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
ТМРО	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	Clorf35	MED19	RPS6
WDR48	AHSA1	WDR5	BCCIP	RPS3A
EXOSC9	NUDC	RRS1	SNRNP40	NPM1
BYSL	LYAR	EIF4A1	HNRNPA0	CSNK2A1
POLR1E	TAF4	BXDC2	EEF1A1	RPL7A

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

A	BRDT BRD4 BRD2 BRD3	LKSEDEDNAKPMNYI YESEEEDKCKPMSYI YDSEEEEESRPMSYI .**:*:: **.*	DEKRQLSLNINKLPGDKLGRVVHIIQSREPSISNSNPDEIEIDFET 558 EEKRQLSLDINKLPGEKLGRVVHIIQSREPSIKNSNPDEIEIDFET 658 DEKRQLSLDINKLPGEKLGRVVHIIQAREPSIRDSNPEEIEIDFET 690 DEKRQLSLDINRLPGEKLGRVVHIIQSREPSIRDSNPDEIEIDFET 620 :*******:**:***:************	
	BRDT BRD4 BRD2 BRD3	LKASTLRELEKYVS LKPSTLRELERYVI LKPSTLRELERYVL LKPTTLRELERYVK ** :******:**	ACLRKRP 579 SCLRKKR 679 SCLRKKP 711 SCLQKKQ 644 :**:*:	
В	sp Q14839 sp Q12873 sp Q8TDI0 sp Q6PDQ2 sp 097159 tr Q7ZWN3 NP_0010383	CHD4_HUMAN CHD3_HUMAN CHD5_HUMAN CHD4_MOUSE CHDM_DROME Q7ZWN3_XENLA 323.1 (DANRE)	-TKEGKGPNARRKPKGSPRVPDAKKPKPKKVAPLKIKLGGFGSKRKRSSSEDDD -TKEGKGPGHKRRSKSPRVPDGRKKLRGKKMAPLKIKLGLLGGKRKKGGSYVFQSDEGPE -TKEGKGPGVRKKIKGSKDGKKKGKGKKTAGLKFRFGGISNKRKKGSSSEED -TKEGKGPNARRKPKGSPRVPDAKKPKKVAPLKIKLGGFGSKRKRSSSEDDD EEEEEKKPRRKRSGRGKKGRRPSGKVPTLKIKLLGKRKRDSSDEEQDASGAS -TKEGKGPNARKKPKVVRPPETKKTPKAKKVAPLKIKLGGFGSKRKRSSSEED -TKEGKGPNARKKSKPAPKP-QEKKVKTKKVAPLKIKLGGFNSKRKRSSSEED :* * * :: : * **::: ***::	314 318 294 307 295 304 301
С	sp P51532 sp P51531 sp Q3TKT4 tr Q7ZSY3 tr Q4VQ79	SMCA4_HUMAN SMCA2_HUMAN SMCA4_MOUSE Q7ZSY3_DANRE Q4VQ79_XENLA	SVRQKIEKED-DSEGEESEEEEGEEEGEEEGSESESRSVKVKIKLGRKEKAQDRLKGGRRRPS SARQKIAKEEES-EDESNEEEEEDEEESESEAKSVKVKIKLNKKDDKGRDKGKGKKRPN SVRQKIEKED-DSEGEESEEEEEGEEEGSESESRSVKVKIKLGRKEKAQDRLKGGRRRPS SVRQKIEKEEEESEGDDSEEEEDDVDEGSESESRSVKVKIKLSRKDKAERGKGRRRTG SVRQKIEKEE-ESEGDESEEEEEVEEEGSESESRSVKVKIKLGRKEKGQERMKG-RRRTS *.**** **: . *.:****: :* ****::**	1617 1559 1583 1597 1568
D	sp Q9C086 sp Q99PT3 tr F1QV73 tr A5PKP5	IN80B_HUMAN IN80B_MOUSE F1QV73_DANRE A5PKP5_XENLA	KKKHKKKHHQEEDAGPTQPSPAKPQIKLKIKIGGQVLGTKSVPTFTVIPEG KKKHKKKHHQEEEAGPTLQTPAKPQIKLKIKIGGQVLGTKSVPTFTVIPEG KKH-KKKHHRDEGHGFSSEALESDSGLLILKPPQIKLKIKIGGQVLGTKSVPTFTVLPDS KKH-KKKHHQDGGTPGLWPESTPKAQAKPQIKLKIKIGGQVLGTKSVPTFTVIAEA **: *****:: ***	94 111 89 76
E	sp Q9BZ95 sp Q96L73 sp 096028 sp Q6P2L6 XP_0181090 XP_0051553	NSD3_HUMAN NSD1_HUMAN NSD2_HUMAN NSD3_MOUSE 061.1 (XENLE) 379.1 (DANRE)	-TVIPKKTGSPEIKLKITKTIQNGRELFESSLCGDLLNEVQASEHTKSKHESR MKQEPSCNNSPELQVKVTKTIKNGFLHFENFTCVDDADVDSEMDPEQPVTEDESIEEIFE PNTTPIKNGSPEIKLKITKTYMNGKPLFESSICGDSAADVSQSEENGQKPENK -TVIPKKTGSPEIKLKITKTIQNGRELFESSLCGDLLNEVQASEHTKSKHESR TTTPQKKTGSPEIKLRITKTIQNGREMFESSLCGDLLHEFQASEMTRKKHERR ITTTPKKTSSPEIKLKIIKTYQNGRELFESSLCGDLLQEFQAGEDSRRQHEQK ***::::: ** ** ** ** * * *	193 185 164 193 196 269

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.