

Figure S1. HSV-1 infection induces the release of P-TEFb from the 7SK snRNP. Related to Figures 1-3.

MRC5 cells were infected with HSV-1 at the indicated MOI for 2 h. Cells were lysed and subjected to differential extraction to obtain Free and 7SK snRNP bound CDK9 fractions. (A) Western blot of CDK9 and loading control GAPDH (7SK snRNP fraction). (B) Quantitation of bound and free CDK9 shown as a percentage of total CDK9 from 2 independent experiments.

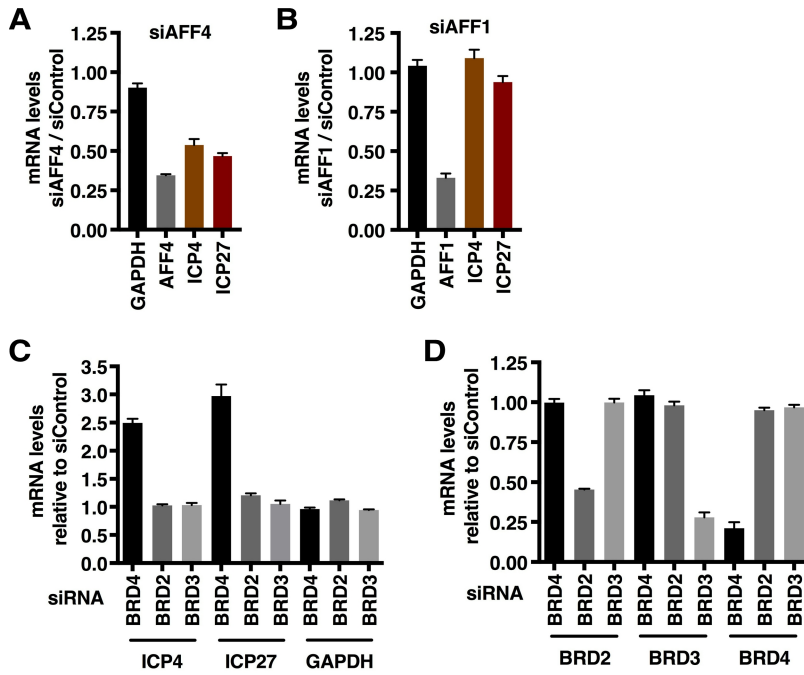


Figure S2. Depletion of AFF1, BRD2 or BRD3 does not impact HSV IE gene expression. Related to Figure 3.

(A-C) MRC5 cells were transfected with siControl or siRNAs to AFF4, AFF1, BRD2, BRD3, or BRD4. Cells were infected with HSV-1 for 2 h and mRNA levels of the siRNA targets, control cellular (GAPDH), and viral IE (ICP4, ICP27) genes were determined. Data is shown as levels relative to those in control siRNA cells. (D) mRNA levels of BRD2, BRD3, and BRD4 in cells transfected with the indicated BRD siRNA. Data are means \pm s.e.m., $n > 4$.

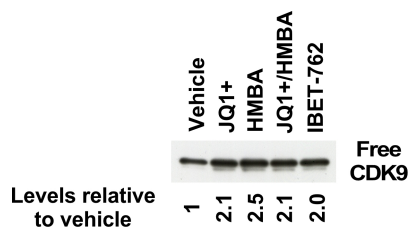


Figure S3. BRD inhibitors induce the release of P-TEFb from the 7SK snRNP. Related to Figure 3. Western blot of free CDK9 fractions obtained by differential extraction of MRC5 cells treated with vehicle or the indicated compound for 3 h.

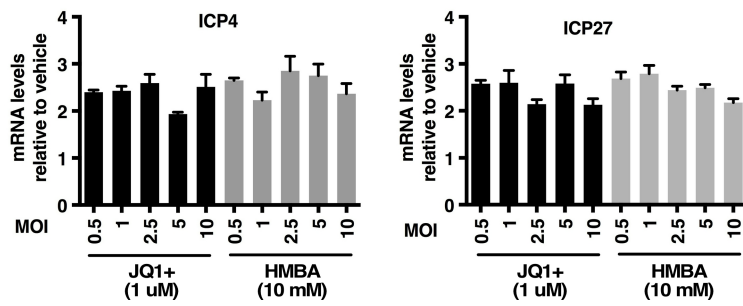


Figure S4. Induction of HSV-1 IE transcription by BRD inhibitors is not MOI dependent. Related to Figure 3.

mRNA levels of HSV-1 IE genes (ICP4, ICP27) in cells treated with vehicle, JQ1+ or HMBA and infected at the indicated MOI. Levels were normalized to cellular control GAPDH and shown relative to those in vehicle treated cells. Data are means \pm s.e.m., $n = 3$.

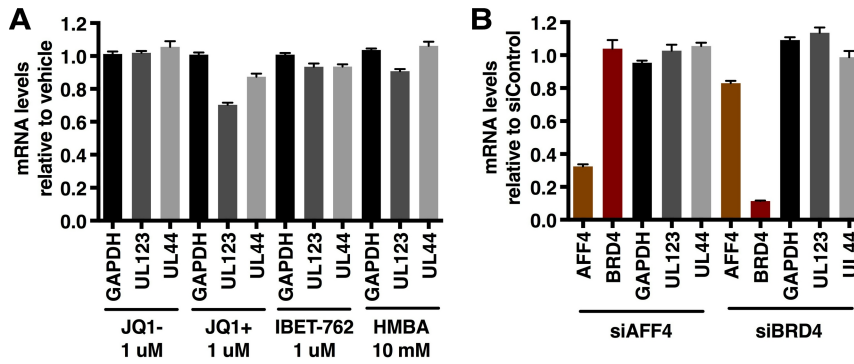


Figure S5. hCMV IE gene expression is not dependent on AFF4 or BRD4. Related to Figure 3.
 (A) HFF cells were treated with control vehicle, control JQ1-, or the BRD inhibitors JQ1+, IBET-762, or HMBA and infected with hCMV for 2 hrs. The levels of control cellular GAPDH and viral IE mRNAs are shown relative to the levels in vehicle treated cells. (B) MRC5 cells transfected with siRNAs to AFF4, BRD4, or control siRNAs were infected with hCMV for 2 hrs. The levels of target, control GAPDH, and hCMV IE mRNAs are shown relative to levels in siControl cells. Data are means +/- s.e.m., n = 2.

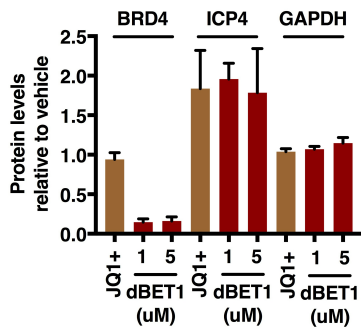


Figure S6. dBET1 mediated turnover of BRD4 enhances IE mRNA levels. Related to Figure 3. MRC5 cells treated with vehicle, JQ1+, or dBET1 and infected with HSV-1 for 2 h. Quantitation of western blot of ICP4, BRD4, and control GAPDH protein levels shown in Figure 3. Data are means +/- s.e.m., n = 3.

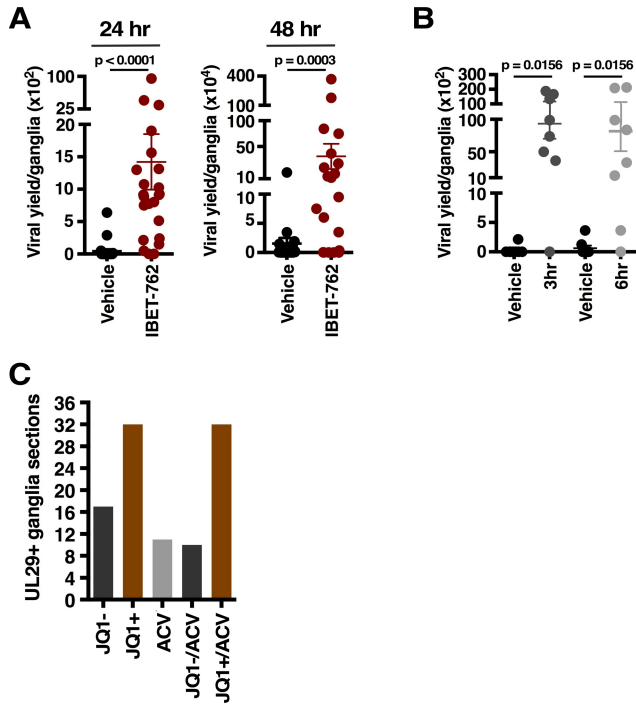


Figure S7. BET inhibitors induce HSV reactivation in a ganglia explant model system. Related to Figure 6.

(A) Viral yields from HSV-1 latently infected trigeminal ganglia explanted into culture in the presence of the BRD4-BET inhibitor IBET-762 or control Vehicle for 24 or 48 h ($n \geq 20$). (B) Viral yields from HSV-1 latently infected trigeminal ganglia explanted into culture in the presence of JQ1+ or Vehicle for 3 or 6 h, followed by removal of the drugs and continued incubation for a total of 36 h post explant ($n = 8$). (C) The number of ganglia sections exhibiting UL29+ cells.

Table S1. Selected HCF-1 associated proteins in mock and HSV infected cells. Related to Figure 1.

HCF-1; HCFC1	HCFC1	Gene name	Accession	MW	AA	MRC5				
						Spectral counts Mock	Spectral counts 2hpi*	SAINT Mock	SAINT 2hpi	
		<i>HCF-1</i>	<i>P51610</i>	209	2035	393	393	1	1	
Protein class	Protein name	Description								
Mediator Complex	Mediator of RNA polymerase II transcription subunit 1	MED1	Q15648	168	1581	4	110	1	1	
	Mediator of RNA polymerase II transcription subunit 23	MED23	Q9ULK4	156	1368	6	61	1	1	
	Mediator of RNA polymerase II transcription subunit 14	MED14	O60244	161	1454	0	61	NA	1	
	Mediator of RNA polymerase II transcription subunit 24	MED24	O75448	110	989	0	58	NA	1	
	Mediator of RNA polymerase II transcription subunit 16	MED16	Q9Y2X0	97	877	0	44	NA	1	
	Mediator of RNA polymerase II transcription subunit 17	MED17	Q9NVC6	97	877	0	40	NA	1	
	Mediator of RNA polymerase II transcription subunit 13	MED13	Q9UHV7	239	2174	0	38	NA	1	
	Mediator of RNA polymerase II transcription subunit 13-like	MED13L	Q71F56	243	2210	0	34	NA	1	
	Mediator of RNA polymerase II transcription subunit 4	MED4	Q9NPJ6	30	270	0	31	NA	1	
	Cyclin-dependent kinase 8	CDK8	P49336	53	464	0	21	NA	1	
	Cyclin-C	CCNC	P24863	33	283	0	20	NA	1	
	Mediator of RNA polymerase II transcription subunit 6	MED6	O75586	28	246	0	12	NA	1	
	Mediator of RNA polymerase II transcription subunit 31	MED31	Q9Y3C7	16	131	0	11	NA	1	
	Mediator of RNA polymerase II transcription subunit 12	MED12	Q93074	243	2177	0	11	NA	1	
	Mediator of RNA polymerase II transcription subunit 18	MED18	Q9BUE0	24	208	0	7	NA	1	
	Mediator of RNA polymerase II transcription subunit 27	MED27	Q6P2C8	35	311	0	6	NA	1	
	Mediator of RNA polymerase II transcription subunit 26	MED26	Q95402	65	600	0	6	NA	1	
	Mediator of RNA polymerase II transcription subunit 20	MED20	Q9H944	23	212	0	5	NA	1	
	Mediator of RNA polymerase II transcription subunit 7	MED7	O43513	27	233	0	5	NA	1	
	Mediator of RNA polymerase II transcription subunit 22	MED22	Q15528	22	200	0	4	NA	1	
Transcription Elongation	AF4/FMR2 family member 4	AFP4	Q9UHB7	127	1163	13	22	1	1	
	Protein ENL	MLLT1	Q03111	62	559	0	8	NA	1	
	RNA polymerase II elongation factor ELL	ELL	P55199	68	621	0	5	NA	1	
	RNA polymerase II elongation factor ELL2	ELL2	O00472	72	640	0	5	NA	1	
	Cyclin-dependent kinase 9	CDK9	P50750	43	372	6	4	1	1	
	RNA polymerase II-associated factor 1 homolog	PAF1 Complex	PAF1	Q8N7H5	60	531	14	18	1	1
	Parafibromin	PAF1 Complex	CDC73	Q6P1J9	61	531	6	13	1	1
	RNA polymerase-associated protein CTR9 homolog	PAF1 Complex	CTR9	Q6PD62	134	1173	10	9	1	1
	RNA polymerase-associated protein LEO1	PAF1 Complex	LEO1	Q8WVC0	75	666	4	6	1	1
	WD repeat-containing protein 61	PAF1 Complex	WDR61	Q9GZS3	34	305	5	6	1	1
	FACT complex subunit SPT16	FACT Complex	SUPT16H	Q9Y5B9	120	1047	37	60	1	1
	FACT complex subunit SSRP1	FACT Complex	SSRP1	Q08945	81	709	21	27	1	1
	La-related protein 7	7SK snRNP Complex. Associates with and inhibits the P-TEF-b Complex	LARP7	Q4G0J3	67	582	4	4	1	1
	Histone-lysine N-methyltransferase SETD2	H3K36 Histone methyltransferase	SETD2	Q9BYW2	288	2564	24	25	1	1
RNAPII subunits and Transcription Initiation factors	DNA-directed RNA polymerase II subunit RPB1	RNAPII subunit	POLR2A	P24928	217	1970	42	40	1	1
	DNA-directed RNA polymerase II subunit RPB2	RNAPII subunit	POLR2B	P30876	134	1174	20	51	1	1
	DNA-directed RNA polymerases I, II, and III subunit RPABC1	RNAPII subunit	POLR2E	P19388	25	210	0	4	NA	1
	Transcription initiation factor TFIID subunit 5	Transcription Initiation factor	TAF5	Q15542	87	800	0	4	NA	1
	TATA-binding protein-associated factor 2N	Transcription Initiation factor	TAF15	Q92804	62	592	4	17	1	1
Chromatin Modification/Protein Modification	Histone-lysine N-methyltransferase 2A	MLL1-2/COMPASS like Complex; H3K4 methyltransferase	KMT2A	Q03164	432	3969	9	12	1	1
	Histone-lysine N-methyltransferase 2E	MLL5 Complex; H3K4 methyltransferase	KMT2E	Q8IZD2	205	1858	14	11	1	1
	Histone-lysine N-methyltransferase 2C	MLL3-4/COMPASS like Complex; H3K4 methyltransferase	KMT2C	Q8NEZ4	541	4911	3	4	1	1
	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase 110 kDa subunit	O-Linked N-Acetylglucosamine (GlcNAc) Transferase	OGT	O15294	117	1046	158	198	1	1
	Ubiquitin carboxyl-terminal hydrolase BAP1	Polycomb repressive deubiquitinase (PR-DUB) complex	BAP1	Q92560	80	729	59	77	1	1
	Histone-lysine N-methyltransferase SETD1A	Set1A-B/COMPASS Complex; H3K4 methyltransferase	SETD1A	O15047	186	1707	153	143	1	1
	Protein dpy-30 homolog	Set1A-B/COMPASS Complex/also present in other hCOMPASS like Complexes	DPY30	Q9C005	11	99	4	6	1	1
	Set1/Ash2 histone methyltransferase complex subunit ASH2	Set1A-B/COMPASS Complex/also present in other hCOMPASS like Complexes	ASH2L	Q9UBL3	69	628	82	85	1	1
	CpG-binding protein	Set1A-B/COMPASS Complex	CXXC1	Q9P0U4	76	656	63	84	1	1
	WD repeat-containing protein 5	Set1A-B/COMPASS Complex/also present in other hCOMPASS like Complexes	WDR5	P61964	37	334	43	42	1	1
	Retinoblastoma-binding protein 5	Set1A-B/COMPASS Complex/also present in other hCOMPASS like Complexes	RBBP5	Q15291	59	538	41	52	1	1
	WD repeat-containing protein 82	Set1A-B/COMPASS Complex	WDR82	Q6UXN9	35	313	16	14	1	1

*Spectra counts were normalized based on HCF-1 spectral counts in uninfected cells

Table S2. Primer sets used in this study. Related to Experimental Procedures.

PRIMERS

qRT-PCR

Target

Target	Sequence
ICP4	GAAGTTGTGGACTGGGAAGG GTTGCCGTTTATTGCGTCTT
ICP22	TTTGGGGAGTTTGACTGGAC CAGACACTTGC GG TCTTCTG
ICP27	GCATCCTTCGTGTTTGTCAATTCTG GCATCTTCTCTCCGACCCCG
UL30	AGAGGGACATCCAGGACTTTGT CAGGCGCTTGTGGTGTAC
gC	TGATTATCGGCGAGGTGAC ACAAACTCCACGGGGTTACG
hCMV.UL123	GCCTTCCCTAAGACCACCAAT ATTTCTGGGCATAAGCCATAATC
hCMV.UL44	GCCCCGATTCAATATGGAGTTTCAG CGGCCGAATTCTCGCTTTC
HPRT	ATTGTAATGACCAGTCAACAGGG GCAITGTTTTGCCAGTGTCAA
TUBB	AAGATCCGAGAAGAATACCTGA CTACCAACTGATGGACGGAGA
GAPDH	TTCGACAGTCAGCCGCATCTTCTT CAGGCGCCAATACGACCAAATC
mGAPDH	CTGACGTGCCGCCTGGAGAAA CCCGGCATCGAAGGTGGAAGAGT
AFF4	AAAGGCCAGCATGGATCAGAA GTGATTTGGAGCGTTGATGTTC
AFF1	CTGAAAATAGGTTGGGAAAGCCG GACGCAGGAGTGTGAATGGA
BRD2	AATGGCACAAACGCTGGAAAA CACTGGTAACACTGCCCTG
BRD3	TGCAAGCGAATGTATGCAGGA CATCTGGGCCACTTTTTGTAGAA
BRD4	GAGCTACCCACAGAAGAAACC GAGTCGATGCTTGAGTTGTGTT

Short RNA Amplification

Target

Target	Sequence
ICP4 Promoter-Proximal (+57) Forward	GCCAGAGACAGACCGTCAGA
ICP27 Promoter-Proximal (+81) Forward	CCACCACCAGAGGCCATATC
miR 16 Forward	TAGCAGCACGTAATATTGGCG
SNORD44 Forward	GCAAATGCTGACTGAACATGAAG

Chromatin Immunoprecipitation

Target

Target	Sequence
ICP4 (-324 to -254)	GCGGGGCTCGTATCTCATT CCGCATGGCATCTCATTACC
ICP4 Promoter-TSS (-37 to +73)	GCCCCTGGGACTATATGAGC GCGTCTGACGGTCTGTCTCT
ICP4 (+1425 to +1563)	GATCACGCGGCTGCTGTA ATGAAGGAGCTGCTGTTGCG
ICP0 (-268 to -119)	TTCGGGAAGGCGGGAAG CCCAAAGAATATCATTAGCATGCAC
ICP0 (+249 to +392)	ATGTCTGGGGCGCCA GTTCCACGCCCTAACAT
MYC (+83 to +192)	TTCTCAGAGGCTTGGCGGGAAA CTGCCTCTCGCTGGAATTACTACA
MYC (+1170 to +1266)	AACCTGGGTCTCTAGAGGTGTTAGGA TCAACGATTCCAGGAGAATCGGAC
MYC (+2242 to +2362)	ACTCGGTGCAGCCGTATTTCTACT GCAGCAGCTCGAATTTCTCCAGA