#### **Supplementary Materials**

#### **Supplementary Figure Legends**

**Fig. S1.** A close-up stereo view of the BRD2-BD2 dimer interface region. The intermolecular interactions occur through buried water molecules.

**Fig. S2.** A close-up view of the H4K5ac/K12ac complex in the BRD2-BD2 complex. (A) A close-up view of the H4K5ac/H12ac tail Q with its 2mFo-DFc map contoured at 1.0  $\sigma$ . The two BD2 chains A<sub>1</sub> and A<sub>2</sub> which associate with the H4 tail are shown in the background. (B) A close-up view of the H4K5ac/H12ac tail R with its 2mFo-DFc map contoured at 1.0  $\sigma$ . The two BD2 chains B<sub>1</sub> and B<sub>2</sub> which associate with the H4 tail are shown in the background.

**Fig. S3.** The electrostatic surface potential of the BD2 chains in the BD2 complex. The H4K5ac/K12ac tail bound to the protein is shown as sticks. The surface is colored red and blue for potential values below  $-5k_BT$  and above  $+5k_BT$ , respectively, where  $k_B$  is the Boltzmann constant and T is the room temperature.

**Fig. S4.** Superposition of the two H4 tails, Q and R, in the BRD2-BD2 complex with respect to K5ac.

**Fig. S5.** Sequence comparison of the BET and non-BET bromodomains. The alignment was produced by ClustalW [1] and was manually modified. The red characters indicate identical residues in the BET family (group 1) of BRD2, BRD4 and mBRDT bromodomains. The white characters on the blue background indicate completely conserved amino acids among the BET family (group 1) and non-BET

family (group 2) bromodomains. Red star denotes the residue that interacts with the acetylated histone H4 tail residues (except for the K5ac and K12ac side chain interactions, for clarity). The figure was generated by ESpript [2].

**Fig. S6.** Comparison of the BRD2-BD1 and BRD2-BD2 complexes. (A) Superposition of the BD2 complex over the BD1 complex, with respect to K12ac in the BD2 complex. In this superposition, the H4 tails in the BD1 and BD2 complexes are oriented in opposite directions. (B) Superposition of the BD2 complex over the BD1 complex with respect to K5ac in the BD2 complex. In this superposition, the H4 tails are oriented in the same direction.

#### References

- [1] Higgins, D. G., Bleasby, A. J., & Fuchs, R. (1992). CLUSTAL W: improved software for multiple sequence alignment. *Comput. Applic. Biosci.* 8, 189–191.
- [2] Gouet, P., Courcelle, E., Stuart, D., & Metoz, F. (1999). ESPript: multiple sequence alignments in postscript. *Bioinformatics* **15**, 305–308.





B B2 B2 B1 K5ac R A15

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			وووووو	αZ 2222222			αA 2020202
			350	360	370	380	390 400
1	hsBrd2_BD2/345-467 hsBrd2_BD1/73-195 hsBrd4_BD1/57-178 hsBrd4_BD2/347-470 mBrdt_BD2/260-382 hsCBP71077-1197 hsTAFI_BD1/1371-1490 hsTAFI_BD2/1497-1633 hsgcn5/730-832 Scgcn5/319-439 hsP-CAF/715-832		SEQLKHCN TNQLQYLH TNQLQYLH YSEQLKCS KKIFKPEE DDQVAFS GDQL.YTT FK.RGPHDA DPDQL.YST	GILKELLS. KVVMKALW. RVVLKTLW. GILKEMFA. EILKEMLA. LRQALMPTLJ SILESIIN.I FILDNIVT.( A.IQNILT.) .LKSILQ.(	KKHAAYAWPFY KHQFAWPFQ KKHAAYAWPFY KKHLPYAWPFY EALYRQDPESLFR DMR.DLPNTYPFH QKMMAVPDSWPFH QIKSHPSA.WPFM ELQNHAAA.WPFM	KPVDASALGLHI OPVDAVKLGLPI QPVDAVKLNLPI KPVDVEALGLHI NPVDADALGLHI TPVNA.KV.VKI HPVNK.KF.VPI EPVKK.SE.API QPVDKK.EE.VPI EPVKR.TE.APC	YHDIIKHPMDLSTVKRK YHKIIKOPMDMGTIKRR YYKIIKTPMDMGTIKRR YCDIIKHPMDMSTIKSK YYDVKNPMDLGTIKGK YFDIVKNPMDLSTIKRK YYKIITRPMDLQTLREN YYKVIVNPMDLETIRKN YYEVIRFPIDLKTMTER YYEVIRFPMDLSTMEIK YYEVIRFPMDLKTMSER
		22 222	αB	0000000	αC 222222222	0000000	
		410	420	430	<b>★★★</b> 440	450	460
1	hsBrd2_BD2/345-467	MENRDYRDAQE	FAADVRLM	FSNCYK <mark>YN</mark> PI	PDHDVVAMARKLQ	DVFEFRYAKMPI	EPLEPGPLPVST
2	hsBrd2_BD1/75-195 hsBrd4_BD1/57-178 hsBrd4_BD2/347-470 mBrdt_BD2/260-382 bsCBP/1077-1197	LENNYYWAASE LENNYYWNAQE LEAREYRDAQE MDNQEYKDAYE	CIQDFNIM FGADVRLM FAADVRLM	FINCYIYNKI FTNCYIYNKI FSNCYKYNPI FMNCYKYNPI	PIDDIVLMAQILE PGDDIVLMAEALE PDHEVVAMARKLQ PDHEVVAMARTLQ	KIFLQKVASMPC KLFLQKINELPI DVFEMRFAKMPI DVFELHFAKIPI	EETEIMIVQAKG DEPEEPVVAVSS DEPIES
4	hsTAFI_BD1/1371-1490 hsTAFI_BD2/1497-1633 hsgcn5/730-832 Scgcn5/319-439 hsP-CAF/715-832	VRKRLYPSREE ISKHKYQSRES LRSRYYVTRKL LESNKYQKMED LKNRYYVSKKL	SFREHLELI SFLDDVNLI SFVADLQRV SFIYDARLV SFMADLQRV	VKNSATYNG LANSVKYNG IANCREYNP FNNCRMYNG FTNCKEYNP	PKHSLTQISQSML PESQYTKTAQEIV PDSEYCRCASALE ENTSYYKYANRLE PESEYYKCANILE	DICDEKLKEKEI NVCYQTLTEYDE KFFYFKLKEG KFFNNKVKEIPE KFFFSKIKEAGI	DKLAR SHLTQLEKDICTAKEAAL SYSHLID JDK

