

# Supporting Information

## CRCM5484: A BET- BDII Selective Compound With Differential Anti-Leukemic Drug Modulation

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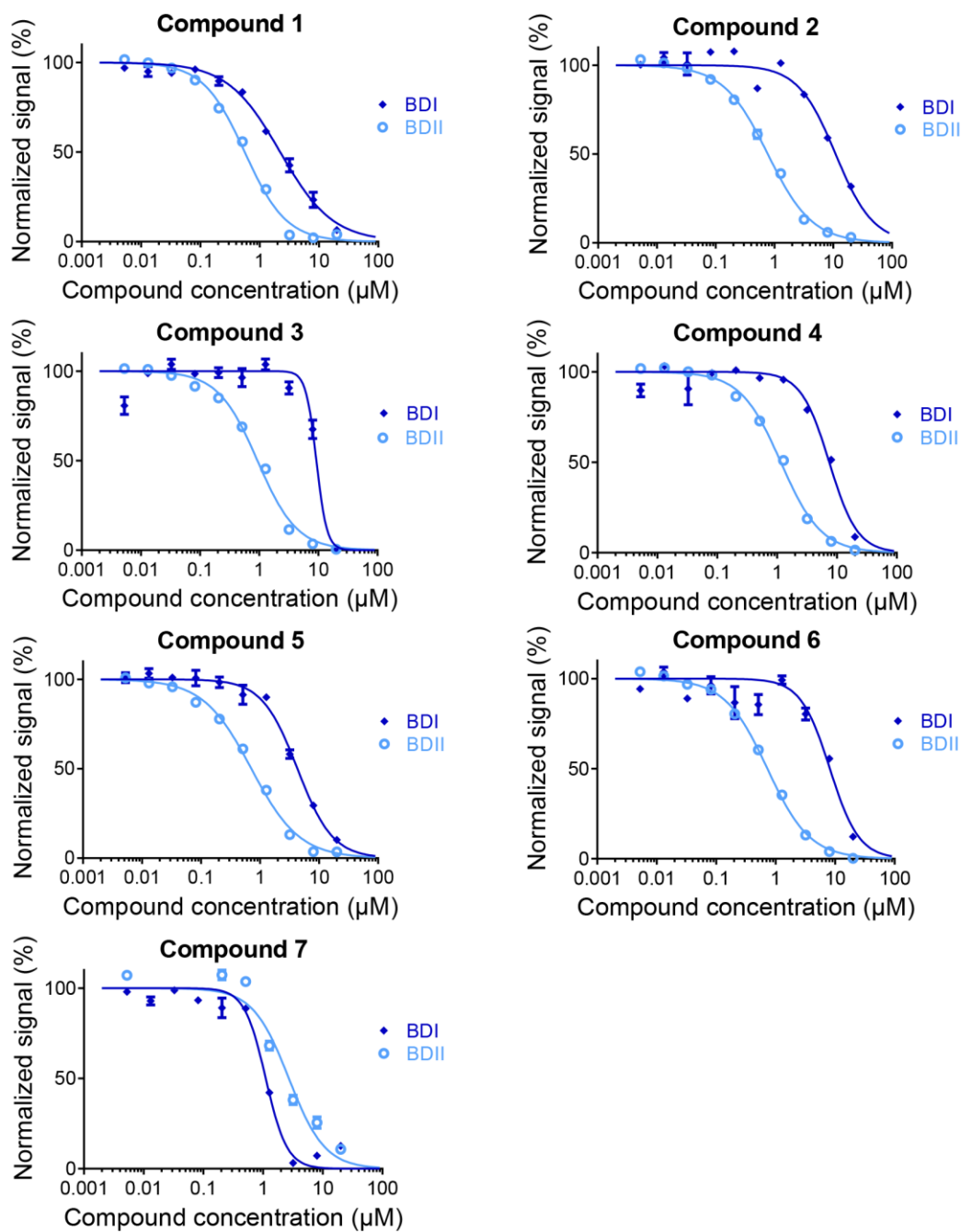
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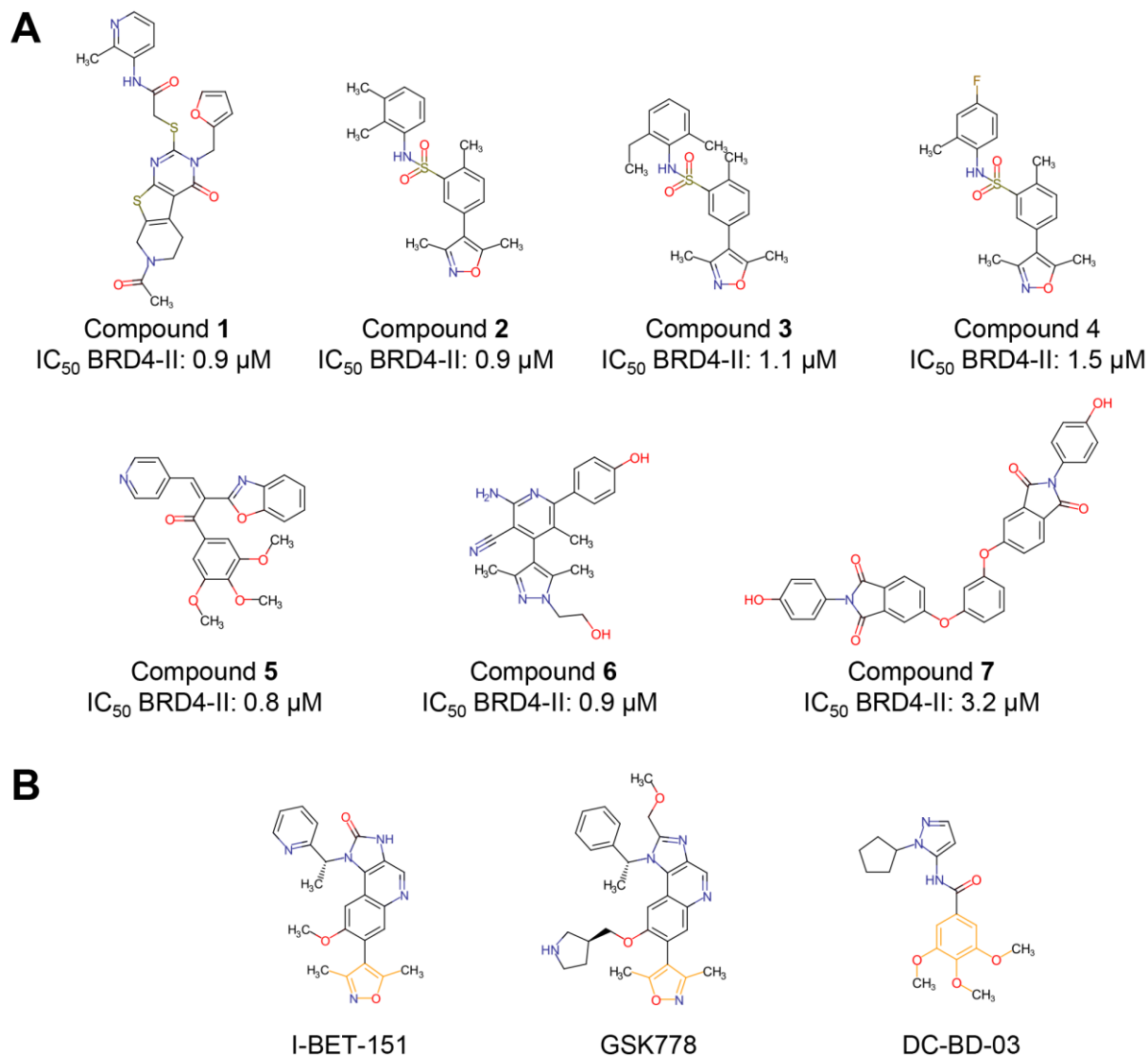
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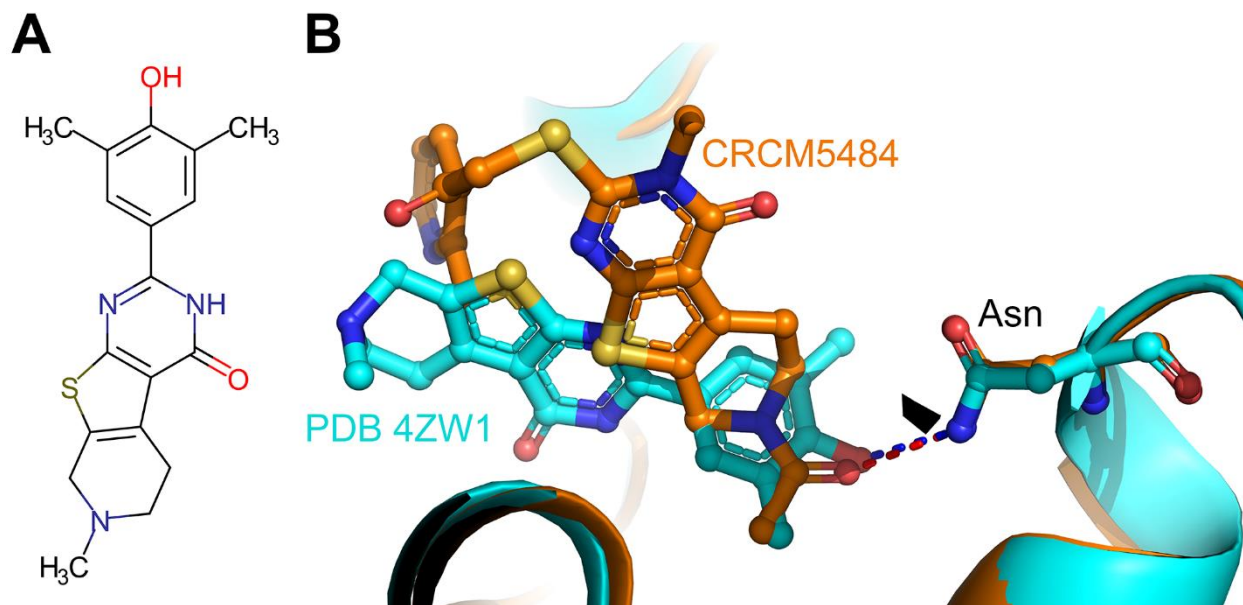
# Supplementary information



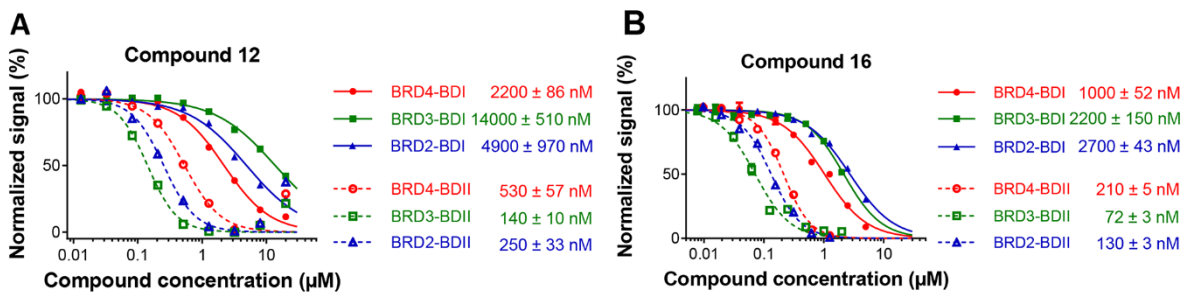
**Supplementary Figure S1.** HTRF evaluation of the FR-PPICChem screening hits. HTRF assay on the BDI (blue lines) and the BDII (cyan lines) domain of BRD3.



**Figure S2. Chemical structures.** (A) Chemical structure of the seven FR-PPICChem screening hits. (B) Compounds already described with a dimethyl isoxazole or a trimethoxyphenyl core (both colored in orange).



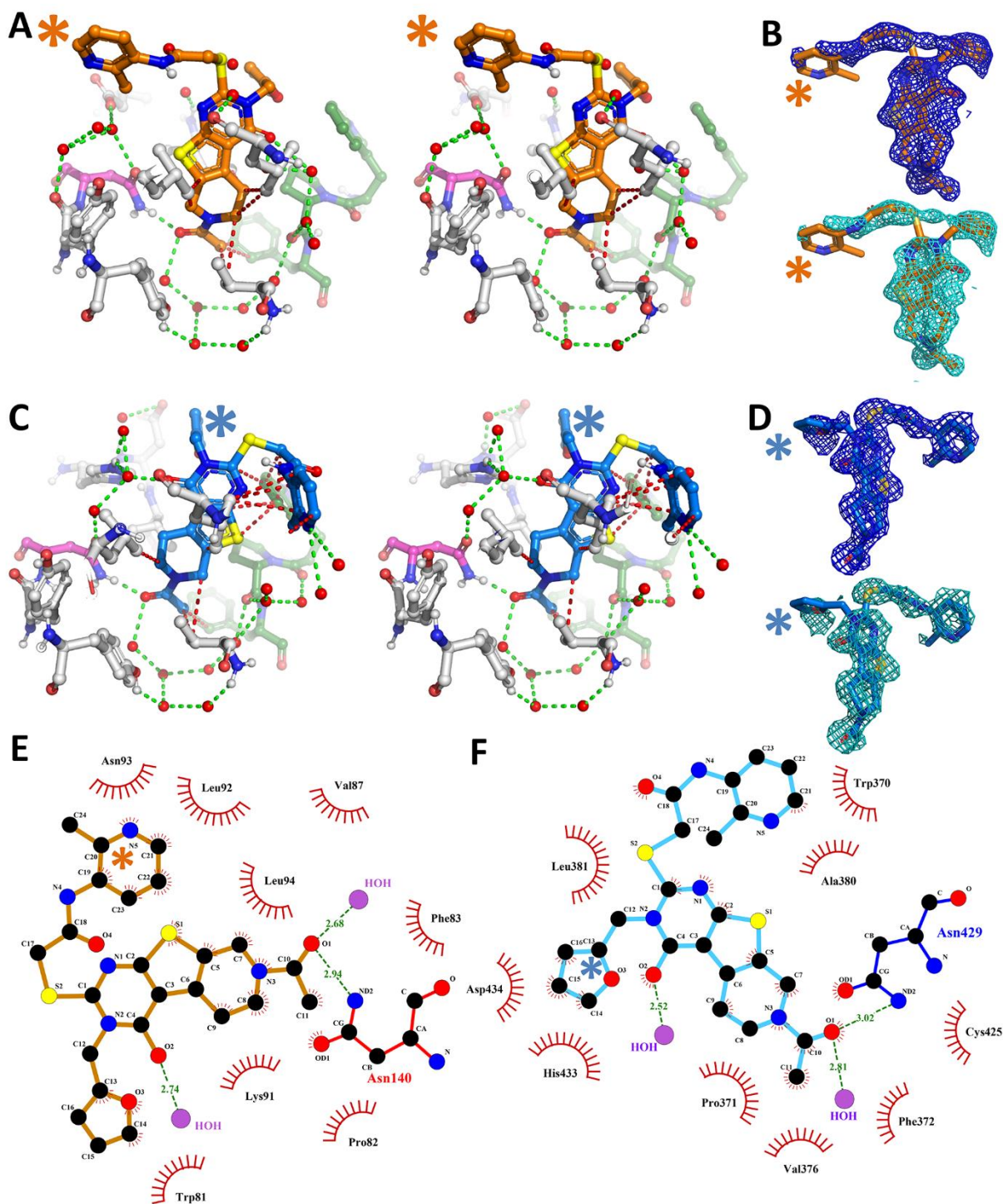
**Figure S3. Binding mode comparison between CRCM5484 and 4ZW1 (PDB ID).** (A) Chemical structure of the 4ZW1 compound. (B) Superposition of the co-crystal structure of CRCM5484/BRD2-BDII (orange) (PDB: 7Q5O) and 4ZW1 (cyan). The hydrogen bond interactions are shown as red dotted lines for CRCM5484/BRD2-BDII and blue dotted lines for 4ZW1.



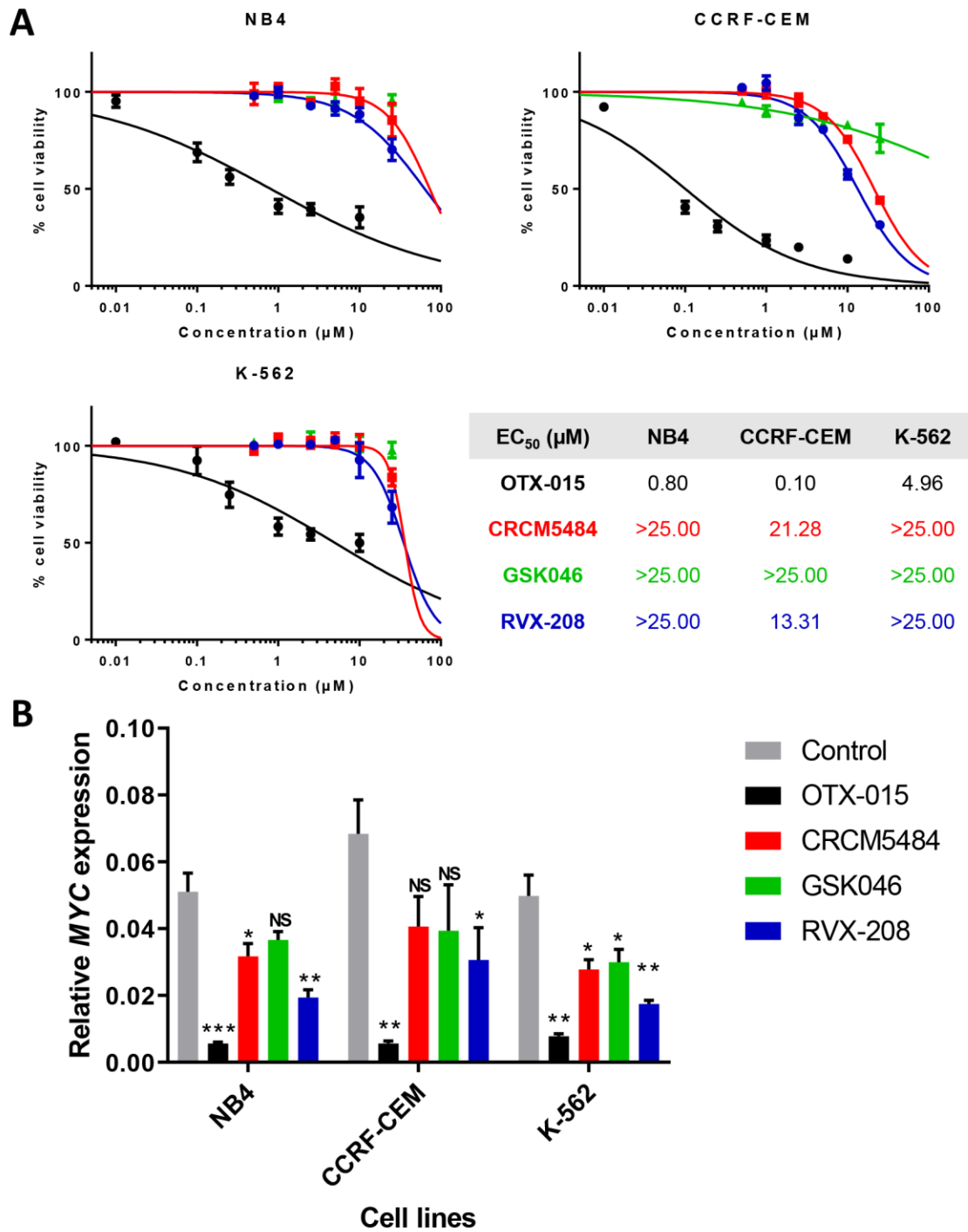
**Figure S4. Selectivity evaluation of compound 12 (A) and compound 16 (B).** HTRF selectivity assay on the BDI (solid lines) and BDII (dashed lines) domain of BRD2 (blue), BRD3 (green) and BRD4 (red).

Table S1. BROMOscan evaluation of CRCM5484.

Target Gene Symbol	CRCM5484 %Ctrl @ 1 $\mu$ M
ATAD2A	100
ATAD2B	90
BAZ2A	95
BAZ2B	100
BRD1	80
BRD2-I	92
BRD2-II	37
BRD3-I	100
BRD3-II	36
BRD4-I	100
BRD4-II	41
BRD7	74
BRD9	88
BRDT-I	96
BRDT-II	78
BRPF1	74
BRPF3	100
CECR2	54
CREBBP	85
EP300	78
FALZ	98
GCN5L2	99
PBRM1-II	100
PBRM1-V	84
PCAF	100
SMARCA2	89
SMARCA4	72
TAFI-II	76
TAF1L-II	98
TRIM24	87
TRIM33	52
WDR9	91



**Figure S5. Binding mode study of CRCM5484.** (A and C) Crossed-eyes stereo views of the co-crystal structures of CRCM5484 with BRD4-BDI (Orange) and BRD2-BDII (Blue) showing the hydrogen bond interactions as green dotted lines, vdW contacts as red dotted lines, the canonical asparagine binding residue as magenta, the WPF shelf as green and the rest of the domain as white balls and sticks. (B and D) 2Fo-Fc (blue) and omitted Fo-Fc map (cyan) of CRCM5484 in the BRD4-BDI (B) and BRD2-BDII (D) X-ray structures. (E and F) 2D Ligplot interaction of the CRCM5484/BRD4-BDI (E) and CRCM5484/BRD2-BDII (F) complexes. The picoline in BRD4-BDI (orange \*) and the furan moiety in BRD2-BDII (blue \*) showed poor electronic density and their positioning were driven by geometry and residual electron density during refinement.



**Figure S6. CRCM5484 compared to other BDII selective compounds.** (A) Cell viability assay on NB4, CCRF-CEM and K-562 leukemic cells performed after 72h incubation with OTX-015 (black), RVX-208 (blue), GSK046 (green) and CRCM5484 (red). (B) Quantification of MYC expression by RT-qPCR following 2h incubation with OTX-015 1µM (black), RVX-208 5µM (blue), GSK046 5µM (green) and CRCM5484 5µM (red). NS: for non-significant.



**Table S2. Maximal concentration used for the 78 evaluated compounds in the drug sensitivity and resistance profiling.** Dose response experiments were done from this condition with three additional 1 log serial dilutions. In these experiments, CRCM5484 was used at the EC<sub>40</sub> concentration measured for each cell type (12 μM for CCRF-CEM; 6 μM for U-937; 5.7 μM for MOLM-14; 0.6 μM for PDX without BMSC-CM; and 4.3 μM for PDX with BMSC-CM).

DSRP drugs	Dose-response maximal dose [μM]	DSRP drugs	Dose-response maximal dose [μM]	DSRP drugs	Dose-response maximal dose [μM]
Luminespid	0.02	OTX015	20	Etoposide	50
Panobinostat	0.02	Ponatinib	20	Everolimus	50
Venetoclax	0.02	Prima1	20	Gefitinib	50
Bortezomib	0.2	Quizartinib	20	GSK126	50
Carfilzomib	0.2	SGI-1027	20	GSK2879552	50
Idarubicin	0.2	Sunitinib	20	Idasanutlin	50
Navitoclax	0.2	Temsirolimus	20	Idelalisib	50
Birinapant	2	Volasertib	20	Lapatinib	50
Cladribine	2	Vorinostat	20	Masitinib	50
Daunorubicine	2	17AAG	50	Nelarabine	50
Rigosertib	2	AC-4-130	50	Nilotinib	50
Selinexor	2	AG-221	50	Nutlin 3	50
Trametinib	2	AGI-5198	50	Olaparid	50
Tretinoin	2	Alisertib	50	Palbociclib	50
Vincristine	2	Arsenic	50	Ruxolitinib	50
Afatinib	12	Azacitidine	50	Selaciclib	50
AraC	20	BKM120	50	SGI-110	50
AT9283	20	Bleomycine	50	SGI-1776	50
Clofarabine	20	Bosutinib	50	Sorafenib	50
DHAP	20	Crenolatinib	50	Tipifarnib	50
Erlotinib	20	Cyclopamine	50	Topotecan	50
Gilteritinib	20	Dasatinib	50	Vermurafenib	50
Imatinib	20	Decitabine	50	VX680	50
JQ1	20	Docetaxel	50	Asparaginase	100
Midostaurin	20	Entinostat	50	Hydroxychloroquine	100
MK2206	20	EPZ5676	50	Metformin	240000

**Table S3. Compounds SMILES, Provider, Provider ID and CAS number.**

#	SMILES	Provider	Provider ID	CAS number
1	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1c(C)ccc(Cl)c1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0348	1185149-44-3
2	<chem>Cc1noc(C)c1-c1cc(S(Nc2c(C)c(C)ccc2)(=O)=O)c(C)cc1</chem>	ChemDiv	F176-0440	1358716-54-7
3	<chem>CCc(cccc1C)c1NS(c1c(C)ccc(-c2c(C)onc2C)c1)(=O)=O</chem>	ChemDiv	F176-0527	1358396-26-5
4	<chem>Cc1noc(C)c1-c1cc(S(Nc(cc2)c(C)cc2F)(=O)=O)c(C)cc1</chem>	ChemDiv	F176-0574	1358583-65-9
5	<chem>CCOc1cc(C/C(/c2nc(cccc3)c3o2)=C/c2ccncc2)=O)cc(OC)c1OC</chem>	Vitas-M Lab	STL071346	2454175-99-4
6	<chem>Cc1nn(CCO)c(C)c1-c1c(C)c(nc(N)c1C#N)-c1ccc(O)cc1</chem>	Chembridge	47081723	1351112-74-7
7	<chem>Oc(cc1)ccc1N(C(c(cc1)c2cc1Oc1cccc(Oc(cc3)cc(C(N4c(cc5)ccc5O)=O)c3C4=O)c1)=O)C2=O</chem>	Vitas-M Lab	STK391617	295348-36-6
8	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1c(C)ccc(Cl)c1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0025	1217090-90-8
9	<chem>CC(N(CC1)Cc2c1c(C(N(CC1OCCC1)C(SCC(Nc1c(C)ccc(Cl)c1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0422	1189972-54-0
10	<chem>CCC(N(CC1)Cc2c1c(C(N(C1CCCC1)C(N1)=O)=O)c1s2)=O</chem>	ChemDiv	V014-6657	922356-59-0
11	<chem>CC(=O)N1CCc2c(C1)sc1nc(SCC(=O)Nc3cccc3)n(-c3cccc3)c(=O)c21</chem>	ChemDiv	G641-0030	1184977-70-5
12	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1cccc1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0355	1215718-74-3
13	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1cc(OC)ccc1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0323	1189643-36-4
14	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc(cccc1)c1Cl)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0325	1215508-33-0
15	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1cc(C)ccc1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0345	1185063-82-4
16	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc(cccc1)c1OC)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0327	1189940-97-3
17	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc(cc1)ccc1OC)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0342	1215387-46-4
18	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc(cc1)ccc1F)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0343	1217036-58-2
19	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1c(C)cccc1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0344	1216802-03-7
20	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1ccc(C)cc1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0326	1215481-48-3
21	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1cccc(Cl)c1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0346	1185044-25-0
22	<chem>CCOc(cccc1)c1NC(CSC(N1Cc2cccc2)=Nc(sc(C2)c3CCN2C(C)=O)c3C1=O)=O</chem>	ChemDiv	G641-0347	1216929-41-7
23	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc(ccc(F)c1)F)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0351	1216742-87-8
24	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc(cccc1)c1F)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0352	1185075-81-3
25	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1cc(F)c(C)cc1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0353	1189947-24-7
26	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1cc(SC)ccc1)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0357	1189692-93-0
27	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc(cc1)ccc1Cl)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0358	1216506-86-3
28	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc(cc(cc1)F)c1F)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0361	1189906-77-1
29	<chem>CC(c1cccc(NC(CSC(N2Cc3cccc3)=Nc(sc(C3)c4CCN3C(C)=O)c4C2=O)=O)c1)=O</chem>	ChemDiv	G641-0362	1189461-87-7
30	<chem>CC(c(cc1)ccc1NC(CSC(N1Cc2cccc2)=Nc(sc(C2)c3CCN2C(C)=O)c3C1=O)=O)=O</chem>	ChemDiv	G641-0366	1189944-52-2
31	<chem>CCOc(cc1)ccc1NC(CSC(N1Cc2cccc2)=Nc(sc(C2)c3CCN2C(C)=O)c3C1=O)=O</chem>	ChemDiv	G641-0368	1189420-68-5
32	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc(cc1)cc(Cl)c1F)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0373	1189437-15-7
33	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc(cc1)cc(F)c1F)=O)=N1)=O)c1s2)=O</chem>	ChemDiv	G641-0374	1189484-35-2
34	<chem>CC(N(CC1)Cc2c1c(C(N(Cc1cccc1)C(SCC(Nc1c(C)nc1)=O)=N1)=O)c1s2)=O</chem>	Synthesized	-	-

Figure S7. CRCM5484 HPLC (A) and LCMS (B) analysis.

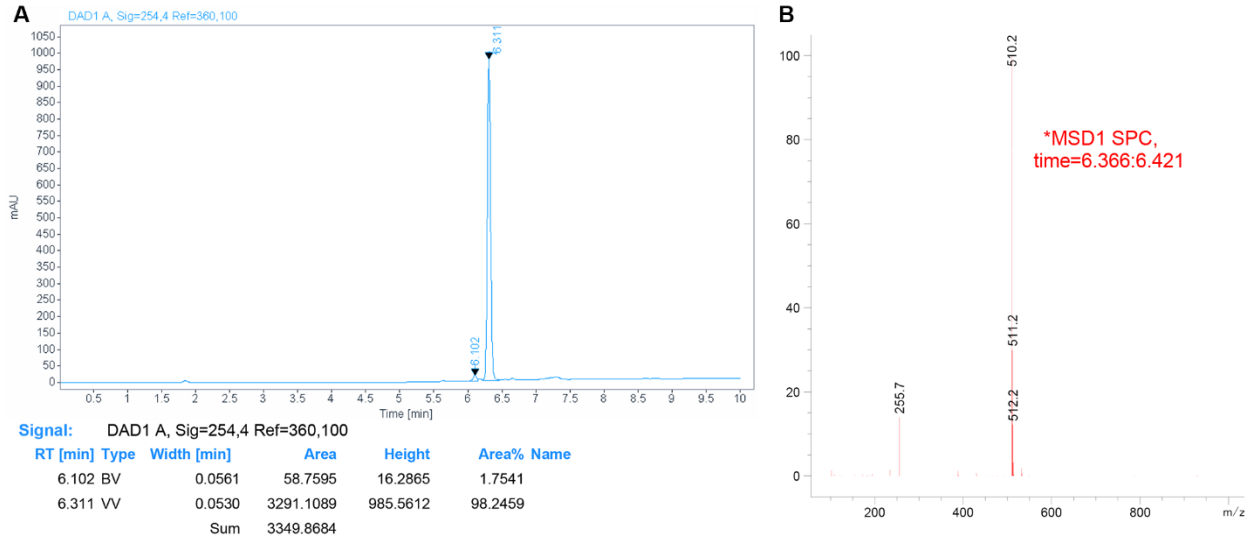


Table S4. HTRF experimental conditions.

HTRF reagents	Details and concentrations [nM]		Provider
BRDs	GST-BRD4-BDI	GST-BRD4-BDII	Internally produced
	5	10	
Donor	MAB Anti GST-EU cryptate		Cisbio
	0.5	0.5	
Acceptor	Streptavidin-d2 (BRD4-BDI)	Streptavidin-XL665 (BRD4-BDII)	Cisbio
	12.5	18.75	
Peptide	SGRG-K(Ac)-GG-K(Ac)-GLG-K(Ac)-GGAK(Ac)-RHRKVGG-K(Biotin) >95% Purity		Genicbio
	15	100	

**Table S5. X-ray data collection and refinement statistics.**

	CRCM5484/BRD4-BDI		CRCM5484/BRD2-BDII	
PDB ID	7Q3F		7Q5O	
<b>Crystal parameters</b>				
Space group	P 21 21 21		P 21 21 2	
Unit-cell dimension				
a, b, c (Å)	43.21	48.72 61.58	52.43	72.49 32.04
$\alpha$ , $\beta$ , $\gamma$ (°)	90 90 90		90 90 90	
<b>Data collection</b>				
Beamline	ESRF - ID30A-1		ESRF - ID30A-1	
Total reflections	148548 (14126)		75416 (6970)	
Unique reflection	40010 (3926)		19190 (1857)	
Completeness (%)	98.07 (88.51)		98.26 (96.43)	
Mean I/ $\sigma$ (I)	8.6 (0.8)		4.6 (1.5)	
R-meas (%)	50 (180)		17 (107)	
CC1/2	99.8 (23.3)		95.5 (4.4)	
<b>Structure Refinement</b>				
Resolution range (Å)	38.207 - 1.210 (1.253 - 1.21)		29.82 - 1.52 (1.574 - 1.52)	
Reflections used in refinement	39573 (3521)		19121 (1801)	
Reflections used for R-free	1969 (185)		876 (76)	
R-work	0.2056 (0.4408)		0.2162 (0.3395)	
R-free	0.2242 (0.4134)		0.2464 (0.2990)	
Number of non-hydrogen atoms	1246		1146	
Macromolecules	1068		940	
Ligand	35		47	
Solvent	143		159	
Average B-factor	28.1		14.90	
Macromolecules	26.4		12.40	
Ligand	48.1		28.00	
Solvent	35.8		25.60	
Ramachandran plot (%)				
Favored regions	99.19		99.07	
Allowed regions	0.81		0.93	
Outlier regions	0.00		0	
RMS deviation				
Bond (Å)	0.0102		0.0024	
Angle (°)	1.18		0.60	