

Figure S1. Crystal structures of human CBP BrD-PHD bound to lysine-acetylated H4 peptides. Related to Figure 1.

(A) Cartoon representation of 3D structure of the tandem BrD (green) and PHD finger (orange) of CBP bound to a histone H4K20ac peptide (shown in sticks with carbon atoms in yellow). The linker of the BrD-PHD module is colored in light cyan and regions that are lack of electron density is indicated by dots. Zn atoms are shown in magenta spheres.

B) Electron density map of H4K20ac and H4K12ac peptides. The Fo-Fc maps are computed after simulated annealing with H4 peptides omitted from the atomic model and shown in mesh (contoured to 1.0σ). H4 peptides are presented in the same orientation as in panel (*A*).

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			10	20	30	40	50	6	0	70	80	90	100	110	_	
		V	1	I	I	I	1		I	\sim	I	I	I	1	\vee	
CBP	1084	IFKPE	ELRQALM	PTLEALYRQD	-PESLPFRQPV	DPQLLGIPI	YFDIVKNPMI	LSTIK	-RKLDTG	QYQEPWQY	DVWLMFNN	AWLYNRKTSF	VYKFCSKLA	EVFEQEIDI	VMQSLG	1197
P300	1048	IFKPE	ELRQALM	PTLEALYRQD	-PESLPFRQPV	DPQLLGIPI	OYFDIVKSPMI	LSTIK	-RKLDTG	Q <mark>Y</mark> QEPWQY\	DIWLMFNN	AWLYNRKTSF	VYKYCSKLS	EVFEQEIDI	PVMQSLG	1161
BRD2_D1	73	GRVTN	QLQYLHK	VVMKALWKHQ	FAWPFRQPV	DAVKLGLPI	OYHKIIKQPMI	MGTIK	-RRLENN	YYWAASECN	QDFNTMFTN	CYIYNKPTDI	IVLMAQTLE	KIFLQKVAS	SMPQEEQ	185
BRD3_D1	33	GRKTN	QLQYMQN	VVVKTLWKHQ	FAWPFYQPV	DAIKLNLPI	YHKIIKNPMI	MGTIK	-KRLENN	YYWSASECN	QDFNTMFTN	CYIYNKPTDI	IVLMAQALE	KIFLQKVAÇ	MPQEEV	145
BRD4_D1	57	KRQTN	QLQYLLR	VVLKTLWKHQ	FAWPFQQPV	DAVKLNLPI	YYKIIKTPMI	MGTIK	-KRLENN	YYWNAQECI	QDFNTMFTN	CYIYNKPGDI	IVLMAEALE	KLFLQKIN	LPTEET	169
BRDT D1	26	GRLTN	QLQYLQK	VVLKDLWKHS	FSWPFQRPV	DAVKLQLPI	OYYTIIKNPMI	LNTIK	-KRLENK	YYAKASEC1	EDFNTMFSN	CYLYNKPGDI	IVLMAQALE	KLFMQKLSQ	MPQEEQ	138
BRD2 D2	344	GKLSE	QLKHCNG	ILKELLSKKH	AAYAWPFYKPV	DASALGLHI	OYHDIIKHPMI	LSTVK	-RKMENR	DYRDAQEFA	ADVRLMFSN	CYKYNPPDHI	VVAMARKLQ	DVFEFRYAR	MPDEPL	458
BRD3_D2	306	GKLSE	HLRYCDS	ILREMLSKKH	AAYAWPFYKPV	DAEALELHI	OYHDIIKHPMI	LSTVK	-RKMDGR	EYPDAQGFA	ADVRLMFSN	CYKYNPPDHE	VVAMARKLQ	DVFEMRFA	MPDEPV	420
BRD4 D2	348	SKVSE	QLKCCSG	ILKEMFAKKH	AAYAWPFYKPV	DVEALGLHI	OYCDIIKHPMI	MSTIK	-SKLEAR	EYRDAQEFO	GADVRLMFSN	CYKYNPPDHE	VVAMARKLQ	DVFEMRFA	MPDEPE	462
BRDT D2	267	VKVTE	QLRHCSE	ILKEMLAKKH	FSYAWPFYNPV	DVNALGLH	NYYDVVKNPMI	LGTIK	-EKMDNQ	E <mark>Y</mark> KDAYKF#	ADVRLMFMN	CYKYNPPDHE	VVTMARMLQ	DVFETHFSE	KIPIEPV	381
BRPF1	627	EMQLT	PFLILLR	KTLEQLQEKD	TGNIFSEPV	PLSEVPI	YLDHIKKPMI	FFTMK	-QNLEAY	RYLNFDDFE	EEDFNLIVSN	CLKYNAKDTI	FYRAAVRLR	EQGGAVLR	ARRQAE	737
BRPF3	588	ELELM	PFNVLLR	TTLDLLQEKD	PAHIFAEPV	NLSEVPI	YLEFISKPMI	FSTMR	-RKLESH	LYRTLEEFE	EEDFNLIVTN	CMKYNAKDTI	FHRAAVRLR	DLGGAILR	IARRQAE	698
BRD1	561	ELRLT	PLTVLLR	SVLDQLQDKD	PARIFAQPV	SLKEVPI	YLDHIKHPMI	FATMR	-KRLEAQ	GYKNLHEFE	EEDFDLIIDN	CMKYNARDTV	FYRAAVRLR	DQGGVVLR	ARREVD	671
GCN5L2	727	LKDPD	QLYTTLK	NLLAQIKSHP	SAWPFMEPV	KKSEAPI	YYEVIRFPII	LKTMT	-ERLRSR	YYVTRKLF\	ADLORVIAN	CREYNPPDSE	YCRCASALE	KFFYFKLKI	EGGLIDK	837
PCAF	722	PRDPD	QLYSTLK	SILQQVKSHQ	SAWPFMEPV	KRTEAPO	YYEVIRFPMI	LKTMS	-ERLKNR	YYVSKKLFN	ADLORVETN	CKEYNPPESE	YYKCANILE	KFFFSKIK	EAGLIDK	832
BPTF	2800	TPLTE	KDYEGLK	RVLRSLQAHK	MAWPFLEPV	DPNDAPI	YYGVIKEPMI	LATME	-ERVQRR	YYEKLTEF\	ADMTKIFDN	CRYYNPSDSE	FYQCAEVLE	SFFVQKLKO	GFKASRS	2910
BAZ1A	1429	OGGVH	ELSAFEO	LVVELVRHDD	SWPFLKLV	SKIOVPI	YYDIIKKPI	LNIIR	-EKVNKC	EYKLASEFI	DIELMFSN	CFEYNPRNTS	EAKAGTRLO	AFFHIOAOH	KLGLHVT	1538
TRIM24	899	KLTPI	DKRKCER	LLLFLYCHEM	SLAFODPV	PLTVPI	YYKIIKNPMI	LSTIKKR	LQEDYS-	MYSKPEDEN	ADFRLIFON	CAEFNEPDSE	VANAGIKLE	NYFEELLKI	ILYPEKR	1009
TRIM33	957	GLSPV	DORKCER	LLLYLYCHEL	SIEFQEPV	PASIP	YYYKIIKKPMI	LSTVKKK	LOKKHSO	HYQIPDDF	ADVRLIFKN	CERFNEADSE	VAQAGKAVA	LYFEDKLTH	EIYSDRT	1068
TRIM66	1041	GLSMY	DOKKCEK	LVLSLCCNNL	SLPFHEPV	SPLAR	IYYQIIKRPMI	LSIIRRK	LQKKDPA	HYTTPEEV	SDVRLMFWN	CAKENYPDSE	VAEAGRCLE	VFFEGWLKE	EIYPEKR	1152
SP140L	467	QMCPE	EQLKCEF	LLLKVYCCSE	SSFFAKIP	YYYY	IREACQGLKE	PMWLDKI	KKRLNEH	GYPQVEGF	ODMRLIFON	HRAS-YKYKI	FGQMGLRLE	AEFEKDFKE	EVFAIQE	575
SP140	754	OMCPE	EOLKCEF	LLLKVYCCSE	SSFFAKIP	YYYY	IREACOGLKE	PMWLDKI	KKRLNEH	GYPOVEGE'	ODMRLIFON	HRAS-YKYKE	FGOMGFRLE	AEFEKNFKI	EVFAIOE	862
TRIM28	695	KLSPA	NORKCER	VLLALFCHEP	CRPLHOLA	TDSTFS	SLDOPGGTLDI	TLIRARL	OEKLSPP	-YSSPOEFA	ODVGRMFKO	FNKL-TEDKA	DVOSIIGLO	RFFETRMN	EAFGDTK	804
BAZ2B	2060	RDDSK	DLALCSM	ILTEMETHED	AWPFLLPV	NLKLVPO	SYKKVIKKPMI	FSTIR	~ -EKLSSG	OYPNLETF	LDVRLVFDN	CETFNEDDSI	IGRAGHNMR	KYFEKKWTI	TFKVS	2168
BAZ2A	1793	RNHHS	DLTFCEI	ILMEMESHDA	AWPFLEPV	NPRLVS		FSTMR	-ERLLRG	~ GYTSSEEF#	ADALLVFDN	COTFNEDDSE	VGKAGHIMR	RFFESRWEI	FYOGKO	1902
BAZ1B	1338	RROSI	ELOKCEE	ILHKIVKYRF	SWPFREPV	TRDEAEI	YYDVITHPM	FOTVO	-NKCSCG	SYRSVOEFI	TDMKOVETN	AEVYNCRGSE	VLSCMVKTE	OCLVALLHE	KHLPGHP	1448
ZMYND8	168	MLTTE	OLSYLLK	FATOKMKOPG	TDAFOKPV	PLE-OHPI	YAEYTFHPM	T.CTT.E	-KNAKKK	MYGCTEAFT	ADAKWTTHN	CTTYNGGNHR	TTOTAKVVT	KICEHEMNE	TEVCPE	277
ZMYND11	112	NTNKO	EMGTYLR	FIVSRMKERA	IDLNKKG	KDNKHPN	YRRLVHSAVI	VPTIO	-EKVNEG	KYRSYEEFE	ADAOLLLHN	TVIFYGADSE	OADIARMLY	KDTCHELDI	ELOLCKN	220
ASH1L	2439	RLAOI	FKEICDG	IISYKDSSRO	-ALAAPLLNLP	PKKKNAI	YYEKISDPLI	LITIE	-KOILTG	YYKTVEAFT	ADMLKVFRN	AEKYYGRKSI	VGRDVCRLR	KAYYNARHI	EASAOID	2550
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Figure S2. Structure-based sequence alignment of representative members of human BrD proteins. Related to Figure 2.

Identical, highly similar and similar residues are colored in red, green and blue respectively. Secondary structure elements of human CBP BrD are assigned by the PROCHECK program (1) and are shown above the sequences: the helices are shown as cylinders. The residues interacting with the PHD finger are labeled with green arrowheads. The alignment was generated using ClustalW (2). The sequences shown are ASH1L (NP_060959), BAZ1A (NP_038476), BAZ1B (NP_115784), BAZ2B (NP_038478), BPTF (NP_872579), BRD1 (NP_055392), BRD2 (NP_005095), BRD3 (NP_031397), BRD4 (NP_490597), BRDT (NP_872579), BRPF1 (NP_001003694), BRPF3 (NP_056510), CBP (NP_004371), GCN5L2 (NP_066564), P300 (NP_001420), PCAF (NP_003875), SP140 (NP_009168), SP140L (NP_612411), TRIM24 (NP_056989), TRIM28 (NP_005753), TRIM33 (NP_056990), TRIM66 (NP_055633), ZMYND8 (NP_898868), and ZMYND11 (NP_006615).

1. Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) PROCHECK: A program to check the stereochemical quality of protein structures. *J Appl Cryst* 26:283–291.

2. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap



Figure S3. NMR analysis of CBP BrD-PHD tandem module binding to histone peptides. Related to Figure 2.

The protein/peptide binding was assessed by 2D 1 H- 15 N-HSQC NMR spectra of the 15 N-labeled protein (0.2 mM) in the free form (black) and in the presence of a histone peptide (1.2 mM) (red) of (**A**) H4K20ac (aa 17-23); (**B**) H3K4me3 (aa 1-18); or (C) H3 (aa 1-18).



	CBI	P-BrD-PHI)	B	RD4-BrD1		BRD4-BrD2			
	IC ₅₀	IC ₅₀ 95% confidence	K_i	IC ₅₀	IC ₅₀ 95% confidence	K_i	IC ₅₀	IC ₅₀ 95% confidence	K_i	
H4K5ac/K8ac	818	731 to 917	383	92.8	82.3 to 104.7	26	424	347 to 518	142	
H4K12ac/K16ac	732	652 to 822	343	952	755 to 1200	270	1139	985 to 1317	380	
H4K20ac	874	708 to 1078	315	508	454 to 571	145	101.3	91.5 to 112.3	34	

Figure S4. Fluorescence anisotropy measurement of binding affinity of the bromodomains of CBP and BRD4 to lysine-acetylated histone H4 peptides. Related to Figure 3.

Upper, fluorescence anisotropy competition plots of the CBP and BRD4 bromodomains binding to various histone H4 peptides. Lower, a Table summarizes the binding affinity values. Ki was calculated as described in Supplementay Information. The errors in IC_{50} values recovered from fitting competition curves reflect 95% of confidence intervals.