Supplementary Information

Dual kinase/bromodomain inhibitors for rationally designed polypharmacology

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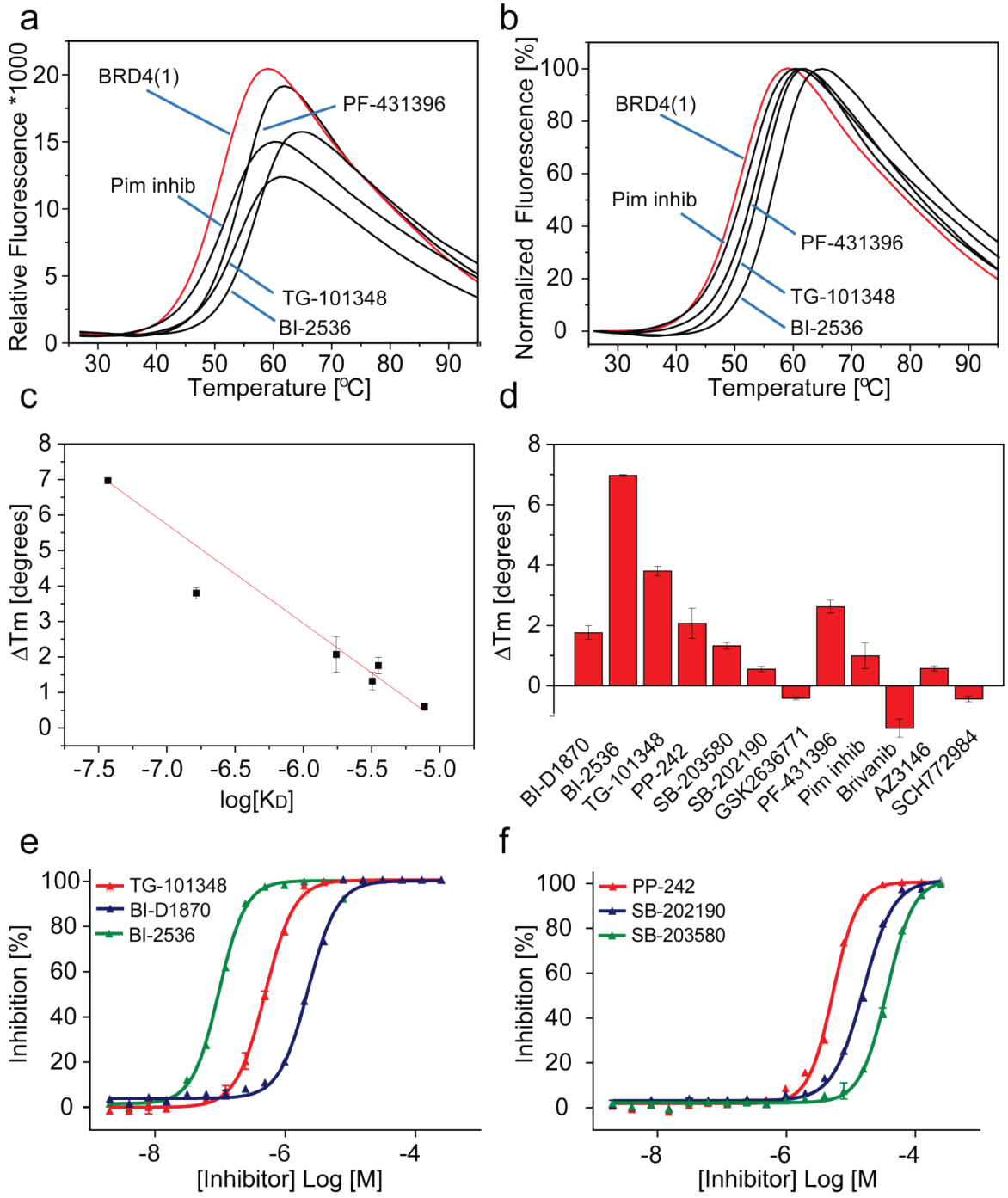
Supplementary Results

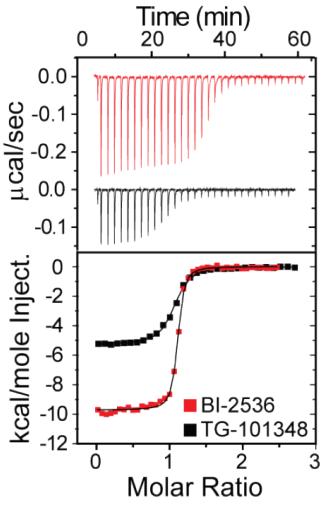
Supplementary Figure 1



Supplementary Figure 1. Validation of primary screening hits in secondary assays. a, Representative temperature shift scans using BRD4(1)**.** BRD4(1) without inhibitor is shown in red. Compounds have been labeled. **b,** Normalized temperature shift data of the dataset shown in panel **a**. The highest fluorescent value has been set to 100%. **c,** Correlation between temperature shift values and dissociation constants (KDs) determined by ITC. **d,** Temperature shift assay data measured on (BRD4(1)) at 10 μM compound concentration on identified hits of the primary screen. **e and f,** Dose response AlphaScreen data (BRD4(1)) on selected hits.

Supplementary Figure 2

BRD4(1)



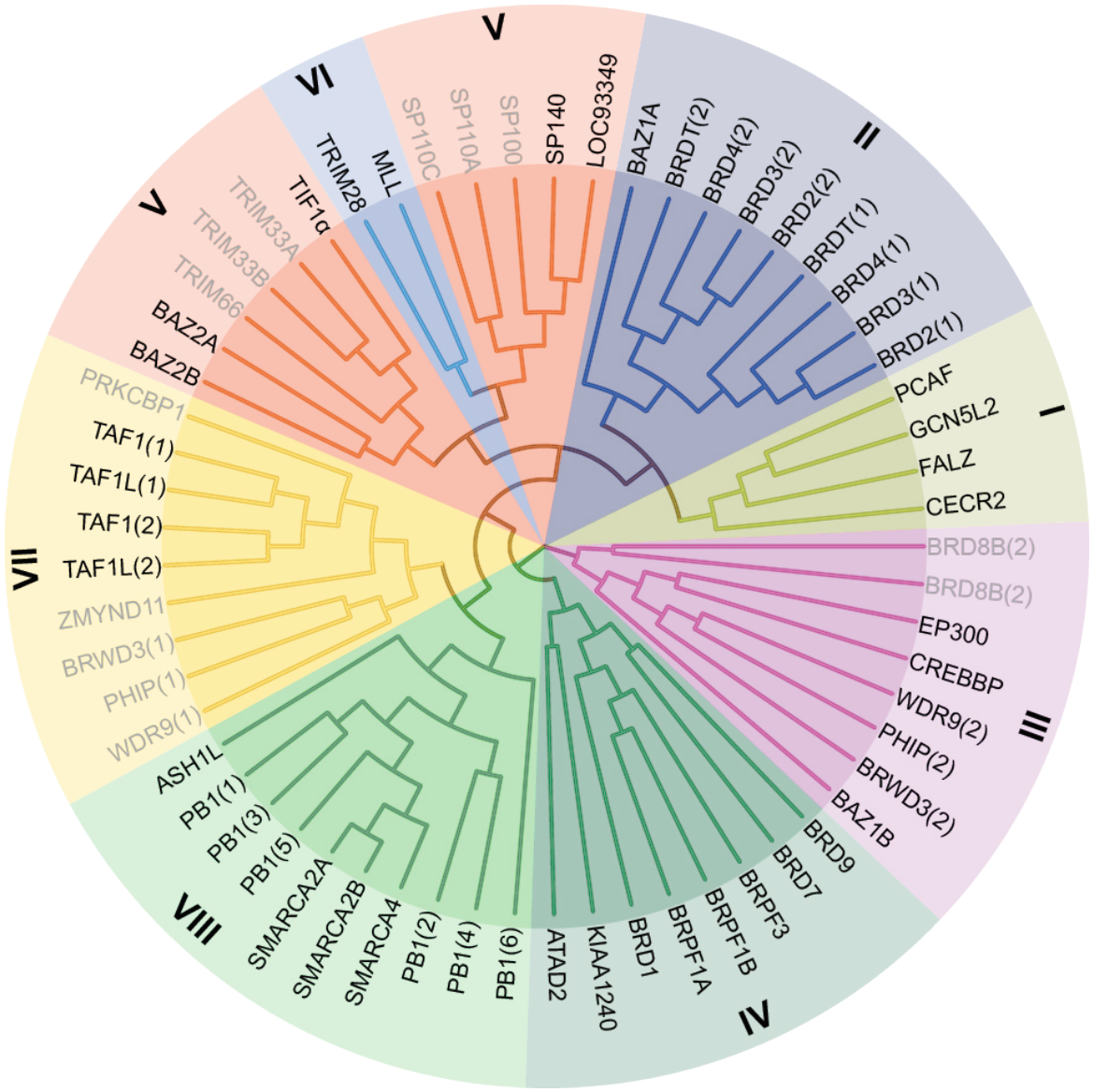
Protein	Inhibitor	K_d (nM)*	ΔH^{obs} (kcal/mol)*	N	$T\Delta S$ (kcal/mol)	ΔG (kcal/mol)
BRD4(1)	**BI-2536**	37 ± 3	-9.7±0.04	1.08±0.02	0.074	-9.79
BRD4(1)	**BI-6727**	79 ± 3	-8.5±0.04	1.02±0.04	0.85	-9.35
BRD4(1)	**BI-D1870**	3546 ± 178	-10.9±0.02	0.965±0.02	-3.80	-7.1
BRD4(1)	**TG-101348**	164 ± 10	-5.28±0.03	1.03±0.04	3.65	-8.94
BRD4(1)	**TG-101209**	123 ± 18	-7.15±0.01	1.16±0.01	1.95	-9.10
BRD4(1)	**PP-242**	1742 ± 76	-7.27±0.02	0.91±0.01	0.31	-7.58
BRD4(1)	**SB-202190**	3424 ± 132	-4.48±0.04	1.03±0.02	2.72	-7.20

Titrations were carried out in 50 mM HEPES pH 7.5 (at 25 °C), 150 mM NaCl at 15 °C while stirring at 1000 rpm. Proteins were titrated into the ligand solution (reverse titration).

*Errors represent errors of the non-linear least squares fit.

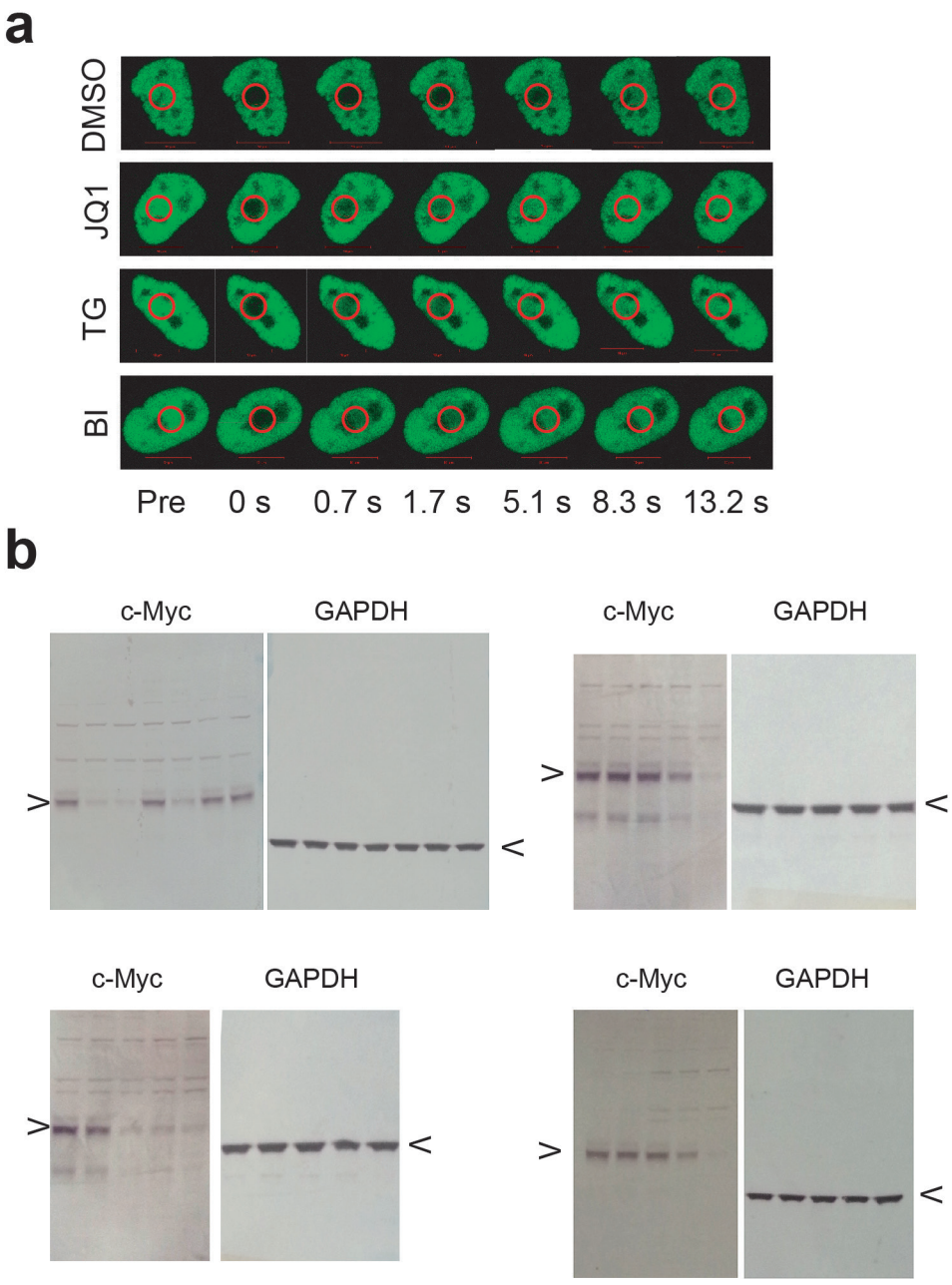
Supplementary Figure 2. Example of two isothermal titration calorimetry (ITC) experiments and fitted parameters for all ITC measurements. Shown are raw binding heats (upper panel) for BI-2536 (red trace) and TG-101348 (black trace) as well as normalized binding heats (lower panel). The fitted functions are shown as a solid line and fitted parameters are incorporated in the table below.

Supplementary Figure 3



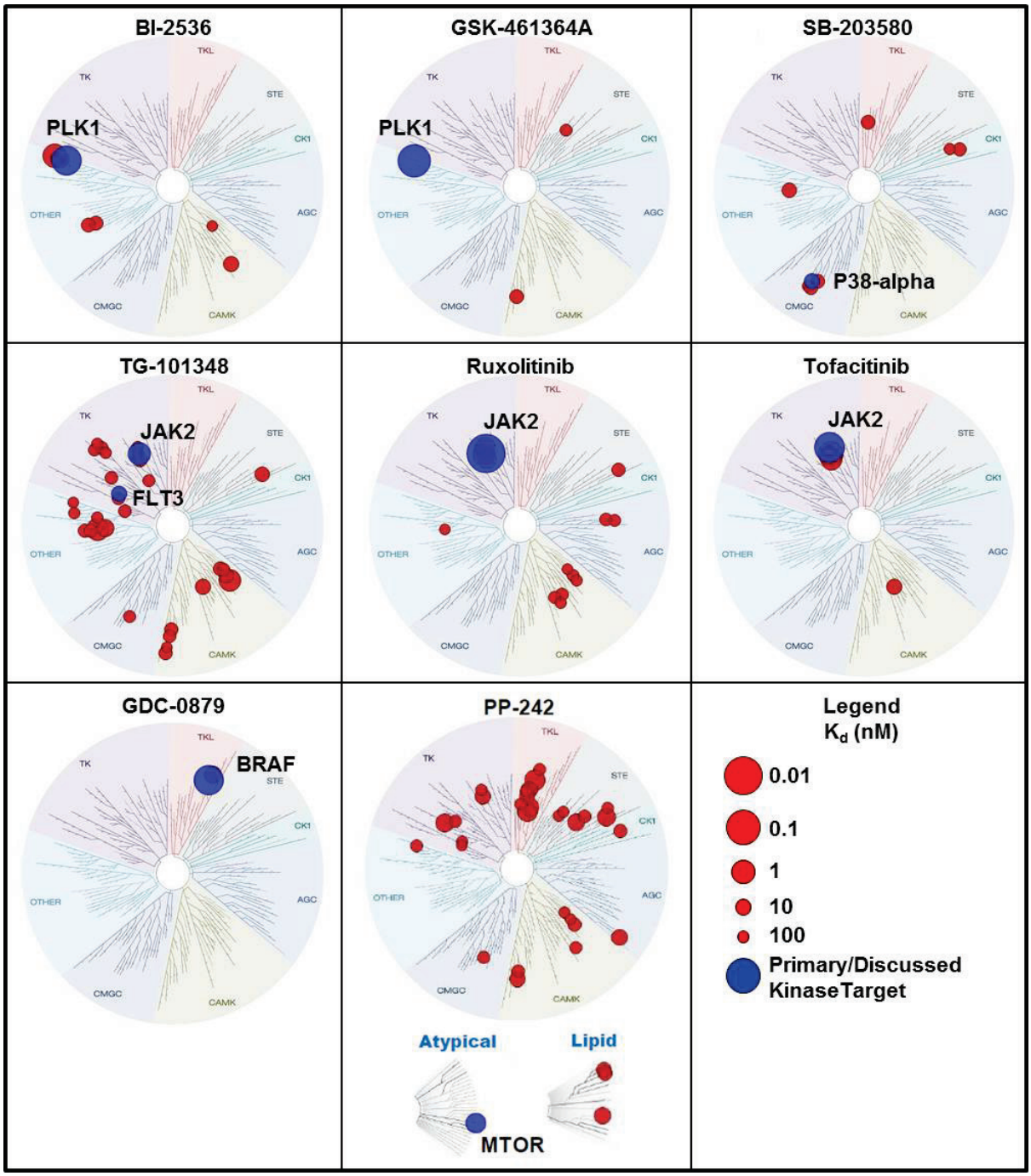
Supplementary Figure 3. Bromodomains addressed in this study. Roman numerals indicate bromodomain families as defined by Filippakopoulos, *et al.* (Filippakopoulos, P. *et al.* Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell* **149**, 214-231, (2012)). Bromodomains included in the selectivity screeing panel are labeled in black.

Supplementary Figure 4



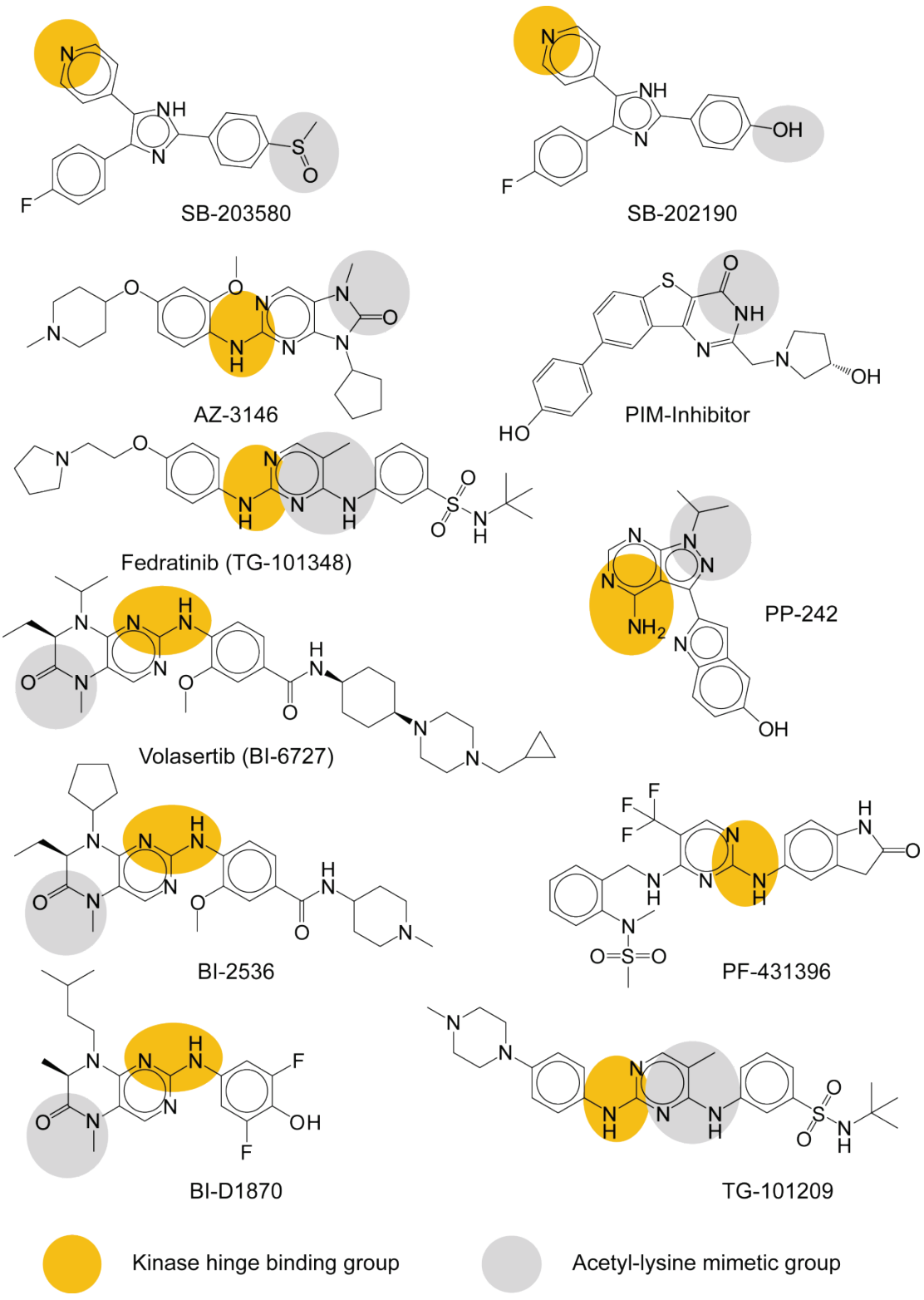
Supplementary Figure 4. BI-2536 and TG-101348 displace BRD4 from chromatin and suppress c-Myc expression. a, Fluorescent Recovery After Photo-bleaching (FRAP) experiments using full-length GFP-BRD4. Nuclei of U2OS cells transfected with GFP-BRD4 that were untreated (top) or exposed to the pan-BET inhibitor JQ1, BI-2536 (BI) or TG-101348 (TG) at 500 nM. Target regions of photobleaching are indicated by a red circle. **b,** Full images of the western blots presented in **Figure 1b** showing down regulation of c-Myc by JQ1 and kinase inhibitors. Blots are arranged as in **Figure 1b** and arrows indicate the bands of interest.

Supplementary Figure 5



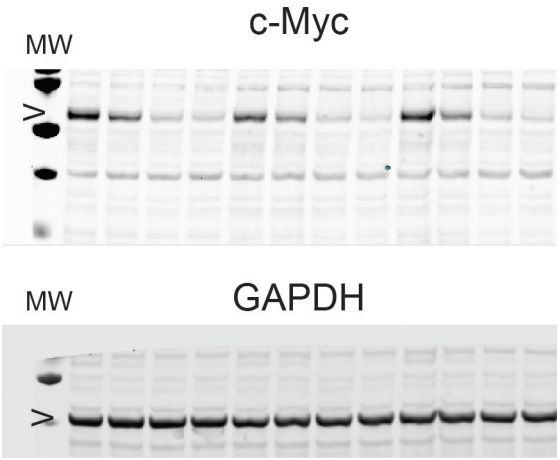
Supplementary Figure 5. Kinome interaction maps for kinase inhibitors addressed in this study. Interaction maps showing all interactions with K_d < 100 nM reported by Davis et al. (Davis, M. I. *et al.* Comprehensive analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **29**, 1046-1051, (2011)). Larger circles indicate higher affinity interactions. Blue circles indicate the intended, primary targets or other high affinity targets discussed in the main text (e.g. FLT3 for TG-101348).

Supplementary Figure 6



Supplementary Figure 5. Structures of kinase/bromodomain dual inhibitors. Acetyl-lysine mimetic and hinge binding groups are highlighted by yellow and grey circles, as indicated, for inhibitors of known binding mode.

Supplementary Figure 7



Supplementary Figure 7. Full images of western blot data displayed in Figure 5c. c-Myc and GAPDH bands are indicated by arrows. Size markers in first lane (not shown in **Figure 5c**) are indicated by MW.

Supplementary Table 1. Temperature shift data. Shown are average measurements of three independent experiments at a screening concentration of 10 μM.

Target	BI-D1870	BI-2536	TG-101348	PP-242	SB-203580	SB-202190
ASH1L	0.25±0.3	-0.11±1.04	0.24±0.17	0.26±0.16	0.31±0.11	0.27±0.9
ATAD2	-0.18±0.07	-0.10±0.17	-0.25±0.15	-0.50±0.46	-1.47±0.21	0.05±0.3
BAZ1A	0.254±0.07	0.2±0.46	0.23±0.16	0.38±0.18	1.03±0.08	0.34±0.1
BAZ1B	0.052±0.07	0.03±0.15	-0.02±0.14	0.37±0.53	0.29±0.21	0.52±0.2
BAZ2A	-0.12±0.10	0.12±0.22	-0.11±0.06	-0.59±0.22	-0.19±0.22	0.16±0.1
BAZ2B	0.046±0.04	-0.03±0.29	-0.13±0.17	0.10±0.15	0.21±0.13	0.12±0.15
BRD1	0.41±0.11	-0.05±0.15	0.51±0.29	1.74±0.45	-0.23±0.21	0.46±0.70
BRD2(1)	1.41±0.11	4.97±0.13	2.40±0.20	2.33±0.93	0.92±0.07	1.18±0.27
BRD2(2)	1.28±0.08	4.12±0.12	2.49±0.07	1.78±0.18	1.40±0.08	0.66±0.02
BRD3(1)	1.24±0.20	4.88±0.25	3.34±0.09	2.13±0.49	1.43±0.06	0.72±0.17
BRD3(2)	1.1±0.10	5.0±0.30	3.4±0.10	2.00±0.2	2.20±0.20	0.8±0.10
BRD4(1)	1.76±0.23	6.97±0.03	3.80±0.16	2.07±0.50	1.32±0.11	0.55±0.09
BRD4(2)	1.03±0.17	5.09±0.07	3.11±0.88	1.53±0.33	1.67±0.10	0.83±0.30
BRD7	0.37±0.17	1.84±0.18	0.22±0.32	0.35±0.23	0.34±0.09	1.29±0.78
BRD9	0.20±0.01	0.51±0.09	0.58±0.40	0.18±0.21	-0.33±0.21	0.31±0.12
BRDT(1)	0.89±0.03	4.03±0.13	3.38±0.16	0.95±0.25	0.85±0.22	-0.47±0.05
BRDT(2)	0.42±0.12	2.08±0.10	2.69±0.15	0.33±0.04	0.59±0.24	0.70±0.26
BRPF1A	-0.38±0.14	0.14±0.45	0.00±0.27	-0.08±0.26	0.66±0.21	-1.22±0.13
BRPF1B	0.95±0.46	1.57±0.42	0.94±0.14	1.72±0.29	2.26±0.08	0.74±0.61
BRPF3A	-0.09±0.07	0.21±0.31	-0.01±0.07	1.06±0.14	0.66±0.10	0.96±0.11
BRWD3(2)	0.21±0.45	1.34±0.20	0.58±0.29	0.09±0.18	0.55±0.21	0.66±0.08
CECR2	0.21±0.18	0.48±0.19	0.12±0.10	0.51±0.17	0.89±0.14	1.35±0.18
CREBBP	0.46±0.21	0.15±0.25	2.06±0.21	0.07±0.31	0.92±0.06	0.47±0.06
EP300	0.52±0.06	0.00±0.14	2.76±0.33	0.29±0.11	0.35±0.21	0.33±0.23
FALZ	0.38±0.38	-0.15±0.88	0.80±0.22	0.45±0.23	0.89±0.3	0.18±0.49
GCN5L2	-0.37±0.21	-0.31±0.53	0.13±0.39	-1.4±0.27	1.05±0.2	-0.05±0.20
KIAA1240	0.01±0.09	-0.28±0.12	-0.06±0.18	-0.06±0.12	0.39±0.11	0.15±0.10
LOC93349*	-0.33±0.08	-0.28±0.26	0.03±0.13	-0.26±0.19	-0.41±0.31	0.28±0.09
MLL	-0.02±0.06	-0.29±0.24	0.01±0.21	-0.24±0.06	0.46±0.22	0.33±0.06
PB1(1)	0.012±0.06	0.24±0.15	0.12±0.09	-0.02±0.06	0.28±0.08	0.00±0.42
PB1(2)	-0.26±0.02	0.20±0.37	0.23±0.10	0.01±0.12	0.4±0.14	0.09±0.07
PB1(3)	-0.41±0.17	-0.04±0.31	-0.07±0.18	0.09±0.17	0.87±0.18	-0.57±0.14
PB1(4)	-0.02±0.13	-0.41±0.16	-0.06±0.26	-0.27±0.08	0.60±0.14	0.78±0.09
PB1(5)	-0.24±0.14	0.04±0.10	0.08±0.10	0.47±0.09	1.33±0.22	0.47±0.05
PB1(6)	0.52±0.25	0.14±0.15	0.58±0.08	0.64±0.14	1.07±0.3	0.43±0.4
PCAF	0.36±0.32	-0.12±0.30	0.29±0.13	-0.01±0.22	0.43±0.21	-0.19±0.3
PHIP(2)	0.24±0.28	-0.48±0.30	0.60±0.09	0.33±0.12	1.14±0.11	0.27±0.4
SMARCA2	0.08±0.12	-0.37±0.37	-0.09±0.07	-0.08±0.09	0.31±0.07	0.56±0.2
SMARCA4	0.02±0.10	-0.24±0.02	0.01±0.08	0.08±0.16	-1.47±0.21	0.04±0.3
SP140	-0.14±0.04	-0.05±0.05	-0.01±0.09	-0.19±0.14	1.03±0.14	0.36±0.1
TAF1A(2)	0.13±0.26	0.79±0.07	0.28±0.05	0.023±0.01	0.29±0.22	-0.06±0.2
Target	**BI-D1870**	**BI-2536**	**TG-101348**	**PP-242**	**SB-203580**	**SB-202190**

TAF1A(1)	-0.02±0.12	-0.49±0.04	0.01±0.07	-0.16±0.03	-0.19±0.1	-0.08±0.1
TAF1L(1)	0.14±0.11	-0.48±0.10	0.41±0.17	0.38±0.25	0.21±0.1	0.17±0.5
TAF1L(2)	0.24±0.29	0.34±0.50	0.91±0.31	-0.32±0.23	-0.23±0.3	-0.32±0.5
TIF1*	-0.20±0.01	-0.47±0.36	0.01±0.14	-0.08±0.24	0.92±0.3	-0.35±0.2
TIF1	-0.05±0.06	-0.43±0.18	0.00±0.08	-0.01±0.10	1.40±0.21	-0.23±0.3
TRIM28	-0.02±0.28	-0.60±0.39	0.08±0.19	-0.52±0.11	1.43±0.23	-0.03±0.5
WDR9(2)	-1.23±0.03	-0.53±0.11	-0.10±0.20	-0.28±0.22	1.32±0.08	-0.73±0.1

*PHD-Bromodomain construct.

Supplementary Table 1, continued from previous page.

Supplementary Table 2. Data collection and refinement statistics (molecular replacement)

	BRD4(1)/BI-2536 PDB ID: 4OGI	BRD4(1)/TG-101348 PDB ID: 4OGJ
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	42.24, 59.64, 115.35	42.16, 59.41, 111.67
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	1.73 (1.82-1.73)*	1.65 (1.74-1.65)*
R_{sym} or R_{merge}	0.094 (0.772)	0.051 (0.271)
$I / \sigma I$	11.9 (2.0)	17.9 (4.6)
Completeness (%)	99.7 (99.5)	99.3 (96.5)
Redundancy	4.6 (4.5)	4.5 (3.7)
Refinement		
Resolution (Å)	1.73	1.65
No. reflections	31083	34298
R_{work} / R_{free}	17.6/21.4	15.1/18.2
No. atoms		
Protein	2130	2174
Ligand/ion	84	86
Water	256	291
B-factors		
Protein	23.09	18.03
Ligand/ion	16.44	14.26
Water	25.96	28.43
R.m.s. deviations		
Bond lengths (Å)	0.015	0.015
Bond angles (°)	1.645	1.765

*Values in parentheses are for highest-resolution shell. One crystal was analyzed for each structure.

Supplementary Table 3. Kinase affinities of dihydropteridinones and pyridinyl imidazoles

Compound:	BI-2536	BI-D1870	Compound:	SB-203580	SB-202190
Kinase	K_d (nM)	K_d (nM)	Kinase	K_d (nM)	K_d (nM)
BMPR2	>3000	65	CSNK1D	37	59
CAMKK1	22	1300	GAK	19	53
CAMKK2	23	n/d	JNK3	35	42
CSNK1A1	3100	66	p38-alpha	25	28
CSNK1A1L	>3000	21	p38-beta	12	9.8
CSNK1E	380	70	p38-gamma	70	32
JAK1(JH2domain-pseudokinase)	>3000	63	RIPK2	24	150
KIT(V559D,V654A)	>3000	37			
MYLK	97	>3000			
PIK3CG	>3000	48			
PIP4K2C	110	n/d			
PLK1	0.19	14			
PLK2	0.81	8.4			
PLK3	4	15			
RPS6KA4	12	300			
RSK1(Kin.Dom.1-N-terminal)	5400	120			
RSK2(Kin.Dom.1-N-terminal)	>3000	60			
RSK3(Kin.Dom.1-N-terminal)	700	12			
RSK4(Kin.Dom.1-N-terminal)	1300	26			
TAOK1	>3000	94			
TNIK	>3000	110			

Supplementary Table 3. Kinase affinities of dihydropteridinones and pyridinyl imidazoles. Kinase K_d values for the dihydropteridinones (BI-2536, BI-D1870) and pyridinyl imidazoles (SB-203580, SB-202190) discussed in **Figure 4**. Data for all kinases with K_d <120 nM for at least one member of each compound pair are shown. Yellow shaded cells indicate inter-compound pair K_d differences of at least 10-fold. Data for BI-2536, SB-203580, and SB-202190 are from Davis *et al*. (Davis, M. I. *et al.* Comprehensive analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **29**, 1046-1051, (2011)) and Karaman *et al*. (Karaman, M.W. *et al*. A quantitative analysis of kinase inhibitor selectivity. *Nat. Biotechnol.* **26**, 127-132, (2008)). 442 kinase assays were tested for the dihydropteridinones and 317 for the pyridinyl imidazoles. Data for BI-D1870 (442 assays) were collected according to Davis *et al*. Interactions not measured are indicated by n/d.