Supplemental Methods

Plasmids and transfection

The plasmid used for transient expression of HA-tagged BmBRD4 (Gene ID 101735474) were generated from pIZ/V5-His **(Supplemental Fig. S3A)**. The plasmids for enhancer activity analysis were generated from pNL1.1 (Promega, N1001) **(Supplemental Fig. S9B)**, and the plasmid served as the internal reference was generated from pGL4.10 (Promega, E6651) by insert a promoter of BmNPV *ie-1* before *luc2* **(Supplemental Fig. S9A)**, which enables stable expression of the reference luciferase in BmN cells. The plasmid PB-luc-Zeo was constructed by assembling inverted terminal repeats (ITR) of *piggyBac*, a transposon with high transformation activity in insects (Handler et al. 1998), and fragments cloned from pNL1.1, pGL4.10 and pIZ/V5-His, which was preserved in our laboratory **(Supplemental Fig. S9C)**. Apart from the coding sequence (CDS) of BmBRD4-HA and the promoters of *KWMTBOMO15542* and *KWMTBOMO16284* synthetized by Tsingke Biotechnology (Hangzhou, China), all the plasmid used in this study was constructed via Gibson assembly (Gibson et al. 2009), and sequences of the primers used for plasmid construction were listed **(Supplemental Table S1)**. All insertion fragments of the plasmids were confirmed by sequencing in both directions.

For plasmid transfection, BmN cells were resuspended and equally added to individual wells of a 6-well plate. Once monolayers had formed, the medium was replaced by 1ml TC-100 medium (CellWorld) before transfection medium consisting of 125 μl TC-100 medium, 5 μl Lipo8000 (Beyotime, C0533), and 3 μg plasmid was added. The cells were incubated for 5 h after addition of the mixture and then replenished with 1.5 ml of SF900 medium supplemented with 3% FBS.

Preparation and sequencing of BmBRD4 ChIP-seq libraries

BmN cells (5×10^7) were transfected with pIZ-BmBRD4-HA, and treated with DMSO or 0.5 μM of JQ-1 at 48 h post transcription. After 48 h of treatment, cells were collected and cross-linked with 1% formaldehyde for 10 min at 25˚C. The

reaction was then quenched by addition of glycine to a final concentration of 125 mM. Afterwards, cells were lysed and chromatin was obtained on ice. Chromatin was sheared to a mean fragment size of 200-500 base pairs. The samples were immunoprecipitated by anti-HA antibody (Abcam, 9110). Sequencing libraries were generated using VAHTS Universal DNA Library Prep Kit for Illumina V3 (Vazyme, ND607) following the instructions. The prepared libraries were sequenced on an Illumina NovaSeq 6000 platform (Allwegene) and paired-end 150bp reads were generated.

Processing and visualization of ATAC-seq and ChIP-seq data

The raw sequencing reads of ATAC-seq and ChIP-seq were trimmed and filtered using Trim Galore! (Version 0.6.6) (Martin 2011) and mapped to the reference *Bombyx mori* genome (SilkBase Nov.2016) (Kawamoto et al. 2019)) using Bowtie 2 (Version 2.4.4) (Langmead and Salzberg 2012), respectively. Afterwards, the resulted SAM files were converted into BAM format using SAMtools (Version 1.12) (Li et al. 2009), and MACS2 (Version 2.2.7.1) was adopted for peaks calling (Zhang et al. 2008).

For visualization of these sequencing data, several tools of deepTools (Version 3.5.1), including bamCoverage, computeMatrix, plotHeatmap and plotProfile were employed to generate the read coverages around transcription start sites (TSS) or enhancers (Ramírez et al. 2014). Detailly, the aligned and sorted BAM files were normalized to genome size as reads per genomic content (RPGC) and were converted to bigWig or bedGraph format using bamCoverage, computeMatrix was adopted to calculate the read coverages while plotHeatmap and plotProfile were used to generate the heatmaps and summary plots. As for the genomic views of the sequencing data, the normalized bigWig files were input to IGV (Version 2.12.3) for linear visualization (Robinson et al. 2011), and the bedGraph files were processed by the R package, circlize (Version 0.4.15) for circular genome-wide visualization (Gu et al. 2014).

Generation of in situ Hi-C libraries and analysis of sequencing data

The Hi-C libraries were constructed as described in previous studies (Rao et al.

2014). Briefly, BmN cells (1×10^7) with indicated treatments were cross-linked for with 1% formaldehyde and then lysed in lysis buffer, which contains 10 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.2% NP-40, and complete protease inhibitors (Roche) to obtain the nuclei for each sample. Later, the DNA in the nuclei was digested by restriction enzyme MboI and the cohesive ends were marked with biotin-14-dCTP. Subsequently, T4 DNA ligase were used to start proximity ligation, and the cross-linking was reversed by 200 μg/mL Proteinase K (Invitrogen). Purified DNA was obtained through QIAamp DNA Mini Kit (Qiagen) and sheared to a length of about 400 bp. DNA fragments with biotin-marked point ligation junctions were pulled down by Dynabead™ MyOne™ Streptavidin C1 (Invitrogen) according to the instructions. The resulted final library was sequenced on the Illumina HiSeq X Ten platform (San Diego, CA, United States) with 150PEmode.

For Hi-C analysis, the raw data was filtered using Trimmomatic (version 0.38) (Bolger et al. 2014) and then mapped to *Bombyx mori* genome (SilkBase) using Bowtie 2. ICE was used to filter unusable data, construct Hi-C maps and correct the generated interaction matrix (Imakaev et al. 2012). A Hi-C map is a list of DNA-DNA contacts produced by a Hi-C experiment. The valid pairs after pooling were binned into 10 kb nonoverlapping genomic intervals to generate contact matrices. The reproducibility between the two replicates was represented by the *Pearson* correlation coefficient and the stratum-adjusted correlation (Yang et al. 2017). The chromosome interactions of each sample were input to Fit-Hi-C (Version 2.0.8) to calculate the corresponding *p*-value and false discovery rate (FDR) (Ay et al. 2014). After calculation, the interactions in which both the p-value and FDR were less than 0.01, and contact count was more than 2 were identified as significant interactions. The distribution of all significant interaction regions at each genomic feature was generated by R package ChIPseeker (Version 1.24.0) (Yu et al. 2015). HiCPlotter (Version 0.8.1) and JuiceBox (Version 2.15.7) was used to visualize the contact matrices and compare interaction matrices between various conditions (Akdemir and Chin 2015; Durand et al. 2016).

RNA sequencing and data analysis

BmN cells (1×10^6) with indicated treatments were harvested and total RNA of the cells was extracted. A total amount of 1.5 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB) following the instructions. Library quality was assessed on the Agilent Bioanalyzer 2100 system. The prepared libraries were sequenced on an Illumina NovaSeq 6000 platform (Allwegene) and paired-end 150bp reads were generated.

As for data analysis, raw reads generated from 3 independent libraries of each treatment were cleaned using Trim Galore! (Version 0.6.6) and mapped to *Bombyx mori* genome (SilkBase) using HISAT2 (Version 2.2.1) (Kim et al. 2019). Mapped reads at the exons of each gene were counted via featureCounts, which is a part of the Subread (Version 2.0.1) (Liao et al. 2014). The read counts were normalized as fragments per kilobase of exon model per million mapped fragments (FPKM) using custom R script **(Supplemental Code)** (R Core Team 2022). Differential expression analysis of two groups was performed using R package DESeq2 (Version 1.28.1) (Love et al. 2014). Gene set enrichment analysis (GSEA) was accomplished by R package clusterProfiler (Version 3.16.1) (Yu et al. 2012). The volcano plot and heatmaps were generated by R package ggplot2 (Version 3.3.6) (Wickham 2016) or pheatmap (Version 1.0.12) (Kolde 2019), respectively.

Apoptosis detection and cell viability assay of BmN cells

Apoptosis state was determined using the Annexin V-FITC/PI Apoptosis Detection Kit (Vazyme, A211) according to the instructions. Briefly, BmN cells treated with JQ-1 at different concentrations were centrifuged and resuspended using 100 μl 1 × Binding Buffer. Later, 5 μl Annexin V-FITC and 5 μl PI Staining Solution were added. After incubation at room temperature for 10 minutes in the dark, 400 μl 1 × Binding Buffer was added and emitted fluorescence was quantitated by flow cytometer (BD, FACSVerse). The output data was processed by FlowJo (Version 10.5.3) and the abnormal cells were identified as early apoptosis (Annexin V-FITC+/PI-), late apoptosis (Annexin V-FITC+/PI+), and necrosis (Annexin V-FITC-/PI+).

Cell viability was determined using Cell Counting Kit-8 (Beyotime, C0038) according to the protocol provided by the manufacturers. In brief, BmN cells with indicated treatments in 96-well plate were added with corresponding reagent. The absorbance of each well was measured at a test wavelength of 450 nm.

Gene Silencing

To silence the genes of interest, we used RNAi by generating dsRNAs synthesized *in vitro* using the T7 RNAi transcription kit (Vazyme, TR102) according to the instructions. T7 promoter sequences were incorporated in both forward and reverse primers designed to amplify the DNA fragments of target genes as transcription templates for dsRNA. The control template in this kit was used to generate dsCtr as a negative control. For transfection of the dsRNA, BmN cells were resuspended and equally added to individual wells of a 6-well plate. Once monolayers had formed, the medium was replaced by 1ml TC 100 medium before transfection medium consisting of 125 μl TC-100 medium, 5 μl LipoRNAi (Beyotime, C0535), and 5 μg dsRNA was added. The cells were incubated for 5 h after addition of the mixture and then replenished with 1.5 ml of SF900 medium supplemented with 3% FBS.

Quantitative real-time PCR (qRT-PCR) analysis

BmN cells (1×10^6) with indicated treatments. Total RNA of the cells was extracted using MiniBEST Universal RNA Extraction Kit (Takara, 9767) to synthesize the first-strand cDNAs with the PrimeScript™ RT reagent Kit (Takara, RR047). Reactions were performed using Hieff® qPCR SYBR Green Master Mix (Yeasen, 11203) and run on a real-time PCR thermal cycler (Bio-Rad). *Bmrpl32* (Gene ID 778453) was used for normalizing the data. Three technical replicates for each of the three biological replicates per treatment were analyzed using the $2^{-\triangle\triangle Ct}$ method to calculate the relative expression levels of selected genes. Two-tailed *t*-test was used for significant difference analysis between two samples. The statistical analysis and figure generation were completed by GraphPad Prism (Version 8.0.1).

Analysis of Viral Growth Curve and Genome Copies

Viral growth curve and genome copies analysis was conducted as described previously (Zhao et al. 2022). In brief, BmN cells were infected in triplicate with

BmNPV. At the given time points, cells were centrifuged and supernatants containing the BV were harvested. The titers of BV were determined by $TCID_{50}$ end point dilution assay in BmN cells (Kärber 1931). Three biological replicates were analyzed for each group. To quantify viral genomic DNA (gDNA) copies, total DNA of sediments obtained from the centrifugation was extracted using Universal Genomic DNA Extraction Kit (Takara, 9765). Viral gDNA copies were determined by quantification of a viral gene *gp41* (Gene ID 1488698) by qPCR. DNA concentrations were measured by a NanoDrop instrument (Thermo Fisher Scientific), and 30 ng total genomic DNA was used for each qPCR reaction. *Bmrpl27* (Gene ID 692703) served as an internal normalization control, and the 0 h sample was used as input to normalize the data. Three technical replicates for each of the three biological replicates per treatment were analyzed and the differences in means between two sets of data at a certain time point were compared by *t*-test. GraphPad Prism was used for statistical analysis and figure generation.

Luciferase assays

Dual luciferase reporter assay was performed using Nano-Glo® Dual-Luciferase® Reporter Assay System (Promega, N1610) according to the instructions. Briefly, BmN cells (1×10^6) were co-transected with the reference luciferase vector pGL4.10-Pie1 and pNL1.1-Target harboring target promoter and cloned enhancer region, or the integrated plasmid PB-luc-Zeo or PB-Ctr-Zeo containing the *piggyBac* ITR. After 48 h of transfection, cells were resuspended and equally added to individual wells of an opaque 96-well plate to minimize cross-talk between wells. ONE-Glo™ EX Reagent and NanoDLR™ Stop & Glo® Reagent of the kit were added successively to measure the firefly luminescence driven by *ie-1* promoter and the NanoLuc® luminescence driven by target promoters. The luminescent signal was measured by Microplate Reader (Bio Tek) and the relative light unit were normalized using the firefly luminescence to indicate the promoter activity. Two technical replicates for each of the three biological replicates per treatment were analyzed.

Immunofluorescence

BmN cells transfected with pIZ-BmBRD4-HA on the coverslips were fixed with

4% paraformaldehyde, permeabilized in 0.1% Triton X-100, blocked with the PBS solution containing 5% BSA, and then incubated with anti-HA anti-body (Abcam, 9110). Afterwards, cells were subsequently incubated with secondary FITC-conjugated antibody (Sangon, D110068). Finally, cells were incubated with DAPI (Beyotime, C1005), and examined with the ZEISS LSM 780 confocal scanning laser microscopy (CSLM). Mock transfected cells were served as the control.

Western blot

BmN cells of indicated treatments were harvested at the designated time points and lysed in cell lysis buffer (Beyotime, P0013) for 30 min on ice. Protein concentrations in lysates were determined using Bicinchoninic Acid (BCA) Protein Assay Kit (Takara, T9300A). For each sample, 20 μg of protein extract was separated by a 12% Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE) gel and transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% non-fat milk in TBST and incubated with anti-HA antibody (Abcam, 9110). Thereafter, secondary HRP-conjugated antibodies (Sangon, D110065) were used for enhanced chemiluminescence. α-Tubulin was served as the reference gene.

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