## SUPPLEMENTARY MATERIAL

## Enhanced cellular death in liver and breast cancer cells by dual BET/BRPF1 inhibitors

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[GSK6853] (µM)	0	1.56	3.12	6.25	12.5	25	50
JQ1 EC50 (µM)	> 50	> 50	> 50	> 50	> 50	$6.6 \pm 6.0$	n.d.*

Supplementary Table 1. JQ1 EC<sub>50</sub>s in co-treatment at fixed concentrations of GSK6853.

\*excessive death caused by GSK6853.

Supplementary Table 2. GSK6853 EC<sub>50</sub>s in co-treatment at fixed concentrations of JQ1.

[JQ1] (µM)	0	1.56	3.12	6.25	12.5	25	50
GSK6853 EC50 (μM)	32.9 ± 3.5	28.7 ± 6.1	$25.8 \pm 6.0$	$22.8 \pm 4.8$	$16.8 \pm 12.1$	$16.9 \pm 2.4$	19.0 ± 5.4

Gene Symbol	Kd (µM)
ATAD2A	>100
ATAD2B	>100
BAZ2A	$72 \pm 10$
BAZ2B	>100
BRD1	>100
BRD2(1)	$9.7 \pm 1.4$
BRD2(1,2)	$6.1 \pm 1.1$
BRD2(2)	$7.9 \pm 0.2$
BRD3(1)	$6.1 \pm 0.3$
BRD3(1,2)	$4.2 \pm 0.2$
BRD3(2)	$4.8 \pm 1.5$
BRD4(1)	$5.2 \pm 0.2$
BRD4(1,2)	$3.4 \pm 0.5$
BRD4(2)	$4.4 \pm 0.3$
BRD4(full-length, short iso.)	$3.6 \pm 0.5$
BRD7	$12 \pm 1$
BRD8(1)	>100
BRD8(2)	>100
BRD9	$55 \pm 2.5$
BRDT(1)	$14 \pm 1.5$
BRDT(1,2)	$18 \pm 2$
BRDT(2)	$20 \pm 0.1$
BRPF1	$1.4 \pm 0.6$
BRPF3	>100
CECR2	$95 \pm 10$
CREBBP	51 ± 5
EP300	$51 \pm 10$
FALZ	96 ± 1.5
GCN5L2	>100
PBRM1(2)	>100
PBRM1(5)	>100
PCAF	>100
SMARCA2	>100
SMARCA4	>100
TAF1(2)	>100
TAF1L(2)	>100
TRIM24(bromo)	>100
TRIM24(PHD,Bromo)	>100
TRIM33(PHD,Bromo)	>100
WDRR9(2)	>100

Supplementary Table 3. Binding affinities for compound 1 as measured by BROMOscan

Numbers in parentheses indicate the bromodomain tested for proteins containing multiple bromodomains.

	Cmp 1	Cmp <b>2</b>	Cmp 3				
Data Collection							
Space group	P3221	P3221	P3221				
Unit-cell parameters (Å, °)	a = 60.49	a = 60.54	a = 60.55				
	b = 60.49	b = 60.54	b = 60.55				
	c = 62.23	c = 63.26	c = 62.51				
Wavelength (Å)	1.00	1.00	1.00				
Resolution (Å)	62.23-1.42	63.26-1.45	62.51-1.40				
	(1.45-1.42)	(1.47-1.45)	(1.42-1.40)				
$R_{\text{merge}}$ (%)	4.1 (160.1)	6.2 (115.5)	3.9 (156.7)				
$R_{\text{meas}}$ (%)	4.2 (164.5)	6.4 (118.7)	4.0 (161.4)				
$R_{\rm pim}$ (%)	1.0 (37.7)	1.5 (27.5)	1.0 (38.3)				
< <i>I</i> /σ( <i>I</i> )>	31.0 (2.0)	24.2 (2.9)	33.8 (2.1)				
CC <sup>1/2</sup>	1.000 (0.821)	0.998 (0.893)	1.000 (0.768)				
Completeness (%)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)				
Multiplicity	18.0 (18.8)	18.1 (18.5)	18.2 (17.6)				
	Refinement						
Resolution (Å)	52.42-1.42	52.47-1.45	52.48-1.40				
$R_{\rm work}/R_{\rm free}$ (%)	16.8/19.6	17.9/20.8	16.3/19.5				
R.m.s. deviations							
Bond lengths (Å)	0.008	0.009	0.008				
Bond angles (°)	0.93	0.99	0.91				
PDB entry	8QB2	8QB0	8QAZ				

Supplementary Table 4. Data Collection and Refinement Statistics for BRPF1 structures.

Supplementary Table 5. Data Collection and Refinement Statistics for BRD4 structures.

	Cmp 1	Cmp <b>2</b>	Cmp 4	Cmp <b>5</b>			
Data Collection							
Space group	P212121	P212121	P212121	P212121			
Unit-cell parameters	a = 37.06	a = 37.10	a = 37.21	a = 37.32			
(Å, °)	b = 44.48	b = 44.37	b = 44.67	b = 44.38			
	c = 78.11	c = 78.00	c = 77.73	c = 78.42			
Wavelength (Å)	1.00	1.00	1.00	1.00			
Resolution (Å)	39.05-1.25	44.37-1.40	38.87-1.30	78.42-1.50			
	(1.27-1.25)	(1.42-1.40)	(1.32-1.30)	(1.53-1.50)			
$R_{\rm merge}$ (%)	9.4 (121.5)	10.9 (144.9)	8.3 (80.7)	15.0 (118.7)			
$R_{\text{meas}}$ (%)	9.8 (126.7)	11.4 (151.1)	8.7 (85.1)	15.6 (124.2)			
<i>R</i> <sub>pim</sub> (%)	2.8 (35.5)	3.3 (42.2)	2.7 (26.6)	4.5 (36.0)			
< <i>I</i> /σ( <i>I</i> )>	14.0 (2.4)	12.5 (2.1)	15.3 (3.6)	9.6 (2.2)			
CC <sup>1/2</sup>	0.999 (0.793)	0.998 (0.767)	0.999 (0.857)	0.994 (0.770)			
Completeness (%)	98.2 (95.2)	100.0 (100.0)	95.7 (92.3)	100.0 (100.0)			
Multiplicity	12.4 (12.4)	12.2 (12.6)	10.0 (10.0)	12.3 (11.7)			
	Refinement						
Resolution (Å)	39.05-1.25	39.02-1.40	38.87-1.30	39.22-1.50			
$R_{\rm work}/R_{\rm free}$ (%)	15.4/17.6	15.1/18.6	14.6/17.1	16.6/20.1			
R.m.s. deviations							
Bond lengths (Å)	0.009	0.008	0.008	0.009			
Bond angles (°)	1.12	0.94	1.05	1.05			
PDB entry	8QAN	8QAP	8QAL	8QAR			



**Supplementary Figure 1: morphological changes of Huh7.** The pictures show the morphological changes of Huh7 treated for 72h with the acetylpyrrole-thiazole compounds and the reference compounds JQ1 and GSK6853. The concentrations are the same showed in Fig. 1D-E. The images were obtained from the Incucyte® S3 (Sartorious), magnification 10x. The images are representative of one of three independent experiments.

TMAD-93	Replicate ID = 1 Kd (nM) = >100000	TN40-03 Replicate ID + 2 ATAD2A Kd (nM) = >100000	TN40-00 Replicate 80 = 1 ATAD28 Kd (nM) = >100000	TN40-F3 Repictive ID = 2 ATAD2B Rd (nM) = >100000	TN40-03 BR03(1)	Replicate ID + 1 Kd (nM) = 5000	TN40-03 Regionate (D = 2 BRD3(1) (Cd (nM) = 6400	11440-03 Reptrate (D + 1 BRD5(1,2) Kd (rM) = 4505	TN40-05 Replicate (D = 2 BRD3(1.2) Kd (nM) = 4400
54-7 46-7 28-7 001	1 HE 1000	59-7 49-7 29-7 29-7 29-7 29-7 29-7 29-7 29-7 2	3+7 2+7 1+7 1+7 1+7 1+7 1+7 1+7 1+7 1+7 1+7 1	14-7 14-7 30-7	5+5 ++ 3+4 3+4 3+4 5+1 001	*** * 188 18000*	100 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	2544 344 1444 444 444 444 444 444 444 444	340 10 00 000 1563 449 557 557 100 000 000 000
TN40-03 BAZZA	Replicate ID = 1 Kd (rM) = 62500	TN40-03 Replicate ID = 2 BAZZA Kd (nM) = 02000	TN40-03 Replicate ID = 1 04228 Kd (nM) + >105000	TN40-03 Repirate ID = 2 84228 Kd inM) = >100000	1140-03 BRD3(2)	Replicatie ID = 1 Kd (nM) = 6405	TN40-03 Replicate ID = 2 9R03(2) K0 (IM) = 3300	Thest 43 Replicate ID = 1 BRD4(1) Kd (nM) = 5100	TN40-03 Replicate (0.*.) 9RD4(1) Kd (nM) = \$400
367 267 167 Bot	· · · · · · · · · · · · · · · · · · ·	447 367 197 307 1 100 80000	244 148 317 317 317 317 317 317 317 317 317 317	384 1 10 10001 -	2+4 5+5 2+5 0.01	1 160 10000	540 540 540 541 541 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5		200 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
TN4D-03 BRD1	Replicate (D + 1 Kd (nM) = >100000	11440-03 Replicate ID = 2 BRD1 Kd (nM) = >100000	TN40-03 Replicate 10 = 1 BR08(1) Rd (nM) = 11050	TN45-03 Replicate 0 = 2 8403(1) Kd (rW) = 8200	BRD4(1,2)	Kil (HM) = 2000	BND4(1.2) Kd (nM) = 3900	there us there us a th	BRD4(2) Kd (M) = 4700
1/36-7 16-7 56-8	1 100 1000	1961 1973 368 369 1971 1 100 1988	845 354 354 1546 1546 167 167 1700 1870 1000	445 344 2544 0 0 0 0 1 1 100 0000 1 100 00000	144 142 142 144 144 1000	**************************************	100 100 100 100 100 100 100 100	064 104 204 001 1 103 13000	144 144 144 144 144 144 144 144
TNID-03 BROAT 2	Repiktate ID = 1 Kd (rM) = 5000	TN40-03 BR02(1-2) Replicato 10 = 2 Kd (rM) = 7200	Thito 83 Ropt care ID = 1 9R02(2) Kit (MI) = 8 too	THES 03 Restant 9-58	BRD4(UR BRD4(UR BRD.)	length.short- 4200	BRD4(hull-length,short- bid.) Kit (mN) 2100	11440-03 Replicate ID = 1 19907 Kd (HM) = 11000	TN40-03 Replicate IO = 2 BRD7 Kd (MM) = 13000
244 1344 1345 145 145 145 145 145 145	· · · · · ·	201 1 00 0000 201 1 00 000	840 640 446 266 0 867 1 330 19990	145 145 146 146 170 0 0 0 1 1 100 1500 1500 100 1	544 454 365 792 0.01		Real Bread B	38+2 68+2 13+7 001 1 113 18000	147 447 247 231 1 1 160 (1000)
TN40-03 BRD9(1)	Replicate ID = 1 Kd (nM) = >100000	TN46-03 Replicate ID + 2 BRD0(1) Kd (1M) = >100000	TN40-03 RupScale ID = 1 BR08(2) Kd (nM) = >100000	TN40-03 Replicate ID = 2 BRD8(2) Rd inN0 = >100000	TN40-03 CECR2	Replicate ID = 5 Kd (nN) + 85000	TN40-03 Replicate ID = 3 CECR2 K# (nM) = >100000	TN40-03 Replicate ID = 1 CREBBP Kd (nM) = 56000	TN40-03 Replicate ID = 2 CREBBP Kit (rM) = 45000
000 000 000 000 000 000 000	· · · · · · · · · ·	8+0 1+2 2+0 3+1 3+1 1 50 13500	8+0 4+2 3+0 9 0 0 0 0000	0+4 1+4 1+4 1+4 1+4 1+4 1+4 1+4 1	1+4 2+2 2+0 2+0 0 01	**************************************	840 940 940 940 941 1 1 166 1800		
TN40-03 BRD9	Feplicate ID = 1 Kd (nM) = 55000	TN40-03 Replicate ID + 2 BRD9 Kd (nM) + 53030	TN40.03 Replicate ID = 1 BRDT(1) Kd (nM) + 15000	T7440-03 Repricate (D = 2 BRDT(1) Kd (mM) = 15600	TN40-03 EP300	Replicator IO = 1 Kd (HM) = 41000	TN40-09 Rep/com/(D = 2 EP300 Kd (rM) = 62000	FAL2 Re (=M0 = 97000	TN40-69 Repficare (0 = 2 FAL2 Kd (HM) = 04000
38-7 16-7	1 30 TANK	28-7 19-7 8 07 1 000 1 000 1 000	28-7 19-7 8-17 19-7 8-17 1 100 33000	2007 1 103 1000	34.7 24-7 14-7 8 01	1 Hit NOO	341 341 341 341 341 341 341 341		1264 144 144 1547 147 147 147 147 147 147 147 1
ERDICI 2	Replicate ID = 1 Kd (nM) = 16000	TN40.03 Replicate ID = 2 BHDT(1.2) Kd (HM) = 20000	TN40-03 RoyAcato (0 = 1 BRDT(2) Kd (nM) = 20000	TH40-03 Replicate (D = 2 BRDT(2) Kd (nM) = 20000	GCNSL2	Kd (NV) = >100000	GCNSL2 K# (n/0 + >103000	PBRM1(2) K6 (N0) = >100000	PBRM1(2) K6 (M) = >100000
34-3 34-4 14-3 001	1 100 19802	42 328 240 100 100 100 100 100 100 100 100 100 1	2.564 344 357 357 301 1 105 0001	299 199 390 199 390 1 199 199 199 199 199 199 199 1	24-7 2+7 14-7 8 01	1 100 1000	447 547 347 347 347 347 347 347 347 3	14-7 5+0 3+1 110 110 110 110 110 110 110 1	1201 447 1544 2643 2645 2645 2645 2645 2645 2645 2645 2645 2645 2645 2645 2
1 1000 1 58 7 8 38 7 0 38 7 0 38 7 0 38 7 0 38 7 0 18 7 0 19 7 0 10 10 10 10 10 10 10 10 10 10 10 10 10 1	1 350 1000 - 400	442 442 344 344 344 344 344 344	BITCPT Ke (n.W) = >100000   1=7 • <td>BARPY3 Kall (MS) **** CODOS   3x1 **** ***** ****   5x1 ***** ***** *****   1x2 ****** ****** ******   3x1 ******** ************ ************************************</td> <td>54-7 44-7 3+7 2+7 1+7 800</td> <td>· *** ****</td> <td>4+7 5+7 5+7 9+7 9+7 9+7 1+7 1+7 1+7 1+7 1+7 1+7 1+7 1+7 1+7 1</td> <td>2557 </td> <td>24-7 9-7 9-1 9-1 9-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 9</td>	BARPY3 Kall (MS) **** CODOS   3x1 **** ***** ****   5x1 ***** ***** *****   1x2 ****** ****** ******   3x1 ******** ************ ************************************	54-7 44-7 3+7 2+7 1+7 800	· *** ****	4+7 5+7 5+7 9+7 9+7 9+7 1+7 1+7 1+7 1+7 1+7 1+7 1+7 1+7 1+7 1	2557 	24-7 9-7 9-1 9-1 9-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 1-1 9-1 9
TN40-03 SMARCAU	Replicate (D = 1 Kd (NV) = >100000	TN40-03 Replicate ID = 2 SMARCA2 Kd (n00 = >10000	TN40-03 Resiste ID = 1 SWARCA4 Ke (mil) = >500000	TN40-03 Register (D = 2 SMARCA4 Kd mM) =>100001					
1960 m 16.0 m 16.0 m	· · · · · · · · ·	1940 1940 1947 1947 1947 1947 1947 1948	31+3 31+3 901 1 100 10000	3+4 3+4 8-1 8-1 8-1 8-1 8-1 8-1 8-1 8-1 8-1 8-1					
TN40-03 TAP1(2)	Replicate ID = 1 Kd (rtN) = =100000	TN40-03 Repikate ID = 2 TAP 1(2) Kd (n00) + +1 00000	TA40-03 Replicate ID = 1 TA41L(2) Kd (nH) = >100000	TN45-03 Registrato (D = 2 TAP (L)2) Kd (mH) = = 100000					
0+5 2+8 2+8 801	1 100 1000	100 100 100 100 100 100 100 100		1.544 (14) (14) (14) (14) (14) (14) (14) (1					
TN40-03 TR0M24(B	Replicate ID = 1 romo.) Kd (rM) = >100000	TRIM24(Brons.) Kd (m0 = >10000	TRIM24(PHD,Brome.) Kd (rMI) = > 100000	TRisk24(PHD,Bromo.) Kd (mb) = 2 TRisk24(PHD,Bromo.) Kd (mb) = >100000					
44 34 34 30	**************************************	246 246 8 21 1 166 10665	2564 1664 164 164 164 164 164 164 164 1690	30.0 10.0					
TN40-03 TR0M331P	Replicate ID o 1 HD,Bromo.) Kd (HM) +	TN40-00 Replicato ID = 2 TRM03(PH0,Brene.) Kd (r/kb =	TN40-03 Residente ID = 1 WORN22 Kd (mH) = >100000	TNAB-03 Regilicate ID = 2 WDR9(2) Kd (MI) +>109101					
655 655 45 45 45 55 55 55 55 55 55 55 55 55 5	5-10000 *********************************	*106000 8+0 5+0 3+0 3+0 3+0 3+0 3+0 3+0 3+0 3+0 3+0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1140 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	144 942 947 947 957 957 1 100 9000					

**Supplementary Figure 2.** BROMOscan binding curves for compound 1. Binding to each bromodomain has been performed in duplicate. Bromodomain tested is indicated in each graph together with calculated  $K_d$ . Compound 1 is named TN40-03 in the graphs (internal library name as communicated to Eurofins).



Supplementary Figure 3. Western blot quantification.  $\beta$ III-tubulin protein level relative to the untreated cells and normalized using the stain free method. The bars represent the mean  $\pm$  SD of 3 independent experiments. Cells were treated for 48 hours.



Supplementary Figure 4. Western blot quantification.  $\beta$ III-tubulin protein level relative to the untreated cells and normalized using the stain free method. The bars represent the mean  $\pm$  SD of 4 independent experiments. Cells were treated for 72 hours.



Supplementary Figure 5: Effect of treatment on extrusions length. Huh7 extrusions were measured using the Incucyte® S3 (Sartorious) software (NeuroTrack analysis) after 48h treatment with the indicated compounds. Each point represents the mean  $\pm$  SD of 3 independent experiments.



**Supplementary Figure 6: Cellular target engagement by NanoBRET**. Dose-response curves for compound 5 (black, left) and control compound IBET-151 (blue, right). Compound 5 is named TN40-41 in the graph (internal library name as communicated to Reaction Biology). Experiments were conducted in duplicate.



BRPF1	MQLTPFLILLRKTLEQLQEKDTGNIFSEPVPLSEVPDYLDHIKKPMDFFTMKQNLEAYRYLNFDDFEEDFNLIVSNCLKY
BRPF2_BRD1	RLTPLTVLLRSVLDQLQDKDPARIFAQPVSLKEVPDYLDHIKHPMDFATMRKRLEAQGYKNLHEFEEDFDLIIDNCMKY
BRPF3	LMPFNVLLRTTLDLLQEKDPAHIFAEPVNLSEVPDYLEFISKPMDFSTMRRKLESHLYRTLEEFEEDFNLIVTNCMKY
	* *: :*****: **:*****::** *.*******:.*.:***** **::.*** * .:.:******: **:**
BRPF1	NAKDTIFYRAAVRLREQGGAVLRQARRQAEKM
BRPF2_BRD1_	NARDTVFYRAAVRLRDQGGVVLRQARREVDSIGLEEASGM
BRPF3	NAKDTIFHRAAVRLRDLGGAILRHARRQAENIG
	** ** * ********* ** *** *** ***

**Supplementary Figure 7: Structural comparison of BRPF family members.** Pro658 in BRPF1 (green) allows for a better accommodation of compound 1 with respect to the corresponding amino acids Ser in BRPF2 (cyan) and Asn in BRPF3 (magenta). The aminoacidic substitution is indicated with a green star in the sequence alignment. All other amino acids interacting with the inhibitor are conserved.



**Supplementary Figure 8: Structural comparison of BET family members.** Gln85 in BRD4 (purple) allows for a better interaction of compound 1 with respect to the corresponding amino acids Arg in BRDT (pink). The aminoacidic substitution is indicated with a red star in the sequence alignment. All other amino acids interacting with the inhibitor are conserved (green stars).



**Supplementary Figure 9:**  $F_0$ - $F_c$  polder OMIT maps contoured at 3  $\sigma$  for: a) cmp 1 bound to BRPF1, b) cmp 1 bound to BRD4, c) cmp 2 bound to BRPF1, d) cmp 2 bound to BRD4, e) cmp 3 bound to BRPF1, f) cmp 4 bound to BRD4 and g) cmp 5 bound to BRD4.