Table S1. Mediator components co-immunoprecipitated with FLAG-Brd3.

	# peptides identified	
Protein	FLAG-Brd3 IP	Control IP
MED1	12	0
MED4	9	0
MED6	4	0
MED8	3	0
MED10	3	0
MED12	5	0
MED13	3	0
MED14	14	0
MED16	5	0
MED17	8	0
MED20	3	0
MED21	2	0
MED22	3	0
MED23	16	0
MED26	7	0
MED27	6	0
MED30	3	0

Table S2. PAF complex components co-immunoprecipitated with FLAG-Brd3.

	# peptides identified	
Protein	FLAG-Brd3 IP	Control IP
CDC73	23	0
CTR9	15	0
LEO1	3	0
PAF1	13	0
SKI8	10	0

Table S3. Transcription elongation factors co-immunoprecipitated with FLAG-Brd3.

	# peptides identified	
Protein	FLAG-Brd3 IP	Control IP
CDK9	10	0
Elongin A	8	0
Elongin B	4	0
Elongin C	2	0
FACT	3	1

Table S4. RNA polymerase II preinitiation complex components co-immunoprecipitated with FLAG-Brd3.

		# peptides identified	
Protein complex	Protein	FLAG-Brd3 IP	Control IP
RNA polymerase II			
	POLR2A	5	0
	POLR2C	3	0
	POLR2E	3	0
TFIIH			
	CDK7	5	0
	ERCC2	4	0
	GTF2H1	5	0
	GTF2H2	5	0
	GTF2H3	4	0
	GTF2H4	6	0
TFIID			
	TAF1	35	0
	TAF2	13	0
	TAF3	12	0
	TAF4	13	0
	TAF6	32	0

	TAF7	11	0
	TAF8	6	0
	TAF9	14	0
	TAF9B	6	0
	TAF10	5	0
	TAF11	4	0
	TAF12	2	0
	TAF13	3	0
	TBP	4	0

Table S5. Genes that co-immunoprecipitate specifically with Flag-Brd3 (twice as many peptides detected vs control)

CHD4	PELP1	LOC732272	PAF1	PSIP1
CHD8	USP7	ADNP	TAF7	HNRNPR
SMARCA4	WHSC1L1	ZMYND8	BRD9	GTF2H1
PBRM1	BRD2	SMARCA5	TAF4	KPNA3
TCOF1	POLR2A	TUBA1C	NCL	CBX4
TAF1	NFRKB	EIF5B	LYAR	ACTR8
GLTSCR1	ARID2	SFRS15	PARP1	PHF10
SMARCC1	IWSI	NCAPD2	ACTR5	BRD2
ATAD5	BRD4	RPLPO	MED17	MED16
PRKDC	MED12	HSPA8	TCEB3	NOLC1
DHX9	SMARCA2	TAF6	PUF60	YBX1
SMARCC2	RFC1	RUVBL2	WHSC1L1	CHD4
MED23	RRP12	HSPA5	KPNA2	SERBP1
CTR9	HTATSF1	GTPBP4	XRCC5	BRD7
MYBBP1A	CHD9	WHSC1	U2AF2	DKC1
NOLC1	MED13	CDC73	GATAD2B	ZNF148
MED14	LEO1	RPL4	MED26	ERCC2
TAF4	CHD6	XRCC6	DDX41	RPA1
TAF2	POLR1a	ACTL6A	HDAC2	BRD9
MED1	SUPT16H	RBM39	HMGXB4	MTA1
GTF3C1	ZNF687	RUVBL1	ARRB2	UBTF
TAF3	AGTPBP1	KBTBD8	SMARCE1	CHAF1B
PRPF40A	DHX37	MTA2	HDAC1	RBBP5
SF3B1	IQGAP1	GNL3	RBBP4	ARCN1
BPTF	RBM25	NCL	GATAD2A	HSP90AB1
INO80	PRPF8	ILF3 (NFAT)	HSP90AA1	RSL1D1

C14orf21	CSNK2A1	TAF8	TBP	LUC7L
RPL3	RPL3	HSPA1A	GATAD2a	MED4
LTV1	CSNK2A2	RNF2	FIBP	PUF60
CSDA	RPLP0	GTF2H4	POLR1C	HNRNPD
LUC7L2	ILF2	MED27	RPSAP12	GLRX3
SMARCD2	RPL5	LUC7L2	BCL7A	MKI67IP
TAF3	RFC5	WDR18	FBL	NFKBIL1
TOP1	RFC2	CDC73	CBX4	RBBP4
STRBP	RFC4	HNRNPA1	EEF1A2	PAK1IP1
TCOF1	RPS3A	EIF2S1	PPP1CC	BRD7
GTF3C5	NCL	ACTL6A	HMGXB4	RPS2
CTPS	WDR61	DRG1	WHSC1	RPP38
CHD8	CDK9	CDK7	INO80E	LOC440577
TMPO	MBD3	HIST1H1C	POLR2C	TUBB1
USP39	RFC3	DEK	HNRNPAB	INO80B
NOC4L	EBNA1BP2	RPS3	WDR82	SMARCE1
CROP	MED4	GTF2H2	SFRS5	RPL3L
INO80B	RPL4	TCEB3	PARP1	RPS3
KPNA4	PHF6	ACTR1A	U2AF1	RPS4X
PAF1	SMARCB1	GTF2H3	NOLC1	RPL7
DDX3X	UCHL5	MNAT1	RUVBL1	TAF9
PIP4K2A	HSPA8	TUBB	BRD2	LY6G5B
HSP90AA2	FEN1	BCL7C	TOP1	RPS9
FUS	HSPA1L	C1orf35	MED19	RPS6
WDR48	AHSA1	WDR5	BCCIP	RPS3A
EXOSC9	NUDC	RRS1	SNRNP40	NPM1
BYSL	LYAR	EIF4A1	HNRNPA0	CSNK2A1
POLR1E	TAF4	BXDC2	EEF1A1	RPL7A

RPL21P19
RPS2
RPL23A
RPS17
RPL23
RPS18
RPL18A
RPL13A
RPLP2
RPLP0
RPL30
RPS13
RPL10A
RPL15
RPS19
RPS15A
RPS16
RPL26L1
RPS7
C17orf49
RPL3
RPL22
RPL12
TUBB2C
RPL18
RPS8
RPL8
RPS7P4
RPL9
CSNK2A2
RPS24
RPL19
SNORA7A
RPL4
RPL27
RPL36
RPL24
RPL13P12
SRP14
RPL6
RPL28
RPS14
TAF9B
TUBA4A
RPL31
HIST1H1C
RPS11
RPS25
RPL34
RPL10P16
EIF6
THOC4
TAF10
RPS15
H1FX
RPS5
NOP16
RPL27A
RPL38
ZNF740
PRDX6
RPS23
RPS10
RPL35A
TAF11
TUBB
TUBA1C
RPL13
YWHAE
ILF2
TCEB2
MED6
RPL10L
RPL26
RPL36AL
C7orf50
MED10
INO80C
C4orf43
ACTB
MED22
MED8
POLR2E
MED20
RPS20
NCL
PSME3
HNRNPK
MED30
TAF4
RPP30
RPS27
TAF13
RPL10AP3
HSPA1L
NHP2
EIF4A1
RPS28
RPS12
GAR1
UBE2M
TCEB1
HSPA1A
WDR61
RPL5
EXOSC2
EEF1A1
RUVBL1
RPL37A
INO80E
GAPDH
HSPA7
NPM3
RPL17
MED21
LLPH
RPL7AP27
RPL35
HSPA8
ACTL6A
FAU
SNRPE
RFC4
TAF12
SFRS3

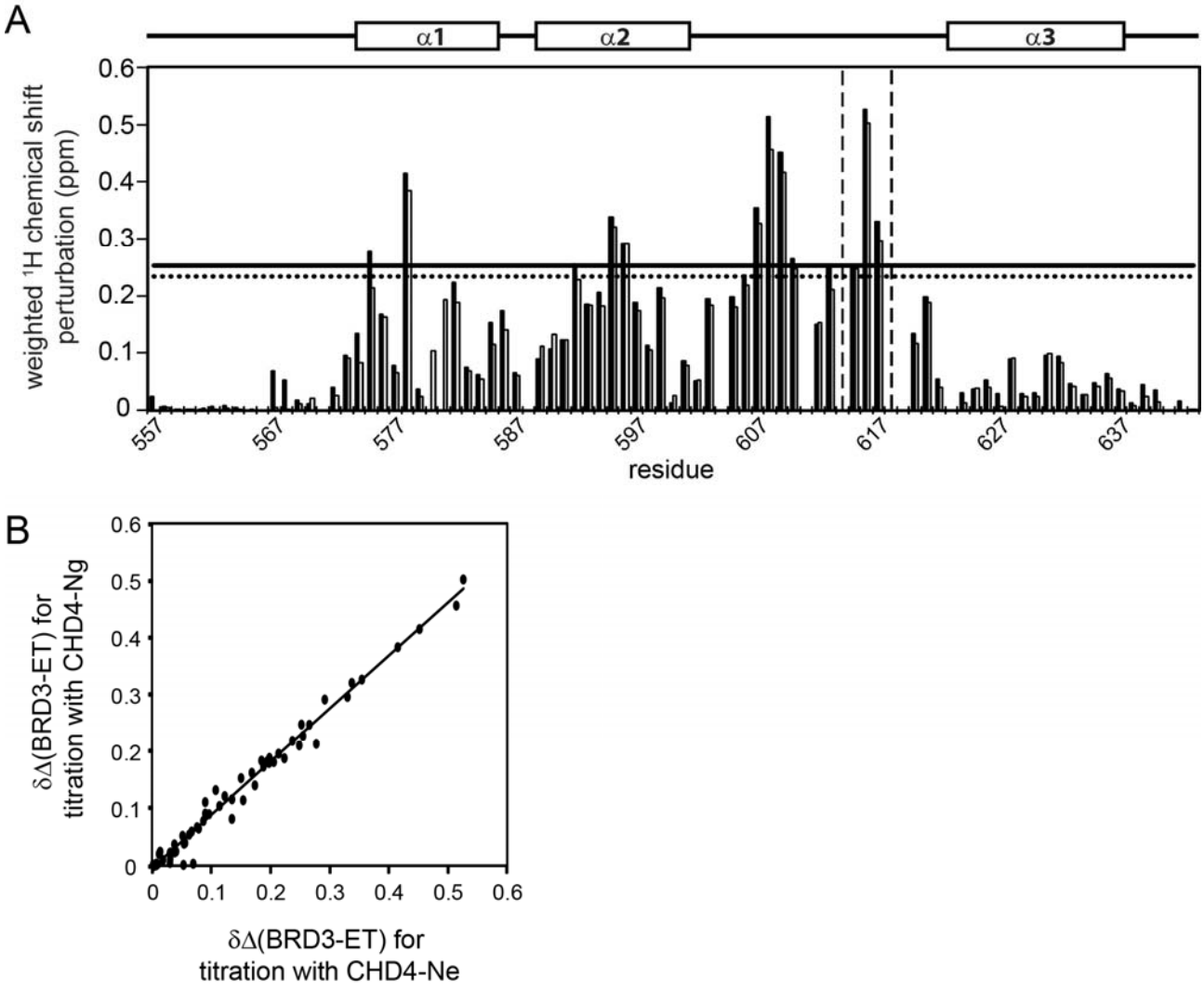

Figure S1. (A) Chemical shift changes induced in BRD3-ET following saturation with either CHD4-Ne (black bars) or CHD4-Ng (white bars). Horizontal solid and dashed lines indicate 1 standard deviation above the mean chemical shift change for CHD4-Ne and CHD4-Ng, respectively. Peaks that disappear by the titration endpoint are indicated by vertical dashed lines. Secondary structure of BRD3-ET is shown above the plot. **(B)** Correlation between the magnitudes of the BRD3-ET chemical shift perturbations induced by CHD4-Ne and CHD4-Ng.

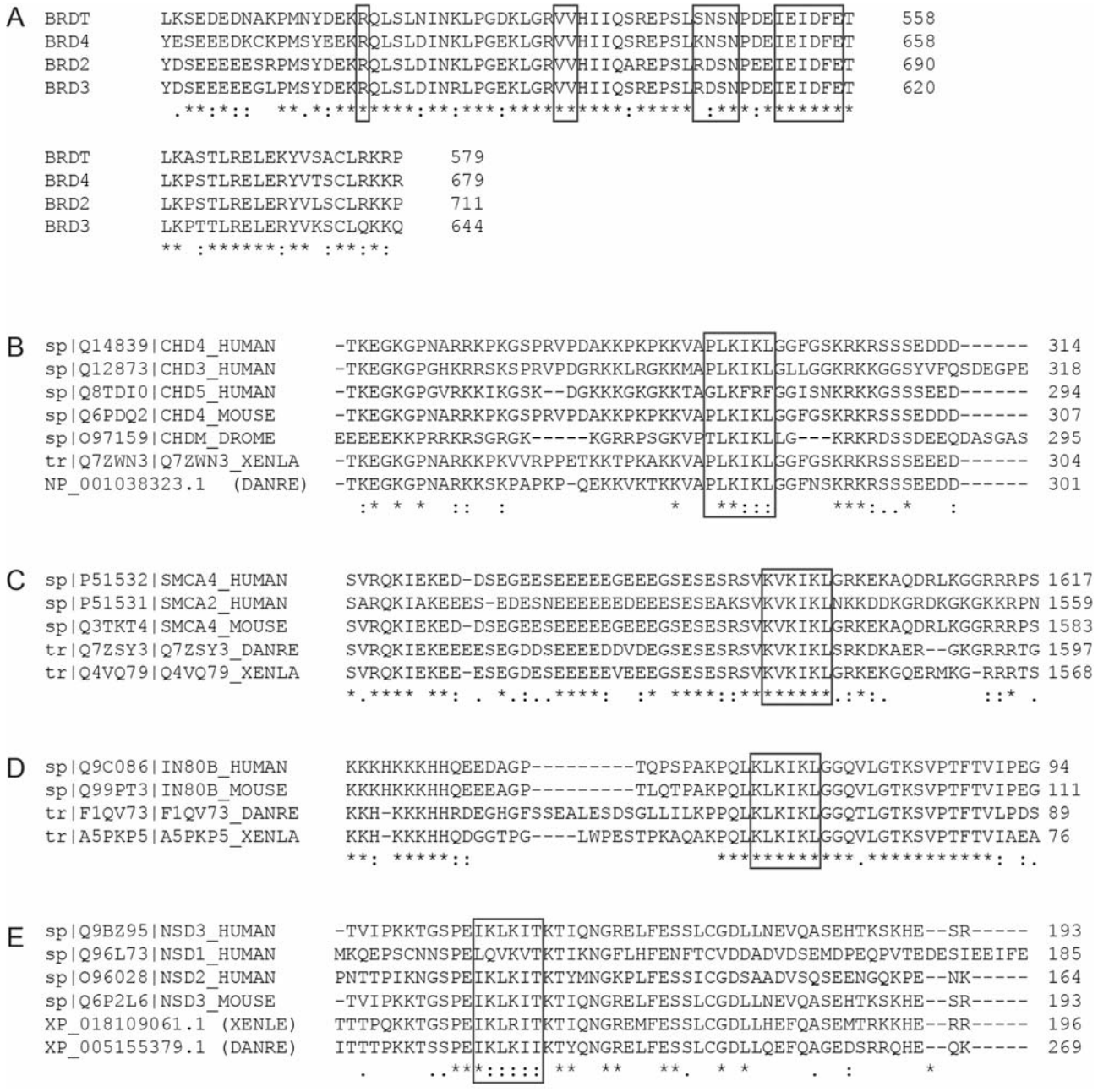

Figure S2. Conservation of ET domains and ET-interacting motifs in transcriptional regulators. (A) Alignment of the ET domains of human BET proteins. ET domain residues involved in the interaction with the PLKIKL motif. Residues with chemical shift perturbations greater than one standard deviation above the mean in titrations of BRD3-ET with CHD4 are boxed. There is almost perfect conservation. **(B)** Sequence alignment of several CHD family chromatin-remodelling ATPases. **(C)** Sequence alignment of SWI/SNF family chromatin-remodelling ATPases. **(D)** Sequence alignment of INO80B family. **(E)** Sequence alignment of NSD family histone lysine methyltransferases. ET domain interaction sites are boxed. Uniprot identifiers are shown for each protein.