

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table 1 | Published affinities of bromodomains for histone peptides.

Bromodomain	Histone	Peptide Sequence Used	Affinity / μM	Ref
BRD2(2)	H4K12	GKGLG <u>K</u> GGAKR	2900 (NMR)	1
BRD4(1)	H4K5	SGRG <u>K</u> GGKGLGKGGAK	810 (NMR)	2
	H4K12	SGRGKGGKGLG <u>K</u> GGAK	650 (NMR)	
BRD4(2)	H4K5	SGRG <u>K</u> GGKGLGKGGAK	1000 (NMR)	
	H4K12	SGRGKGGKGLG <u>K</u> GGAK	1350 (NMR)	
BRD7	H3K14	STGG <u>K</u> APRK	1200 (NMR)	3
	H4K8	SGRGKGG <u>K</u> GLGK	1800 (NMR)	
BRDT(1)	H4K5/8	SGRG <u>K</u> GG <u>K</u> GLGKGGAKRHRK	22 (ITC)	4
CREBBP	H4K20	GGAKRHR <u>K</u> VLRDNIQ	218 (NMR)	5
	H3K36	APATGGV <u>K</u> KPHRYRP	122 (NMR)	
	p53K382	QSTSRHK <u>K</u> LMFKTEG	187 (NMR)/155 (FP)	
PCAF	H3K9	RTKQTAR <u>K</u> STGGKAP	1051 (NMR)	5
	H3K36	APATGGV <u>K</u> KPHRYRP	402 (NMR)	
	H3K14	ARKSTGG <u>K</u> APRKQLA	128 (NMR)	
	H4K16	GLGKGGAK <u>K</u> RHRKVLR	365 (NMR)	
	H4K20	GGAKRHR <u>K</u> VLRDNIQ	247 (NMR)	
	TATK50	LGISYGR <u>K</u> KRRQRRRA	84 (NMR)/212 (FP)	
GCN5	H4K8	SGRGKGG <u>K</u> GLGK	~900 (NMR)	6
	H4K16	A <u>K</u> RHRKVLRDNIQGITKPAI		
TAF1	H3K14	ARKSTGG <u>K</u> APRGC	not quantified (immunoblot)	7
	H4K5/8/12/16	AGG <u>K</u> GG <u>K</u> GMG <u>K</u> VGA <u>K</u> RHSC		
PB1_1	H3K4	ART <u>K</u> QTARKSTGGKAPRKQLATKAA	0.39 (FP)	8, 9
PB1_2	H3K9	ARTKQTAR <u>K</u> STGGKAPRKQLATKAA	0.36 (FP) / 500(NMR)	
PB1_3	H3K9	ARTKQTAR <u>K</u> STGGKAPRKQLATKAA	0.71 (FP)	
PB1_4	H3K23	ARTKQTARKSTGGKAPRKQLAT <u>K</u> AA	0.12 (FP)	
PB1_5	H3K14	ARTKQTARKSTGG <u>K</u> APRKQLATKAA	0.79 (FP)	
PB1_6	H3K23	ARTKQTARKSTGGKAPRKQLAT <u>K</u> AA	9.7 (FP)	
SMARCA2 (SWI/SNF)	H4K8/12	Not specified	not quantified (GSTpull-down)	10
	H3K9/14	ARTKQTAR <u>K</u> STGGKAPRKQLC		
SMARCA4 (BRG1)	H3K14	KSTGG <u>K</u> APRK	1200 (NMR)	11
	H4K8	SGRGKGG <u>K</u> GLGK	4000 (NMR)	
	H4K12	GKGLG <u>K</u> GGAKR	3600 (NMR)	

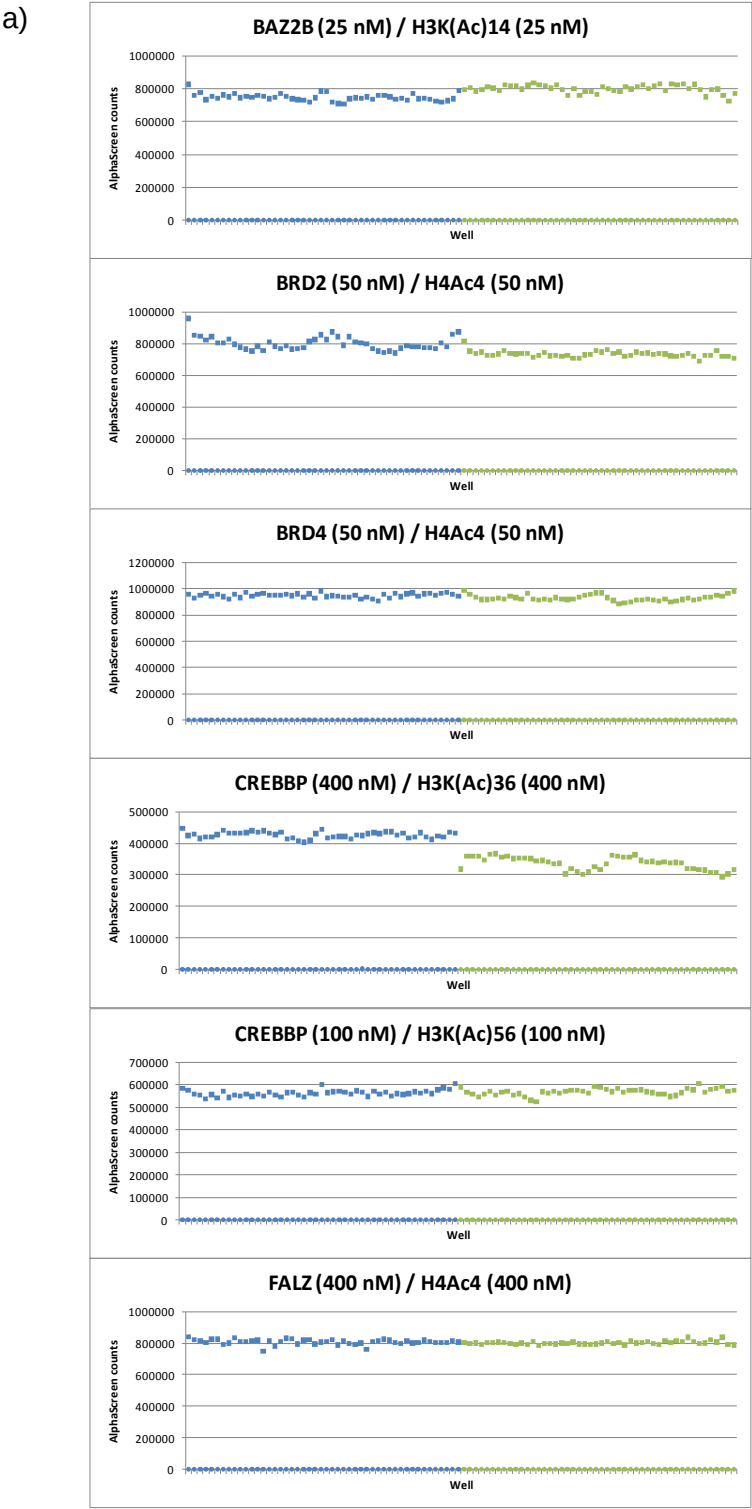
Supplementary Table 2 | Dissociation constants and thermodynamic parameters from ITC measurements characterizing interactions between BRD2(1) or BRD4(1) with H4KAc4, and BAZ2B with H3K14Ac, at 10°C.

Protein	Peptide	[Protein] (μM)	[Peptide] (mM)	N	K _D (μM)	ΔH (kcal/mol)	-TΔS	ΔG
BRD2(1)	H4KAc4	50	1.60	0.575	4.3 ± 0.2	-28.27	21.31	-6.96
BRD4(1)	H4KAc4	54	1.55	0.608	3.1 ± 0.2	-27.08	19.95	-7.13
BAZ2B	H3K14Ac	40	1.40	1.000	6.3 ± 2.0	-6.86	0.12	-6.74

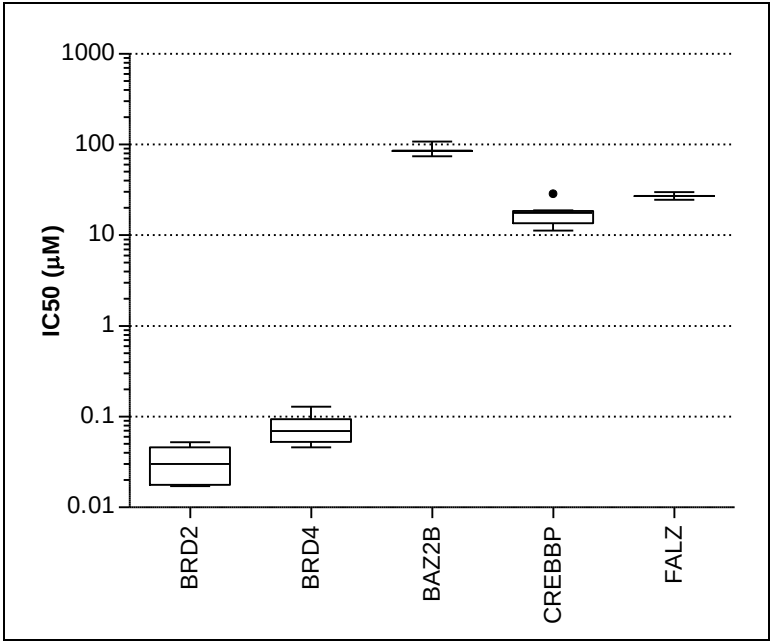
Supplementary Table 3 | Reproducibility of bromodomain AlphaScreens. S/B, S/N and Z' for 48 replicates in two experiments on separate days.

Bromodomain	BAZ2B		BRD2		BRD4		CREBBP		CREBBP		FALZ	
Peptide	H3K(Ac)14		H4Ac4		H4Ac4		H3K(Ac)36		H3K(Ac)56		H4Ac4	
Protein (nM)	25		50		50		400		100		400	
Peptide (nM)	25		50		50		400		100		400	
Experiment	1	2	1	2	1	2	1	2	1	2	1	2
S/B	445	432	456	404	688	516	278	173	366	293	630	652
S/N	34	34	19	39	60	41	43	16	41	37	48	74
Z'	0.910	0.911	0.841	0.921	0.949	0.926	0.928	0.814	0.925	0.918	0.936	0.959

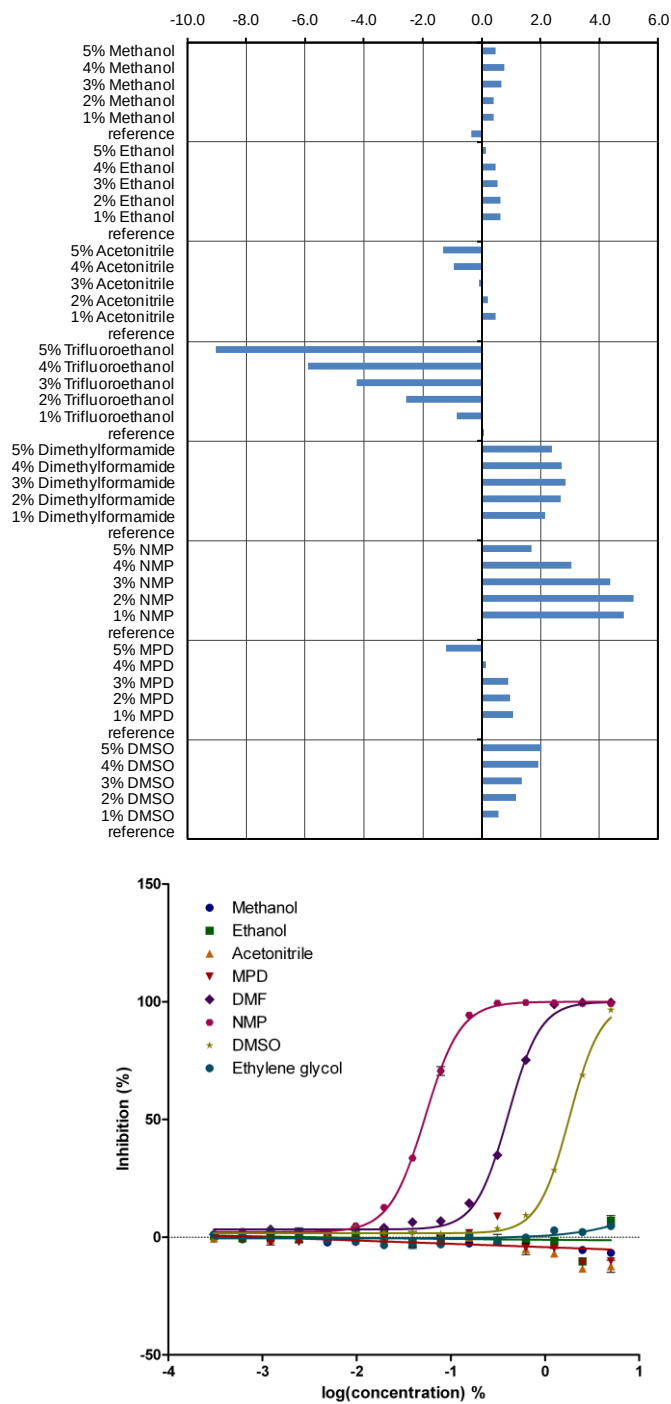
Supplementary Figure 1 | a) Inter-experimental variation of bromodomain AlphaScreen signals testing 48 replicates in two experiments on separate days. See Supplementary Table 2 for S/B, S/N and Z' calculations. **b)** Inter-experimental variation of IC₅₀ values generated on at least two different days for the control inhibitor JQ1/SGCBD01.



b)



Supplementary Figure 2 | a) Concentration-dependent thermostabilization of bromodomains by water miscible solvents, measured by differential scanning fluorimetry assay. Stabilization is determined by shift in denaturation midpoint temperature (T_m shift). b) IC_{50} curves for water miscible solvents in displacement of H4K(Ac)₄ from BRD2 bromodomain using the AlphaScreen assay.



Supplementary References

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