whereas SMC1 Supports MYC Expression and Restricts EBV Lytic Genes

(A) Top: Mean + SEM of FACS analysis of PM gp350 abundance from n=3 replicates in CLIX-FZ Burkitt lymphoma cells, which stably express a doxycycline-inducible conditional HA-BZLF1 allele and also stably express cDNAs encoding GFP or HA-MYC. Cells were mock-induced or induced with 1 µg/ml doxycycline for 24 hours. Bottom: immunoblot of WCL from CLIX-FZ cells used for FACS analysis.

(B) Top: Mean + SEM of FACS analysis of PM gp350 abundance from n=3 replicates in P3HR1-ZHT Burkitt lymphoma cells, which stably express a conditional BZLF1 allele fused to a modified estrogen receptor 4HT binding domain (BZLF1-HT), and which also stably express cDNAs encoding GFP or HA-MYC. Cells were mock-induced or induced with 400nM 4HT for 24 hours. Bottom: immunoblot of WCL from P3HR1-ZHT cells used for FACS analysis.

(C) Top: Distribution of Log2 fold-change (LFC) values of sgRNAs in gp350+ sorted versus input library cells for all Avana library guides at Day 6. Bottom: LFC values for the four SMC1A targeting sgRNAs (red lines), overlaid on gray gradient depicting the overall sgRNA distribution, at CRISPR screen Days 6 versus 9. Average values from two screen biological replicates are shown.

(D) FACS analysis of Akata cells at Day 9 post-transduction with lentiviruses that express either control or SMC1a sgRNAs, as indicated. Left and middle panels show forward and side scatter values, with gates used for live cell analysis indicated. Right panel shows PM gp350 values of Day 9 Akata cells with the indicated sgRNAs. Data are representative of n=3 values.

(E) Immunoblots of WCL from Akata cells expressing control or independent SMC1A sgRNAs.

(F) FACS plots of PM gp350 in P3HR-1 cells expressing control or independent SMC1A sgRNAs, representative of n=3 replicates.

Blots in A, B and E are representative of n=3 replicates.

Figure S4 (related to Figure 4)

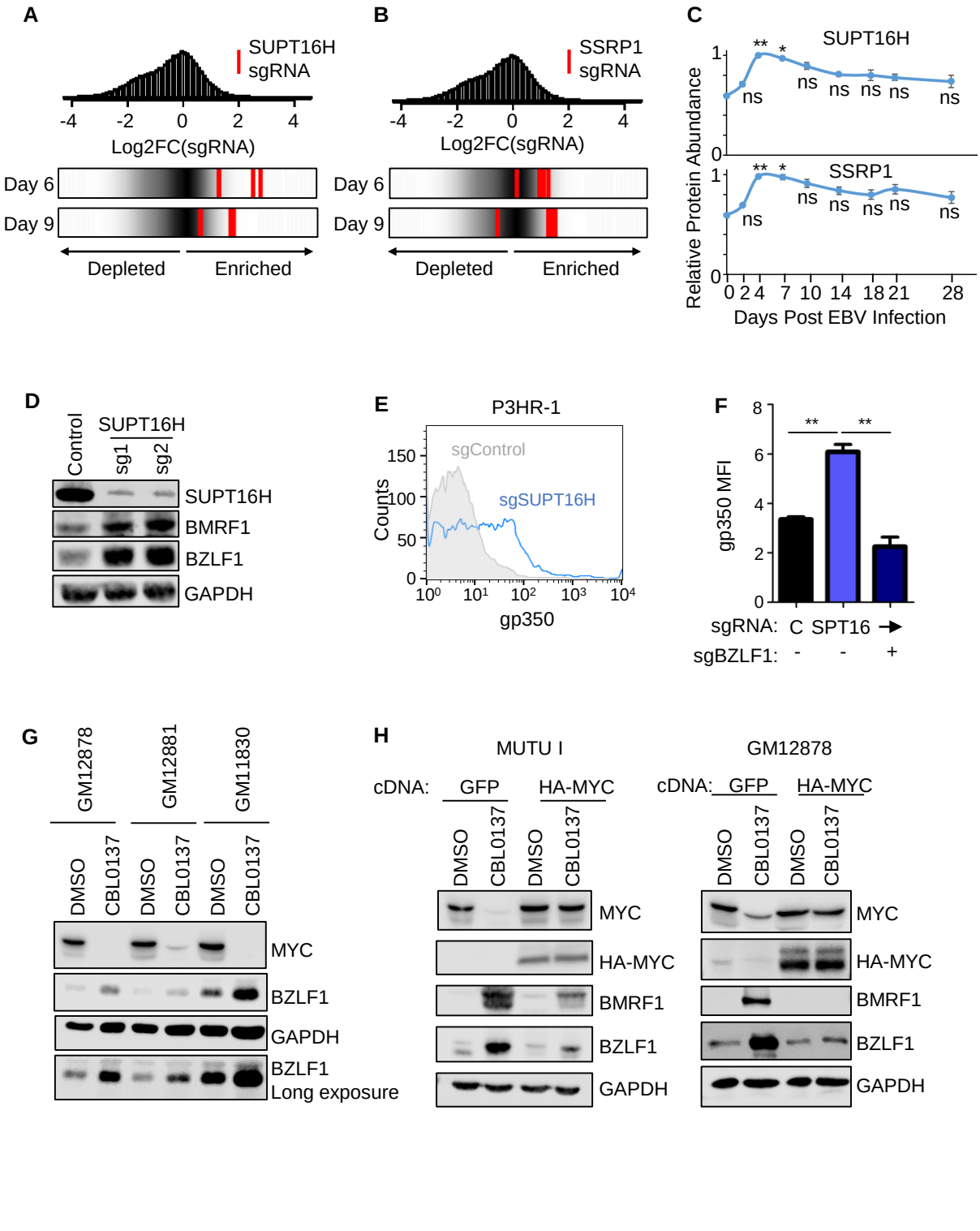


Figure S4, related to Figure 4. FACT Suppresses of EBV Lytic Antigens in BL cells

(A) Top: Distribution of Log2 fold-change (LFC) values of sgRNAs in gp350+ sorted versus input library cells for all Avana library guides at Day 6. Bottom: LFC for the four SUPT16H targeting sgRNAs (red lines), overlaid on gray gradient depicting the overall sgRNA distribution, at CRISPR screen Days 6 versus 9. Average values from two screen biological replicates are shown.

(B) Top: Distribution of Log2 fold-change (LFC) values of sgRNAs in gp350+ sorted versus input library cells for all Avana library guides at Day 6. Bottom: LFC for the four SSRP1 targeting sgRNAs (red lines), overlaid on gray gradient depicting the overall sgRNA distribution at screen Days 6 versus 9. Average values from two screen biological replicates are shown.

(C) Relative SUPT16H and SSPR1 protein abundances in primary human B-cells WCL at the indicated days post infection by B95.8 EBV. Mean +/- SEM values from quadruplicate replicates are shown and were taken from (Wang et al., 2019).

(D) Immunoblots of WCL from Akata cells expressing control or independent SUPT16H sgRNAs.

(E) FACS analysis of PM gp350 abundances in P3HR-1 cells expressing control or independent SUPT16H sgRNAs.

(F) FACS mean + SD PM gp350 MFI values from triplicate experiments with Akata cells that expressed control or BZLF1 sgRNAs followed by control or SUPT16H (SPT16) sgRNAs. ** $p<0.01$.

(G) Immunoblot analysis of WCL from the indicated LCLs treated with DMSO vehicle control or 2.5μM CBL0137 for 6 hours, washed with fresh media three times and then cultured for an additional 42 hours. A long-exposure for the BZLF1 immunoblot is shown at bottom.

(H) Immunoblot analysis of WCL from MUTU BL (left) or GM12878 LCL (right) with stable GFP or HA-MYC expression and treated with DMSO vehicle control or 2.5μM CBL0137 for 6 hours, washed with fresh media three times and then cultured for an additional 42 hours.

Blots in D, G, H are representative of three replicates.

Figure S5 (related to Figure 5)

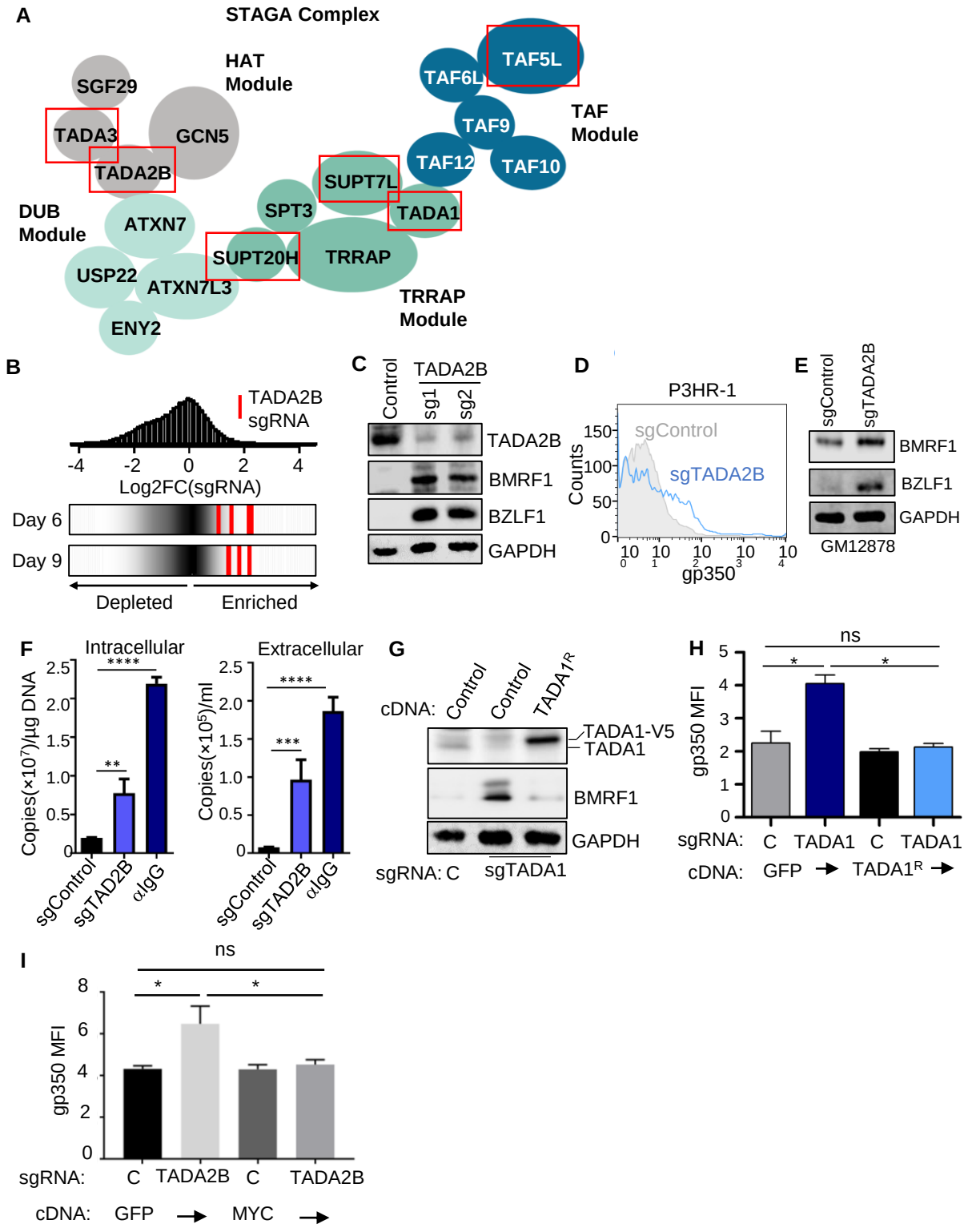


Figure S5, Related to Figure 5. STAGA Suppresses EBV Lytic Antigen Expression in BL.

(A) Schematic model of the STAGA complex, comprised of HAT module (grey), DUB module (light green), TRRAP module (dark green) and TAF module (blue). CRISPR screen hits are highlighted by red boxes.

(B) Top: Distribution of Log2 fold-change (LFC) values of sgRNAs in gp350+ sorted versus input library cells for all Avana library guides at screen Day 6. Bottom: LFC for the four TADA2B targeting sgRNAs (red lines), overlaid on gray gradient depicting the overall sgRNA distribution. Average values from two screen biological replicates are shown.

(C) Immunoblot of WCL from Akata cells expressing control or independent TADA2B sgRNAs.

(D) FACS plots of PM gp350 abundance in P3HR-1 cells expressing control or TADA2B sgRNAs.

(E) Immunoblot of WCL from GM12878 LCLs expressing control or TADA2B sgRNAs.

(F) qRT-PCR analysis of EBV intracellular vs DNAse treated extracellular genome copy number from Akata cells expressing control or TADA2B sgRNAs at Day6 post lentivirus transduction, or 48h post anti-IgG stimulation (10 µg/ml). Mean + SD values from n=3 replicates are shown. ****$p < 0.0001$, ***$p < 0.001$.

(G) Immunoblot analysis of WCL from P3HR-1 cells stably expressing cDNA encoding control GFP or cDNA with silent PAM site mutation encoding V5-tagged TADA1 to abrogate Cas9 editing, together with control or TADA1 sgRNAs, as indicated.

(H) FACS analysis of PM gp350 MFI in P3HR-1 cells stably expressing GFP or V5-tagged TADA1 rescue cDNA, together with control or TADA1 sgRNAs, as indicated. Mean + SD values from n=3 replicates are shown. * $p < 0.05$.

(I) FACS analysis of PM gp350 MFI in P3HR-1 cells stably expressing GFP or HA-MYC cDNAs, together with control or TADA2B sgRNAs, as indicated. Mean + SD values from n=3 replicates are shown. * $p < 0.05$.

Blots in S6C, E, G and I are representative of n=3 values.

Figure S6 (related to Figure 6)

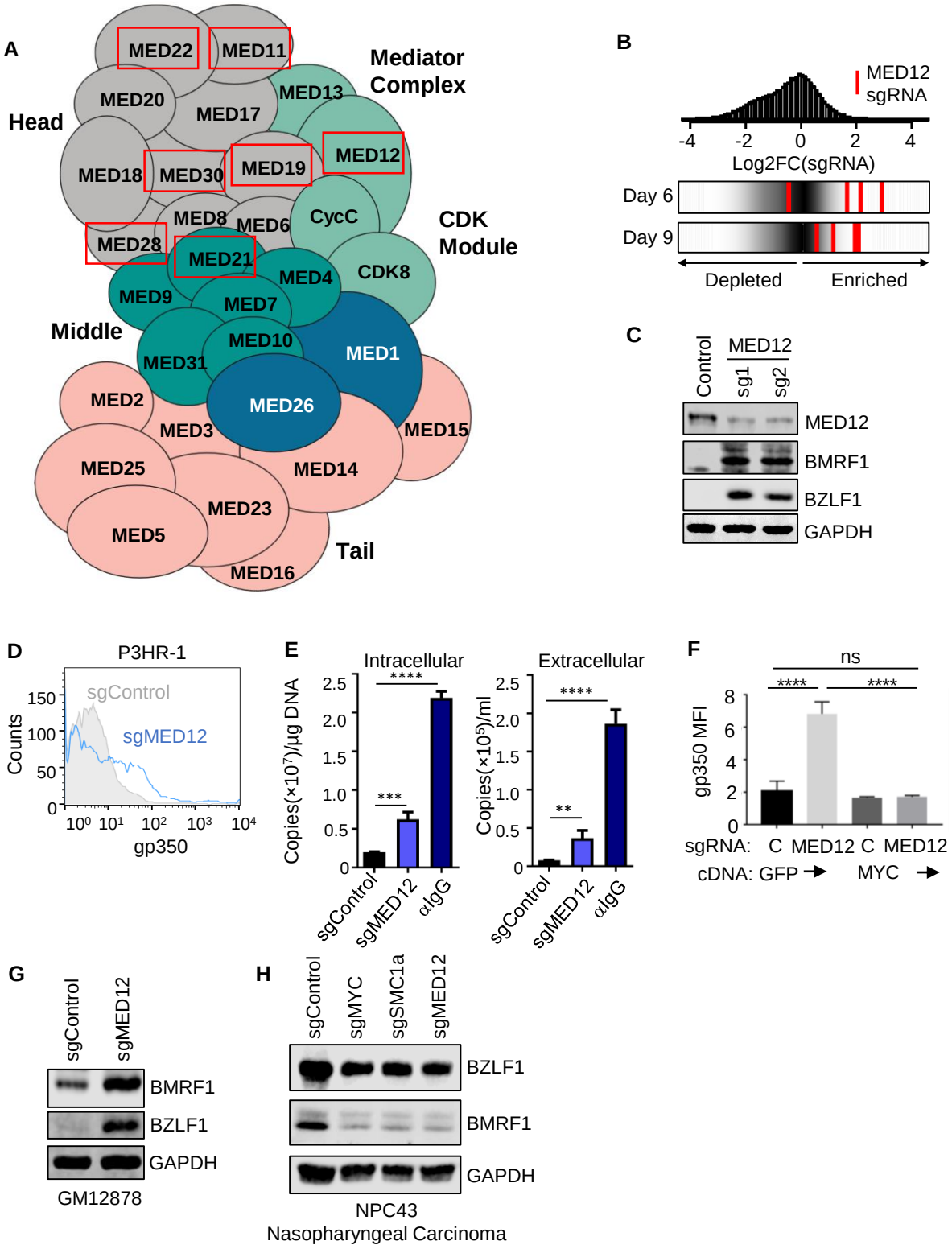


Figure S6, Related to Figure 5. Mediator Suppresses EBV Lytic Antigen Expression in BL

(A) Schematic model of the Mediator complex, comprised of Head (grey), Middle (dark green), CDK (light green) and Tail (peach) modules. CRISPR screen hits are highlighted by red boxes and mapped to three of the four Mediator modules.

(B) Top: Distribution of Log2 fold-change (LFC) values of sgRNAs in gp350+ sorted versus input library cells for all Avana library guides at CRISPR screen Day 6. Bottom: LFC for the four MED12 targeting sgRNAs (red lines), overlaid on gray gradient depicting the overall sgRNA distribution. Average values from two screen biological replicates are shown at screen Days 6 and 9.

(C) Immunoblot of WCL from Akata cells expressing control or independent MED12 sgRNAs.

(D) FACS plots of PM gp350 abundances from P3HR-1 cells expressing control or MED12 sgRNAs.

(E) qRT-PCR analysis of EBV intracellular vs DNAse-treated extracellular genome copy number from Akata cells expressing control or MED12 sgRNAs at Day6 post lentivirus transduction, or 48h post anti-IgG stimulation (10 μg/ml). Mean + SD values from n=3 replicates are shown.$****p < 0.0001$, $***p < 0.001$.

(F) FACS analysis of PM gp350 MFI in Akata cells stably expressing GFP or HA-MYC cDNAs, together with control or MED12 sgRNAs, as indicated. Mean + SD values from n=3 replicates are shown. **** $p < 0.0001$. ns, non-significant

(G) Immunoblot of WCL from GM12878 LCLs expressing control or MED12 sgRNAs.

(H) Immunoblot of WCL from Cas9+ EBV+ NPC43 nasopharyngeal carcinoma cells expressing the indicated sgRNAs.

Blots in S6C, G and H are representative of n=3 values.

Figure S7 (related to Figure 6 and 7)

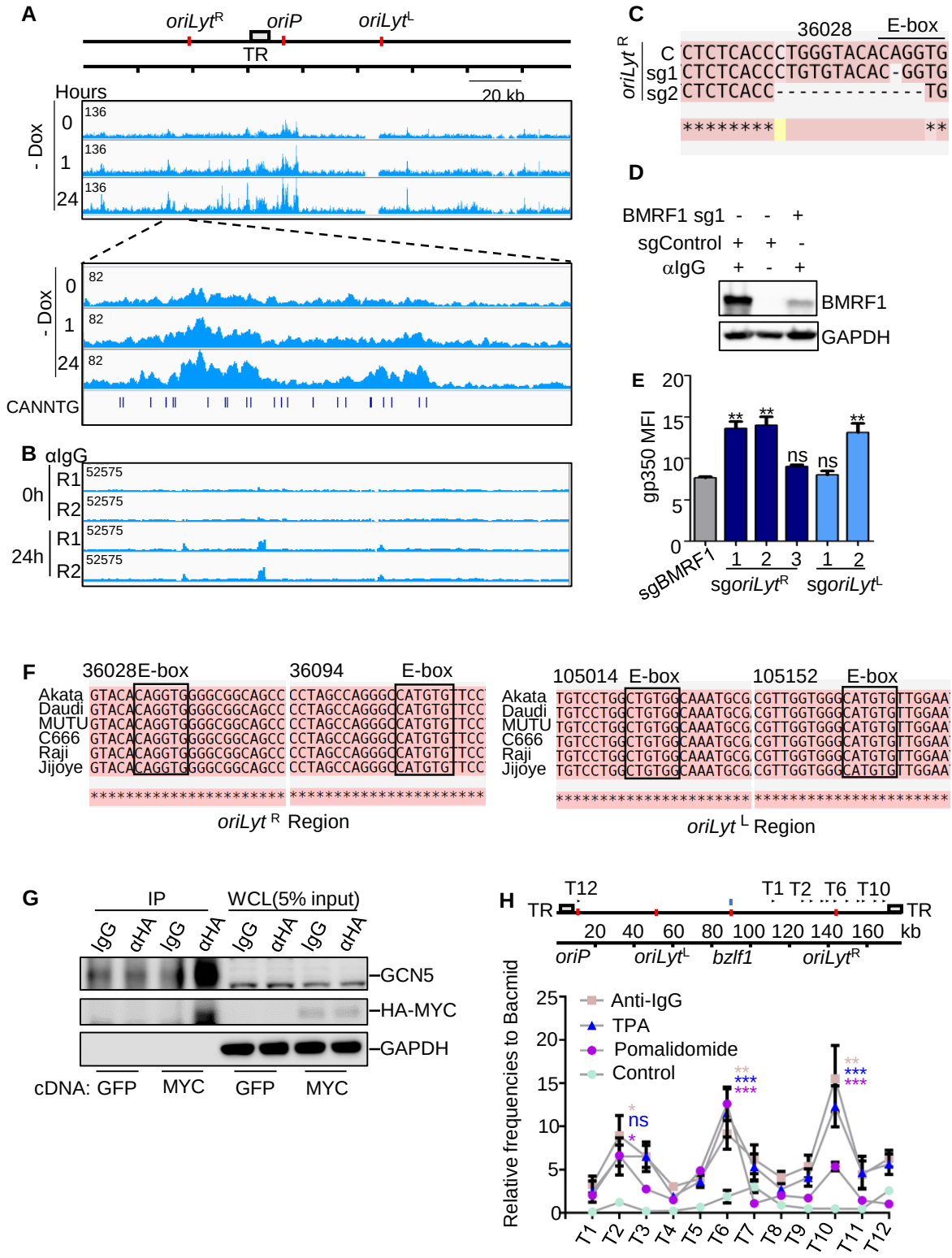


Figure S7, Related to Figure 6 and 7. MYC *OriLyt* Occupancy is Important for EBV Latency in BL.

(A) ChIP-Seq analysis of EBV genome MYC occupancy in p493-6 LCLs. Shown are the p493-6 input and MYC ChIP-Seq tracks from (Lin et al., 2012) and mapped to the EBV genome. P493-6 cells have a conditional MYC allele and were grown at time 0 in the non-permissive condition, in which exogenous MYC expression is repressed by doxycycline. Withdrawal of doxycycline at the zero hour timepoint rapidly induces MYC expression. ChIP-Seq was performed at 0, 1 and 24 hours of MYC induction by doxycycline withdrawal. A zoomed in oriLytR region is shown below the EBV genome track. E-box sites with the canonical CANNTG motif are indicated by blue vertical bars below the zoomed in tracks. Track heights are indicated at top left of each track.

(B) ATAC-seq analysis of IgG-cross-linking effects on Akata BL EBV genomic accessibility. Shown are two biological replicate ATAC-seq tracks (R1 and R2) collected at 0 or 24 hours (h) post stimulation by αIgG (10 µg/ml).

(C) Sanger DNA sequence analysis of EBV genome sequences near the targeted oriLytR E-box site from Akata cells that expressed control (c) or the indicated oriLytR sgRNAs (sg1 vs sg2). Shown are consensus DNA sequences from Sanger sequence analysis of PCR-amplified EBV genome regions from n=3 independent experiments. *indicates identical sequence between cells expressing all three sgRNAs.

(D) Validation of EBV genome BMRF1 CRISPR editing. Akata cells expressing control or BMRF1-targeting sgRNAs were stimulated by IgG cross-linking (10 µg/ml) for 48 hours. WCL were then immunoblotted, as indicated.

(E) FACS analysis of PM gp350 MFI + SD from n=3 independent replicates, from Akata cells expressing either control BMRF1 or oriLyt region targeting sgRNAs, as indicated, as in Fig. 6E.

(F) Alignment of DNA sequences from the indicated EBV type I (Akata, Daudi, MUTU, C666, Raji) and type II (Jijoye) strains highlighting conservation of E-box regions.

(G) Biochemical analysis of MYC and GCN5 association in protein complexes. WCL from Akata cells with control GFP or HA-MYC expression were subject to anti-IgG control or anti-HA-MYC immunopurification. Purified complexes or 5% input were immunoblotted for endogenous GCN5, for HA-MYC or GAPDH load control, as indicated.

(H) 3C assay quantitation of interaction frequency between the BZLF1 anchor primer and test primer regions in Akata cells treat with vehicle control (teal circles), 10 µg/ml anti-IgG (peach squares), 1 µg/ml pomalidomide (magenta circles) or 20 ng/ml TPA (blue triangles), as indicated. Mean + SD values from n=3 replicates are shown. 3C assay frequencies were normalized by Bacmid input values. *$p<0.05$, ** $p<0.01$, *** $p<0.001$.

Blots in S7D and G are representative of n=3 experiments.