Supplementary Data

Supplementary Figure Legends (S1-S7)

Supplementary Figure S1. Control ChIPs. Controls for key ChIPs are shown using uninfected BL31 cells or BL31 infected with B95.8-BAC EBV (WT) to show that the precipitations were reliable. Primers that amplify the promoters of inactive *Myoglobin* and constitutively active *GAPDH* were used, as well as primers for the PRC2 target *IRX4* and non-PRC2-targeted *MCM6*. **A)** The repressive mark H3K27me3 was found associated with the inactive gene encoding Myoglobin but not associated with the active *GAPDH* gene. **B)** H3K4me3 was present at the *GAPDH* gene, but not at the *Myoglobin* gene promoter. **C)** Total histone H3 was associated with both *Myoglobin* and *GAPDH* promoters *Myoglobin* appears to have a higher nucleosome density than *GAPDH* in both infected and uninfected cells. **D)** ChIP analysis shows that JARID2 association with the *BIM* promoter is significant and comparable to known PRC2-target gene *IRX4* (27) with no significant changes associated with the presence of EBV. Background levels were established using primers for the *MCM6* promoter that is not associated with either PRC2 or JARID2 (29). **E)** ChIP for RbAp48 reveals a similar distribution to JARID2 **F)** No RNA Pol II was precipitated from the silent Myoglobin promoter with the pan-specific antibody, but it was abundant at GAPDH. **G)** ChIP for the phospho-Ser5 form of RNA Pol II only showed occupation of the active GAPDH promoter.

Supplementary Figure S2. SUZ12 levels reduced at the *BIM* promoter after SUZ12 and JARID2 knock-down. ChIP analysis showing SUZ12 levels at the *BIM* promoter of cells from which SUZ12 or JARID2 was depleted by shRNA (BL31 WT SUZ12 K/D and JARID2 K/D) relative to control cells

infected with lentiviruses expressing a non-targeting shRNA. Values represent the ratio of chromatin precipitated, after correction for IgG, relative to 5% of input.

Supplementary Figure S3. Binding of initiating and elongating forms of RNA Pol II across the *BIM* promoter. A) An antibody specific for the initiated, phospho-Ser5 form of RNA Pol II revealed a peak around the TSS (primer pair F) and the levels of association are substantially reduced in EBV-infected BL31 WT. **B)** An antibody specific for the elongating, phospho-Ser2 Pol II produced a similar result.

Supplementary Figure S4. ChIPs performed using a panel of BL2 cells show that repression of the *BIM* promoter by EBV is not cell line-restricted. Uninfected BL2 cells, BL2 cells infected with the wild type EBV (WT), EBNA3A knockout (KO), EBNA3C knockout (KO) and a revertant EBV were subjected to ChIP analysis similar to that used for BL31 cells. The trends found are remarkably similar to those in BL31 – **A)** H3K27me3, **B)** H3K4me3, **C)** SUZ12, **D)** Total Pol II and **E)** Phospho-Ser5 Pol II are shown. Values represent ratio of chromatin precipitated, after correction for IgG, relative to 5% of input.

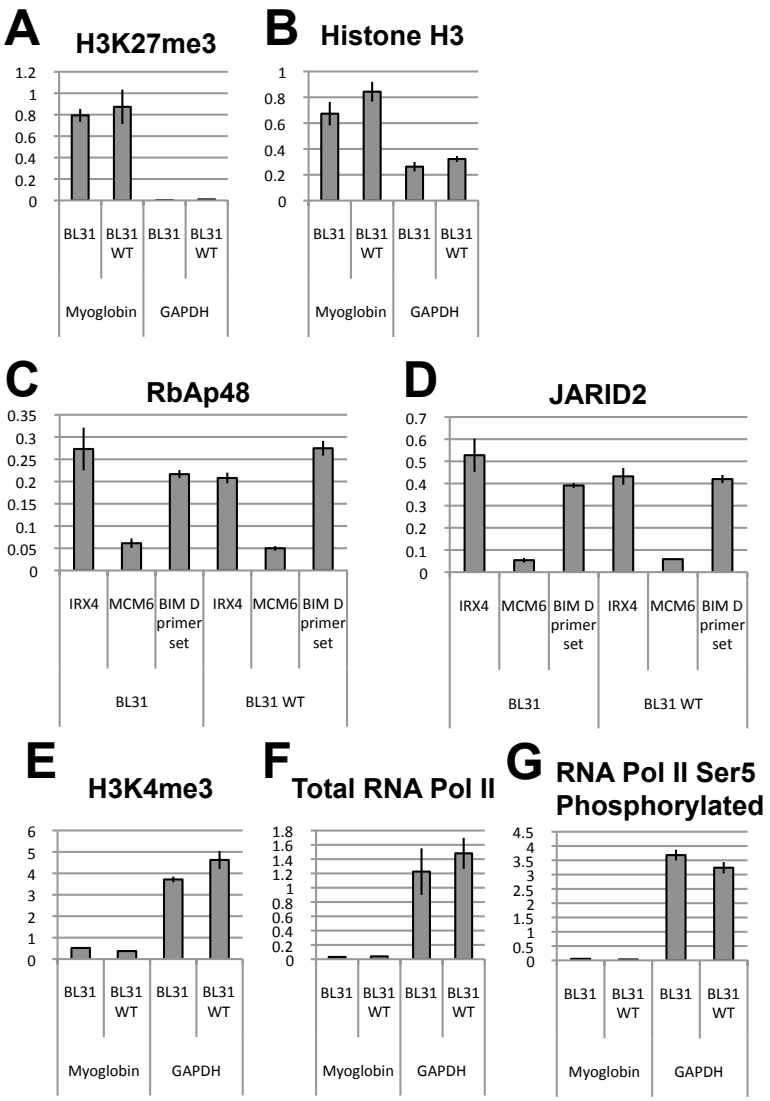
Supplementary Figure S5. The integrity of the EBV-BAC is not compromised after establishment of BL31 cell lines expressing TAP-tagged EBNA3C. Recovered EBV-BAC clones were digested with EcoRI or NotI and analyzed by pulsed-field gel electrophoresis to compare them to BAC DNA used to create the producer HEK293 lines. The bands for the EBV BamH1-W repeats and the terminal repeats (TR) are indicated.

Supplementary Figure S6. Targeting of TAP-tagged EBNA3C in a second BL31 cell line and co-immunoprecipitation of EBNA3C with EBNA3A. A)

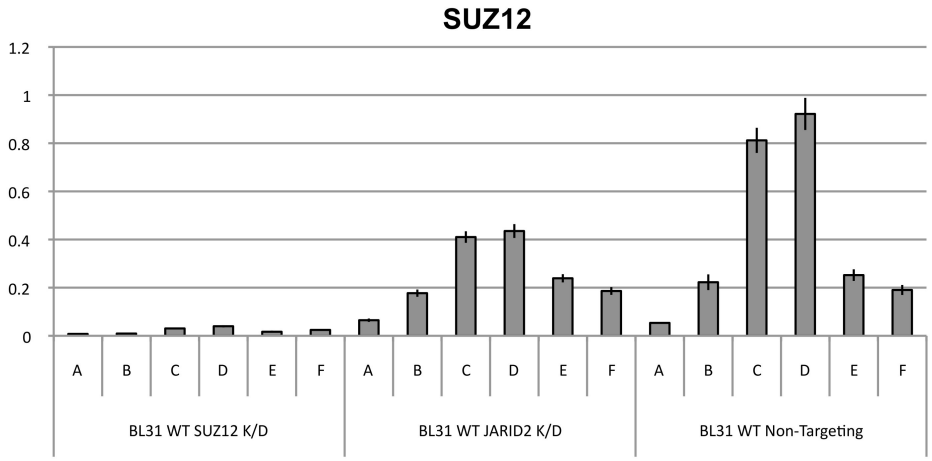
ChIP analysis using an anti-FLAG antibody to precipitate EBNA3C-TAP and chromatin associated with it in BL31 3C-TAP-2 cells to show tagged EBNA3C association with *BIM* and *RASGRP1* (as in Figure 8C), using a cell line produced with an independently generated EBNA3C-TAP-expressing virus. **B)** Immunoprecipitation was performed from an LCL, produced with the B95.8-BAC (WT) virus used throughout this study, with a sheep anti-EBNA3A antibody. Sheep IgG was used for negative control immunoprecipitations. Immunoprecipitates were compared to 10% of input by western blots probed for EBNA3C (WB: EBNA3A) and EBNA3A (WB: EBNA3C). A similar immunoprecipitation was performed on BL31 EBNA3AKO cells as a control for antibody specificity. The western blot was probed with anti-EBNA3C to show that the anti-EBNA3A antibody does not precipitate EBNA3C by direct interaction.

Supplementary Figure S7. Efficient repression of *BIM* requires the interaction of EBNA3A and EBNA3C with CtBP. Cells infected with two independent viruses that express EBNA3A and EBNA3C both of which are incapable of binding CtBP (BL31 E3CTBP1 and 2) (51) were used to assess the requirement of CtBP in the repression of *BIM* by the EBNA3s. These were compared to uninfected BL31, BL31 infected with WT virus and cells infected with the respective binding-mutant revertants BL31 REVCTBP1 and 2 (51). **A)** Western blots of extracted proteins show BIM protein levels for each cell line, and γ-tubulin was used as loading control. **B)** Quantitative real-time RT-PCR was performed to show BIM mRNA levels for each cell line. The histogram bars represent values relative to housekeeping gene *GNB2L1*. The error bars represent standard deviation from three replicate assays.

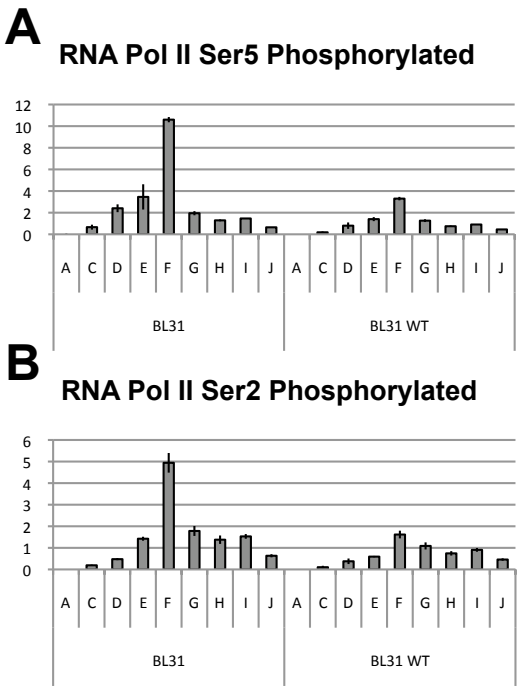
Supplementary Figure S1



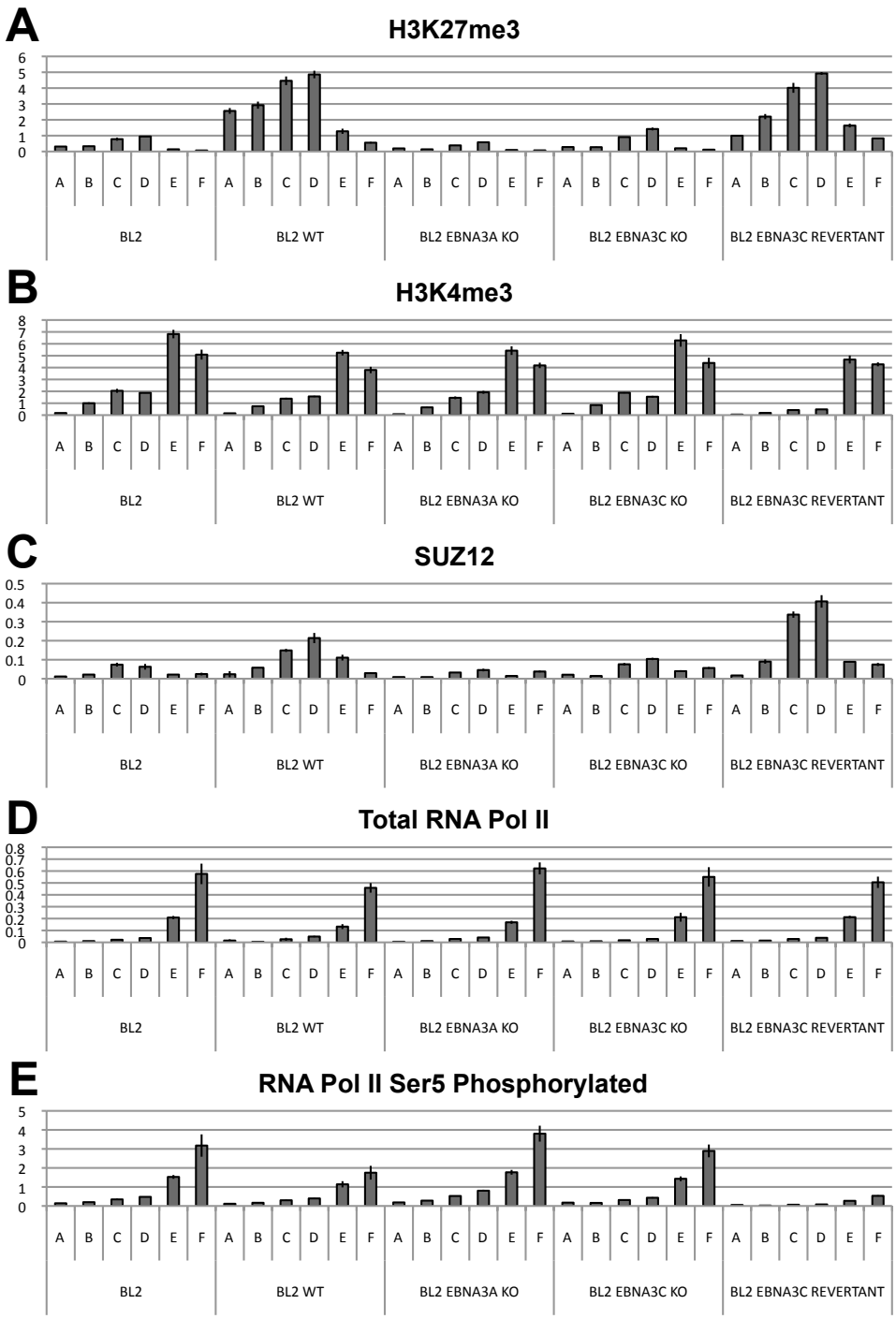
Supplementary Figure S2



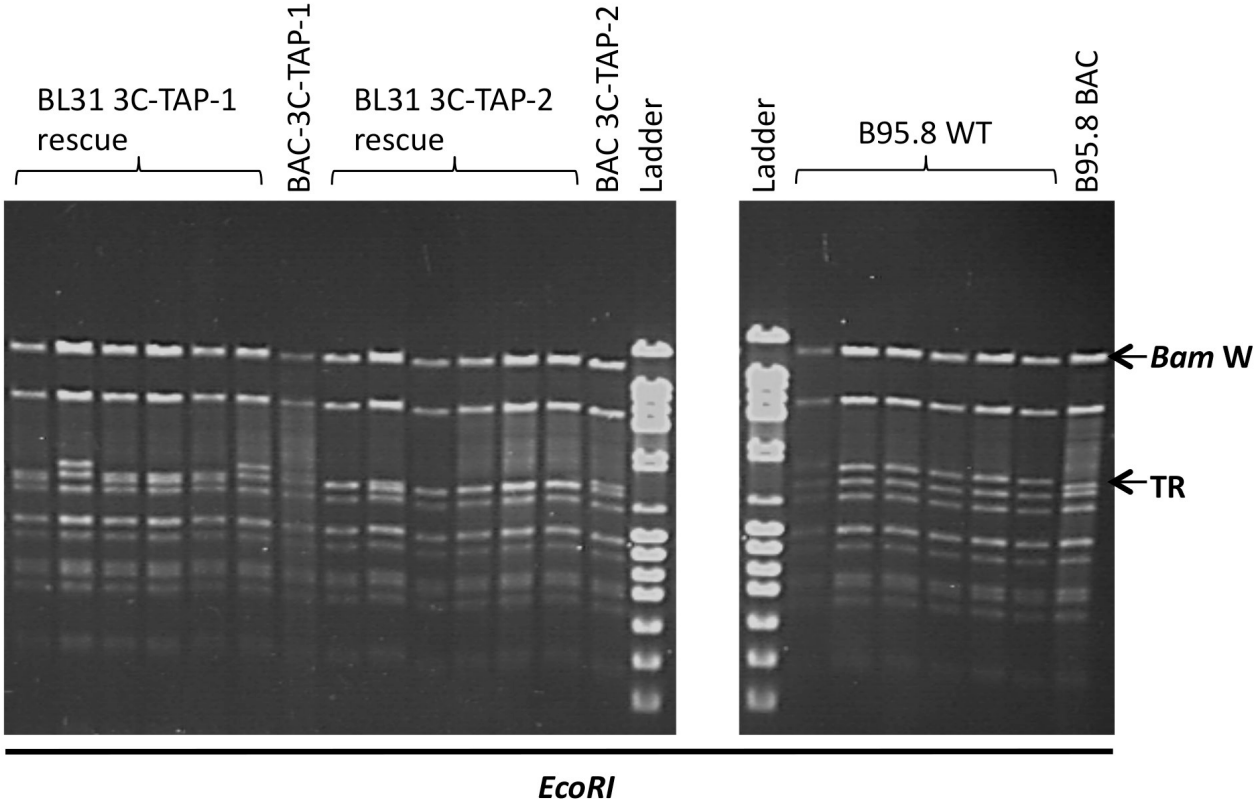
Supplementary Figure S3

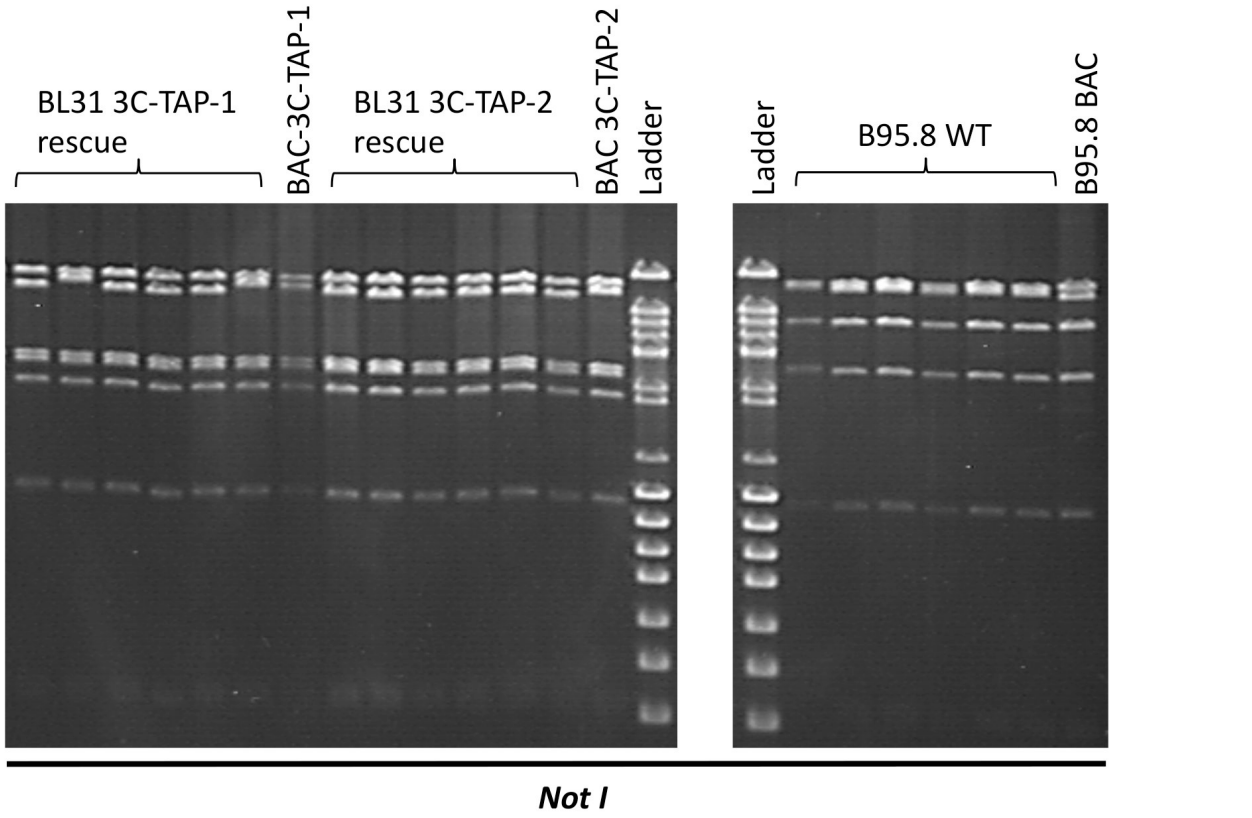


Supplementary Figure S4

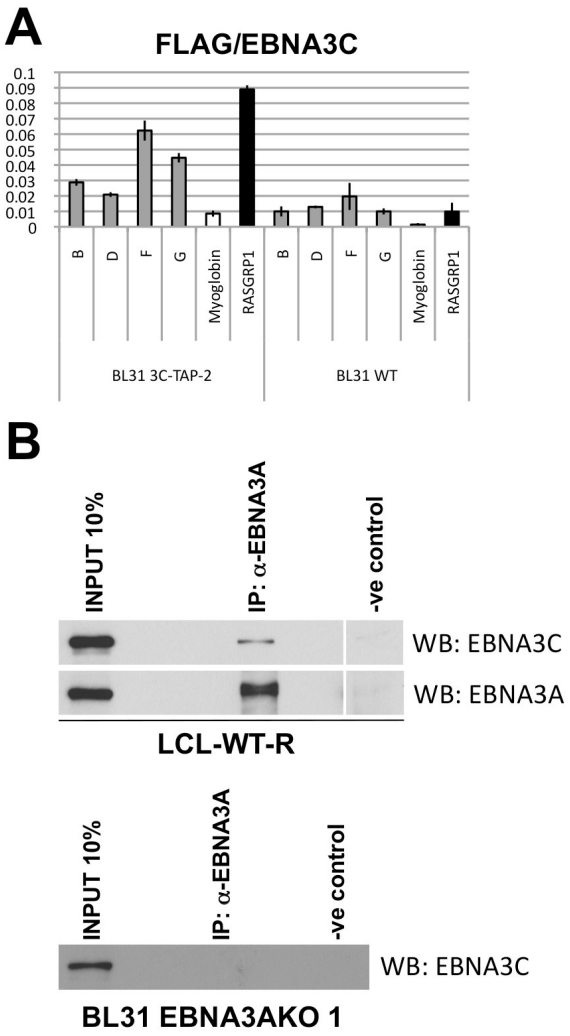


Supplementary Figure S5

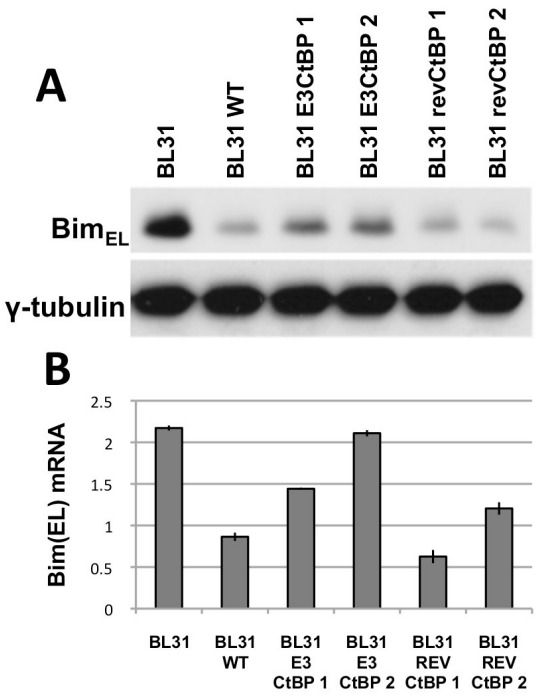




Supplementary Figure S6



Supplementary Figure S7



Supplementary Tables (S1-S3)

Protein Recognized	Application	Product details	per IP(ChIP) or dilution (WB)
BIM	WB	Stressgen; AAP-330	1:1000
BMI1	WB	Abcam; ab14389	1:1000
EED	WB	Millipore; 09-774	1:1000
EZH2	WB	Active Motif; 39639	1:1000
EZH2	ChIP	Cell Signaling; 3147	1:500
FLAG	IP	Sigma; F1804	1:200
FLAG	ChIP	Sigma; F7425	4µg
g-Tubulin	WB	Sigma; T6557	1:8000
H3K27me3	WB	Millipore; 07-449	1:1000
H3K27me3	ChIP	Millipore; 17-625	4µl
H3K4me3	ChIP	Millipore; 17-614	3µl
Histone H3	ChIP, WB	Abcam; ab1791	4µg (ChIP); 1:1000 (WB)
JARID2	ChIP	Abcam; ab48137	4µg
JMJD3	WB	Abcam; ab38113	1:1000
Normal Rabbit IgG	ChIP	Millipore; PP64B	4µg
RbAp48	ChIP, WB	Abcam; ab1765	4µg(ChIP); 1:1000(WB)
RPB1, CTD phospho-Ser5 (RNA pol II S5P)	ChIP	Abcam; ab5131	4µg
RPB1, N-terminus (Total RNA Pol II)	ChIP	Santa Cruz; sc-899; N20	4µg
SUZ12	WB	Santa Cruz; sc-46264 (P-15)	1:1000
SUZ12	ChIP	Abca; ab12073	4µg

WB: western blot
ChIP: Chromatin immunoprecipitation
IP: Immunoprecipitation

Table S1. Antibodies used in this study

Primer pair	Forward primer	Reverse primer	Notes
A	TTTAGAAAGAATCTTGGCAGTCAACTCCTC	CAATGGCTGGTGAAAAGGAGGGTTT	
B	GAAGGACCAGGGAGGAAGGACCAAG	TGACACCTAGCCCAGTGGAAACCCC	
C	CGAGCGGGAAAAAAGGTTTGGTTCA	TAGGCTCCCACTTCCTTCTCCCAGT	
D	AAGAGCAAAGTTCGTCCGCGGTAGG	TATTTCGCTGCAAGAGGGAAAAGGCAC	
E	GACCCTCAGAGGGAGGAGAGCTCAAA	GCCCTGAGTTTCTAAGCCGCTCTGG	
F	CGCCAGCAGGCAGAGTTAC	CAGGCTCGGACAGGTAAAGG	
G	GGTTCCCTCTCGGGCAGGTTCTCTT	CCGATACTACGAGCAGGTCCTCAGC	
H	CCAGGGCGGAGGGTGTGAATTTACT	AACTTCCTCTCTCTCCTGGTGCGCC	
I	CTTTGTCTCCTGCGCTGCTTTCGTG	GGCAATCACACCGAAACTCACGTCC	
J	CGTCCTACCTAACCCCGGGAAGTCA	CACATGCGTTTCCAGAGAAGCAGCT	
GAPDH	CGCTCTCTGCTCCTCC	TTTCTCTCCGCCCGTCCAC	By millipore
IRX4	AGCGTGTCCTTTCATAGGTC	TTTAGTCACCCGAGAACGAG	(27)
MCM6	CTCTTTGGCCATTCCTGATT	GCCCAAGAGAGCGACTTTAC	(29)
Myoglobin	GGAGAAAGAAGGGGAATCACA	GATAAATATAGCCAACGCCACA	(84)

Table S2. List of primers used to analyze precipitated DNA from MeDP and ChIP

Primer Pair	Forward primer	Reverse primer	Notes
BIM(EL)	GCTGTCTCGATCCTCCAGTG	GTTAAACTCGTCTCCAATACG	(25)
BMI1	AATTAGTTCCAGGGCTTTTCAA	CTTCATCTGCAACCTCTCCTCTAT	(85)
GAPDH	Not available	Not available	Qiagen QuantiTect® Primer Assay Hs_GAPDH_2_SG, Cat. No QT01192646
GNB2L1	GAGTGTGGCCTTCTCCTCTG	GCTTGCAGTTAGCCAGGTTC	(86)
SUZ12	GGATCCTGAATGGCTAAGAGAAAAA	TCATCACTTCTTTCTCTCCTTCATTAAC	(87)

Table S3. List of primers used to assay cDNA from mRNA transcripts

REFERENCES (Including supplementary references 84-87)

25. Anderton, E., Yee, J., Smith, P., Crook, T., White, R.E. and Allday, M.J. (2008) Two Epstein-Barr virus (EBV) oncoproteins cooperate to repress expression of the proapoptotic tumour-suppressor Bim: clues to the pathogenesis of Burkitt's lymphoma. *Oncogene*, 27, 421-433.

27. Li, G., Margueron, R., Ku, M., Chambon, P., Bernstein, B.E. and Reinberg, D. (2010) Jarid2 and PRC2, partners in regulating gene expression. *Genes & Development*, 24, 368-380.

29. Peng, J.C., Valouev, A., Swigut, T., Zhang, J., Zhao, Y., Sidow, A. and Wysocka, J. (2009) Jarid2/Jumonji Coordinates Control of PRC2 Enzymatic Activity and Target Gene Occupancy in Pluripotent Cells. *Cell*, 139, 1290-1302.

51. Skalska, L., White, R.E., Franz, M., Ruhmann, M. and Allday, M.J. (2010) Epigenetic repression of p16(INK4A) by latent Epstein-Barr virus requires the interaction of EBNA3A and EBNA3C with CtBP. *PLoS Pathog*, 6, e1000951.

84. Delbarre, E., Jacobsen, B.M., Reiner, A.H., Sorensen, A.L., Kuntziger, T. and Collas, P. (2010) Chromatin environment of histone variant H3.3 revealed by quantitative imaging and genome-scale chromatin and DNA immunoprecipitation. *Mol Biol Cell*, 21, 1872-1884.

85. Nowak, K., Kerl, K., Fehr, D., Kramps, C., Gessner, C., Killmer, K., Samans, B., Berwanger, B., Christiansen, H. and Lutz, W. (2006) BMI1 is a target gene of E2F-1 and is strongly expressed in primary neuroblastomas. *Nucleic Acids Res*, 34, 1745-1754.

86. Zhang, X., Ding, L. and Sandford, A.J. (2005) Selection of reference genes for gene expression studies in human neutrophils by real-time PCR. *BMC Mol Biol*, 6, 4.

87. Chen, H., Gu, X., Su, I.H., Bottino, R., Contreras, J.L., Tarakhovsky, A. and Kim, S.K. (2009) Polycomb protein Ezh2 regulates pancreatic beta-cell Ink4a/Arf expression and regeneration in diabetes mellitus. *Genes Dev*, 23, 975-985.