

Supplementary figures and tables for:

Discovery of CRBN-dependent WEE1 molecular glue degraders from a multicomponent combinatorial library

Authors:

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Supplementary Figures

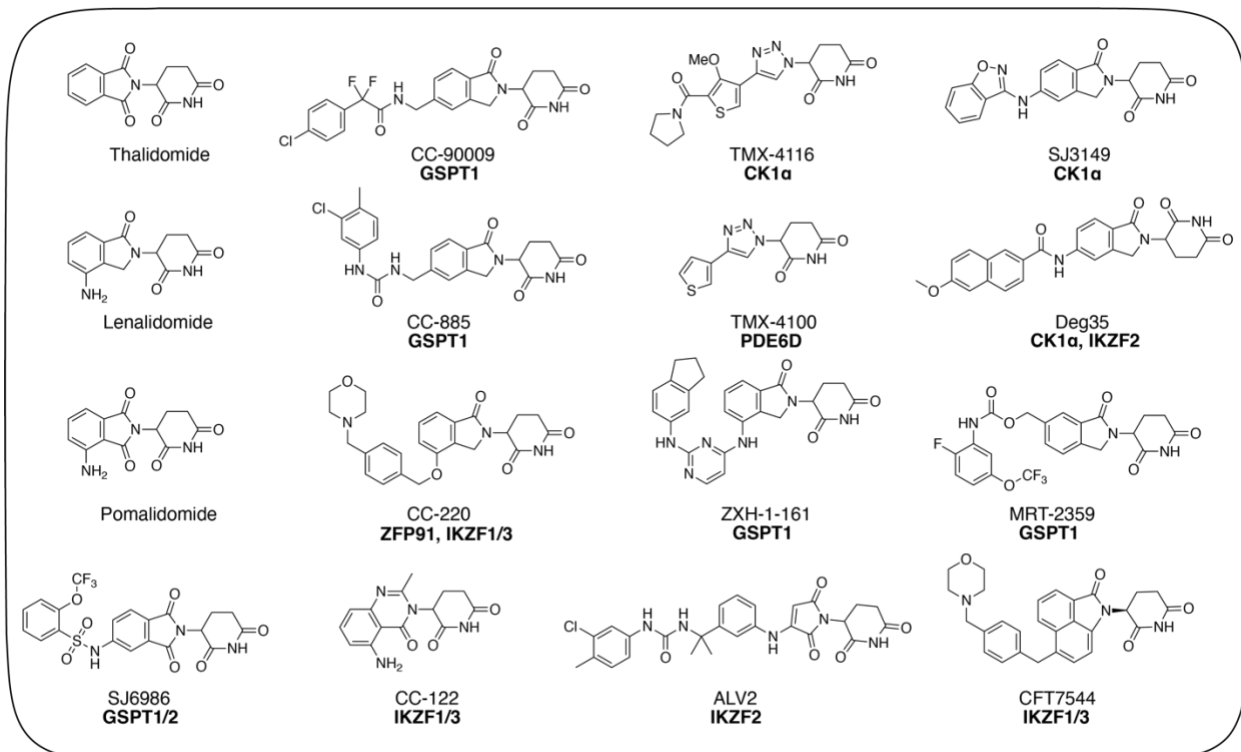


Figure S1. Previously reported CRBN molecular glue degraders and their primary targets.

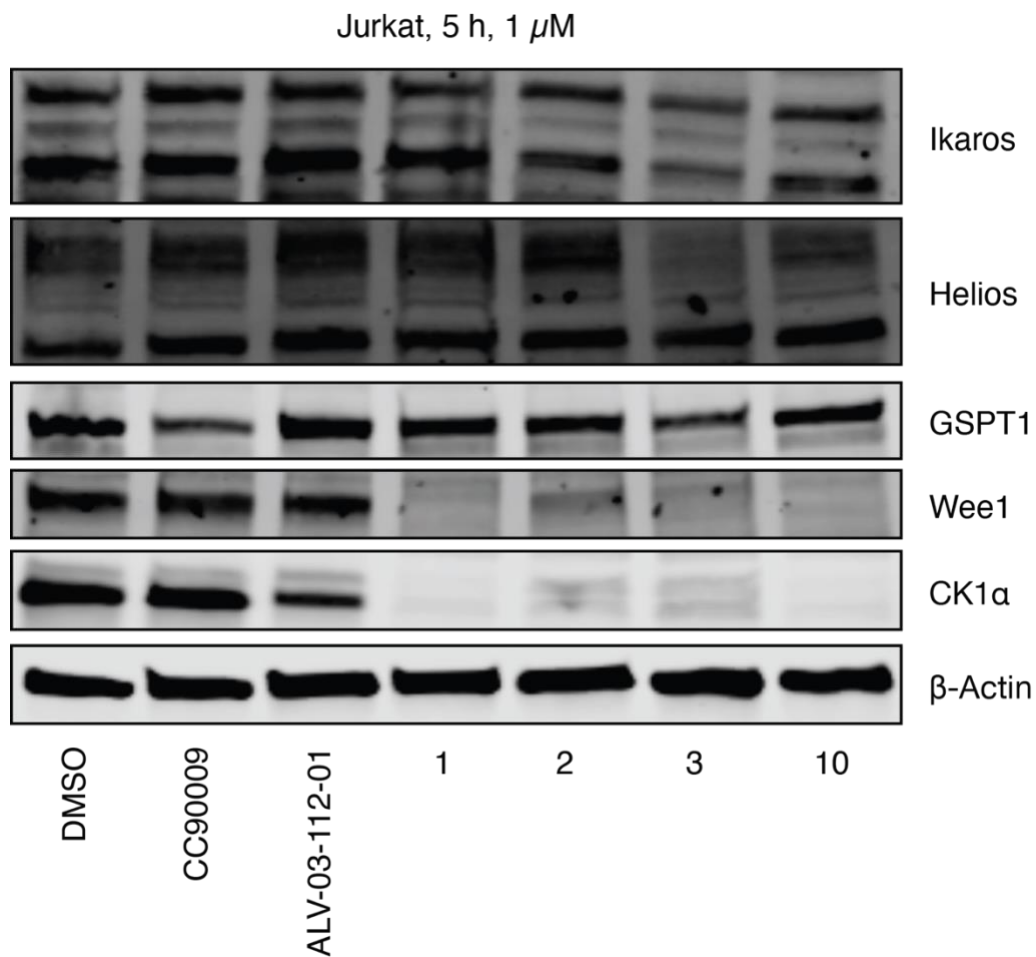


Figure S2. Western blot showing degradation effects of compounds **1-3** and **10** on the known CRBN neo-substrates. Jurkat cells were incubated with 1 μ M of HRZ-1 scaffold hits, Helios degrader ALV-2, or GSPT1 degrader CC-90009 for 5 hours.

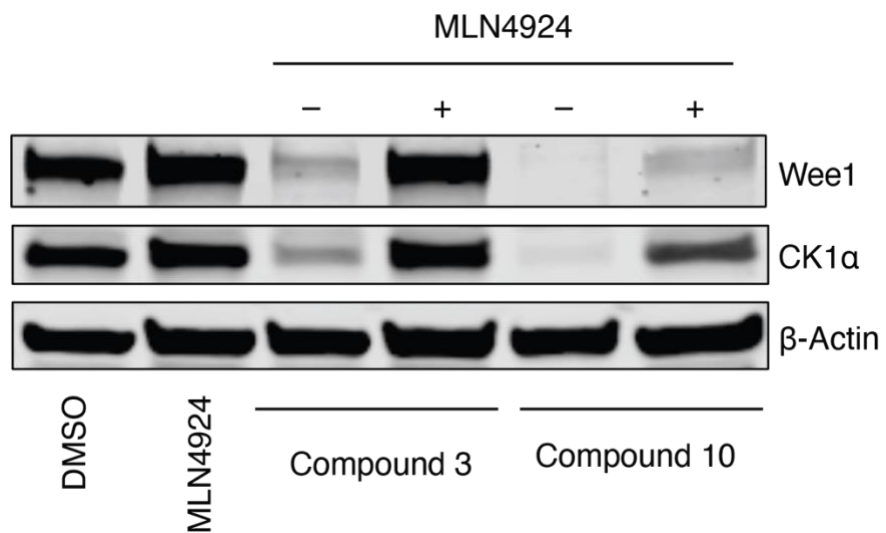


Figure S3. Western blot showing rescue of WEE1 and CK1 α degradation by compounds **3** and **10** under 1 hour 1 μ M pretreatment with MLN4924 that inhibits Cullin-dependent ubiquitination.

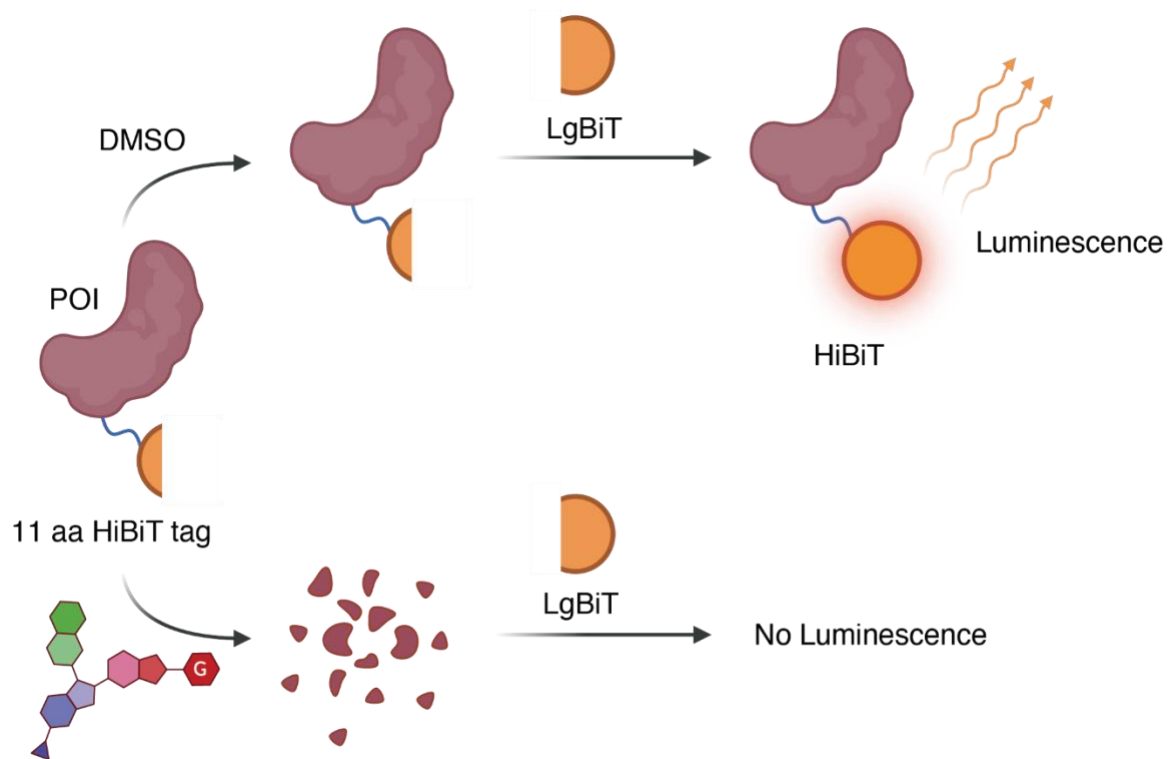


Figure S4. Cartoon depiction of the hit modification process. An N-terminal HiBiT tag was knocked into endogenous proteomics hits: WEE1 and CK1 α . The resulting HiBiT Jurkat cell lines were used to establish selectivity and potency profiles of the HRZ-1 scaffold derivatives. The obtained selective WEE1 degraders were then tested in a proteomics experiment profiling to establish their proteome-wide selectivity profile.

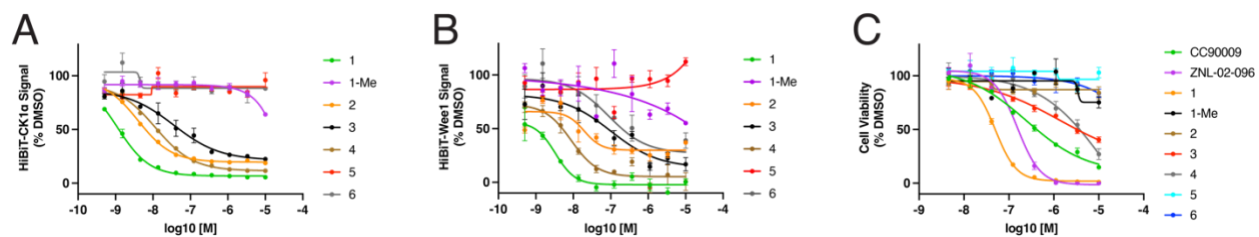


Figure S5. Expanded degradation and anti-proliferative activity data for compounds **1-6** in Table 1. (A, B) HiBiT-CK1 α /Wee1 degradation at a range of concentrations. (C) Anti-proliferative effect of compounds **1-6** at a range of concentrations.

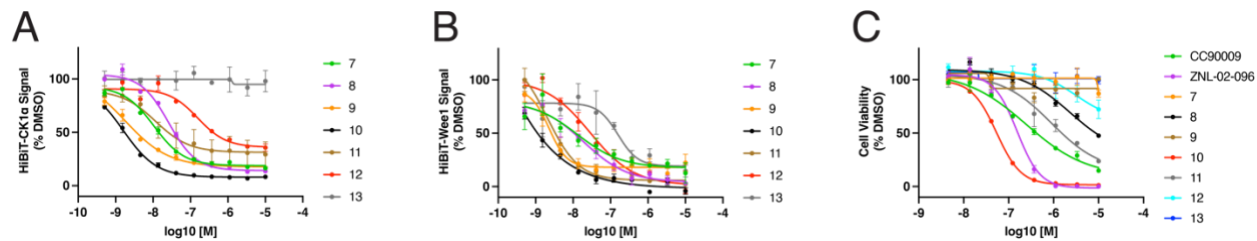


Figure S6. Expanded degradation and anti-proliferative activity data for compounds 1-6 in Table 2. (A, B) HiBiT-CK1 α /WEE1 degradation at a range of concentrations. (C) Anti-proliferative effect of compounds 7-13 at a range of concentrations.

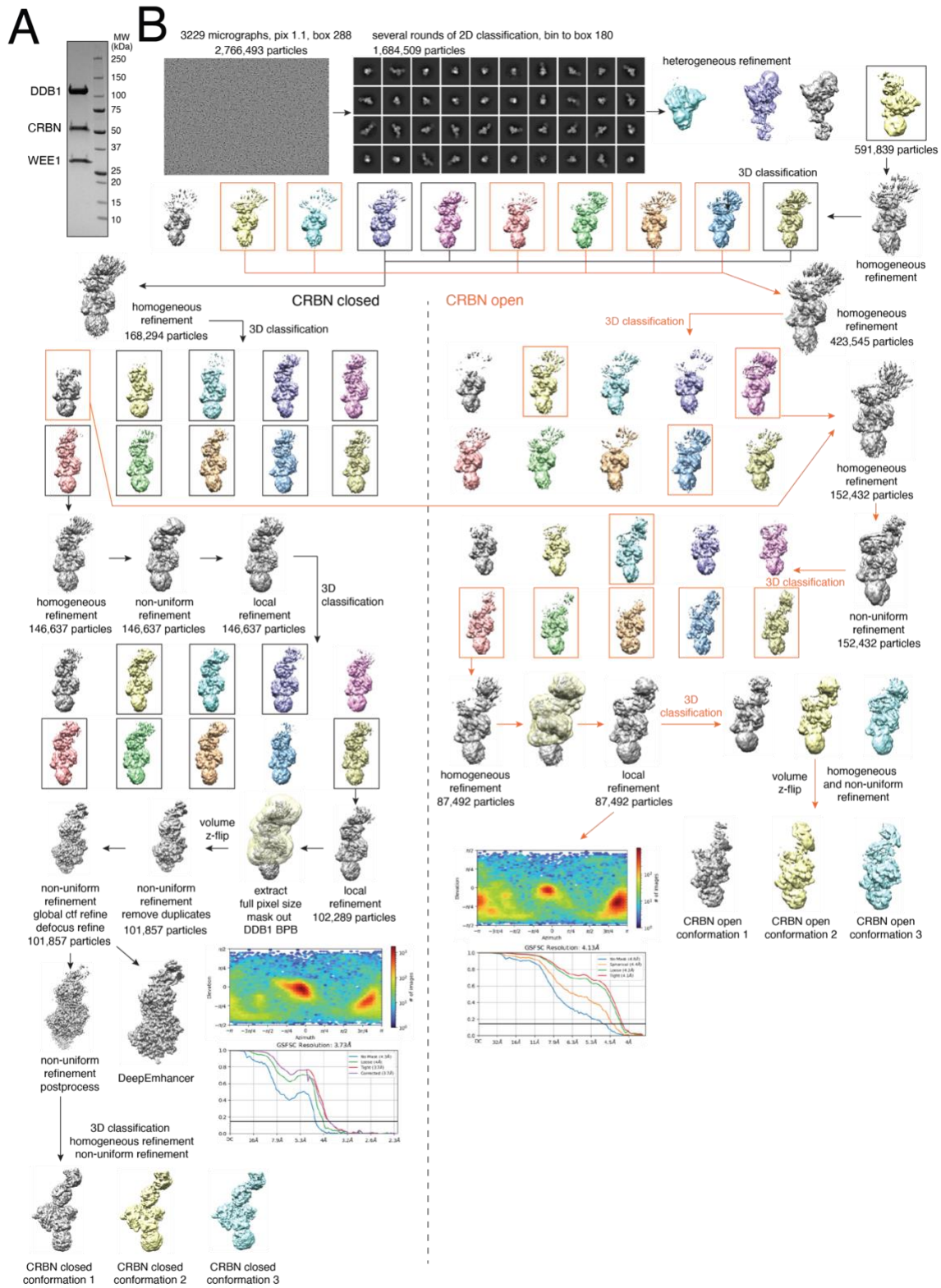


Figure S7. Cryo-EM sample preparation and data processing. (A) Coomassie stain of SDS-PAGE showing WEE1-compound 10-CRBN-DDB1 complex. (B) Cryo-EM processing schematic

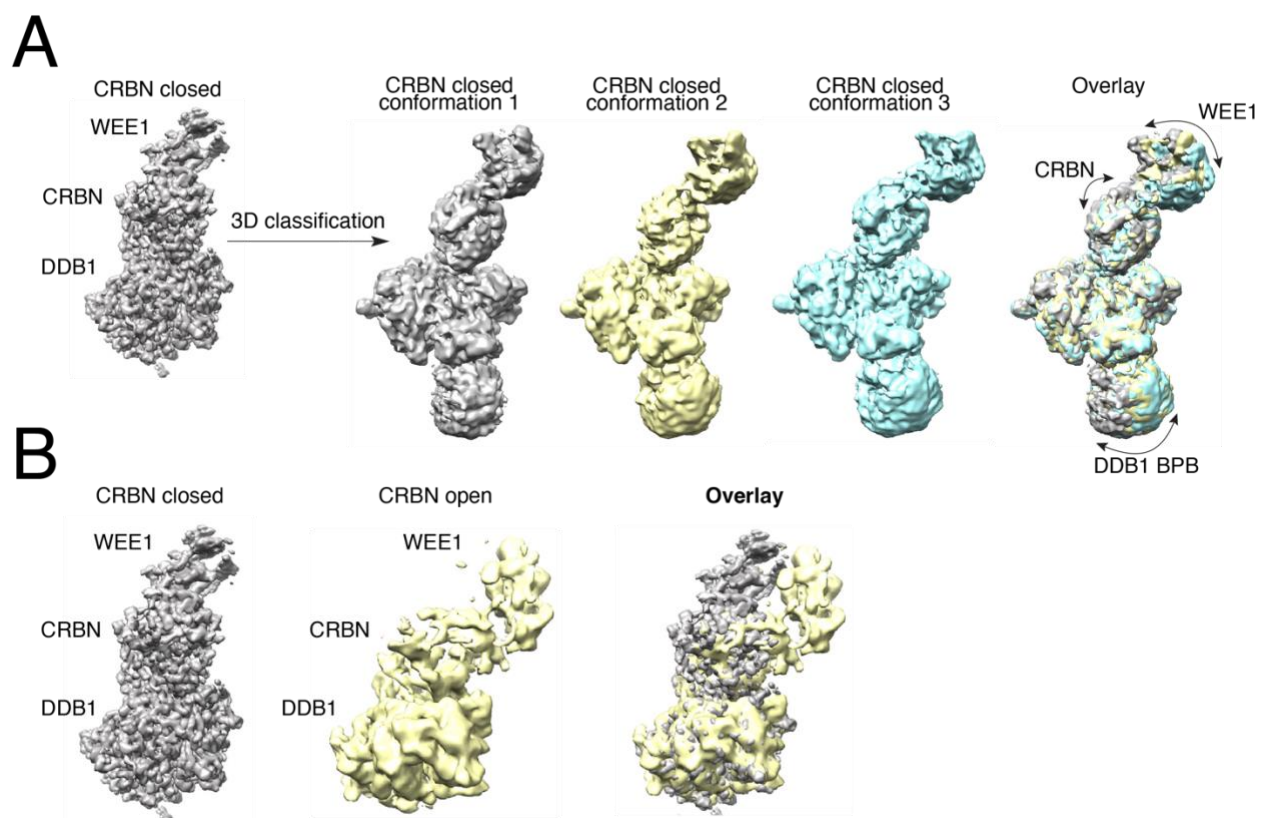


Figure S8. Compound 10 brings CRBN and WEE1 in proximity without excessively rigidifying the ternary complex. Density maps of the ternary complex showing (A) high flexibility with the CRBN in closed confirmation and (B) recruitment of WEE1 by compound 10 to both closed and open CRBN confirmations.

WEE1 vs CK1 α (PDB ID: 8G66)
overlay aligned on CRBN

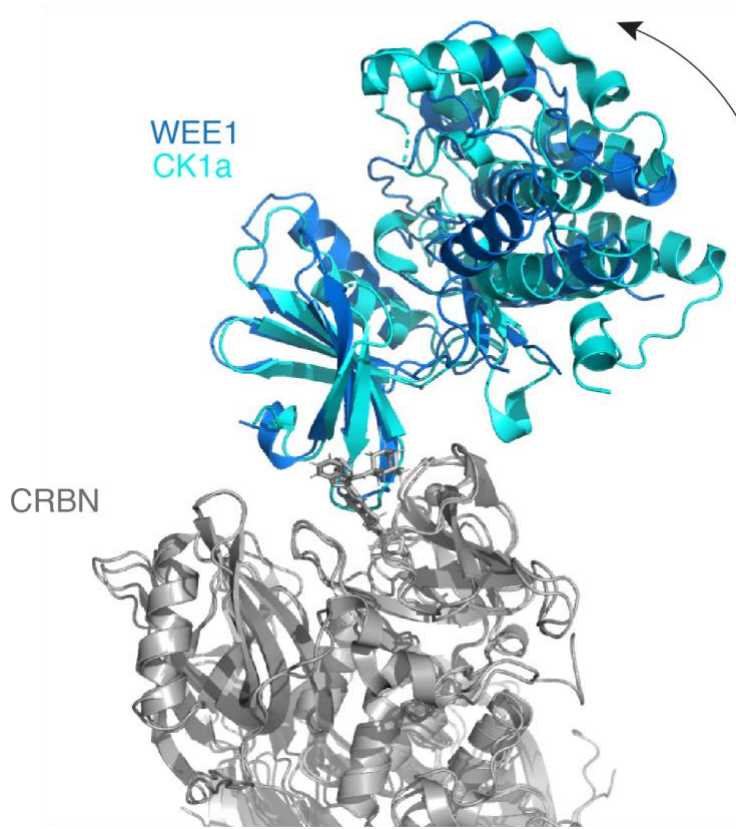


Figure 9. Overlay of the protein complex in Figure 4C with the previously reported DDB1-CRBN-SJ3149-CK1 α aligned on CRBN

Table S1. EC₅₀ values determined from the cellular viability experiments in Figure S5, S6, and S10.

Compound	Jurkat	SUDHL5	Molm14	NB-4	LoVo
1	49	15	570	400	20
1-Me	> 10000	> 10000	> 10000	> 10000	> 10000
2	> 10000	> 10000	> 10000	> 10000	> 10000
3	1700	140	46	21	40
4	> 10000	1600	2000	1000	420
5	> 10000	> 10000	> 10000	> 10000	> 10000
6	> 10000	> 10000	> 10000	> 10000	> 10000
7	> 10000	72	370	> 10000	> 10000
8	2300	104	730	1300	600
9	> 10000	72	> 10000	> 10000	> 10000
10	54	23	35	26	40
11	900	41	420	140	1000
12	> 10000	5800	> 10000	> 10000	> 10000
13	> 10000	> 10000	> 10000	> 10000	> 10000
CC9009	300	170	660	54	71
ZNL-02-096	150	84	220	210	190

Table S2. Maximum antiproliferative effect at 10 μ M values determined from the CTG cellular viability experiments in Figure S5, S6, and S10.

Compound	Jurkat	SUDHL5	Molm14	NB-4	LoVo
1	99	99	100	100	79
1-Me	25	22	-1	-24	9
2	16	77	47	0	46
3	60	76	92	98	82
4	73	97	59	85	69
5	-3	5	-5	-7	-10
6	16	48	15	-8	4
7	13	98	81	-19	47
8	52	89	93	77	54
9	-1	88	35	15	46
10	99	98	99	99	74
11	76	98	89	98	63
12	28	85	29	45	45
13	0	83	23	-6	20
CC9009	85	100	79	99	82
ZNL-02-096	100	84	100	100	83