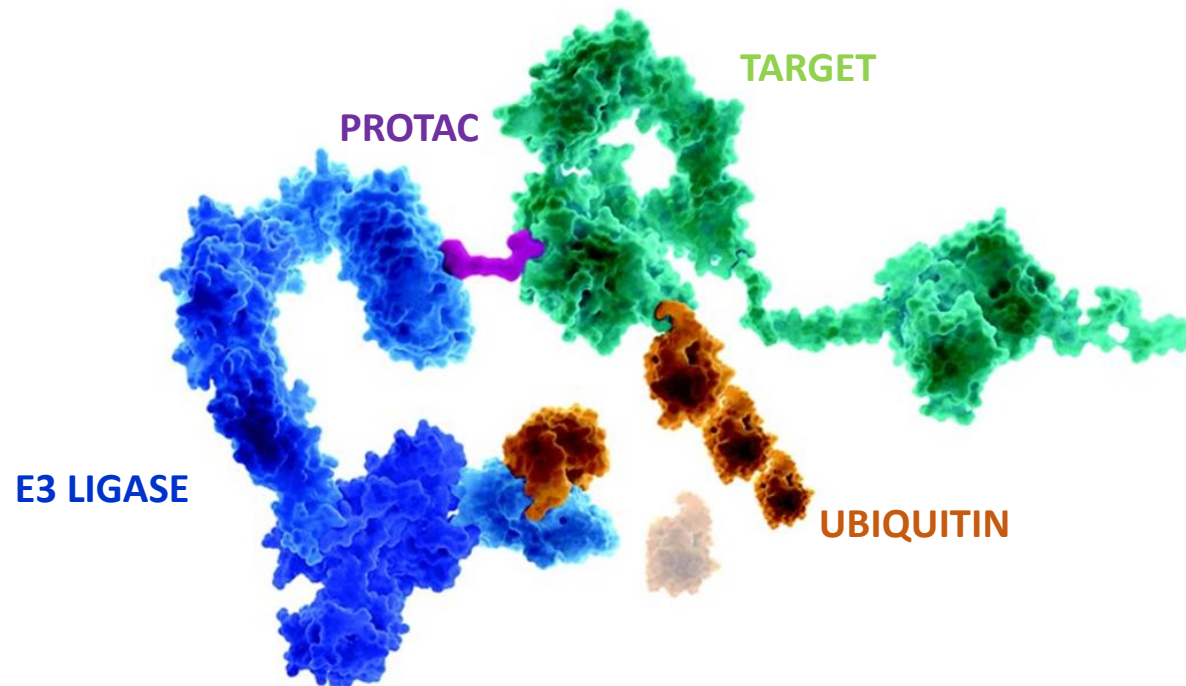
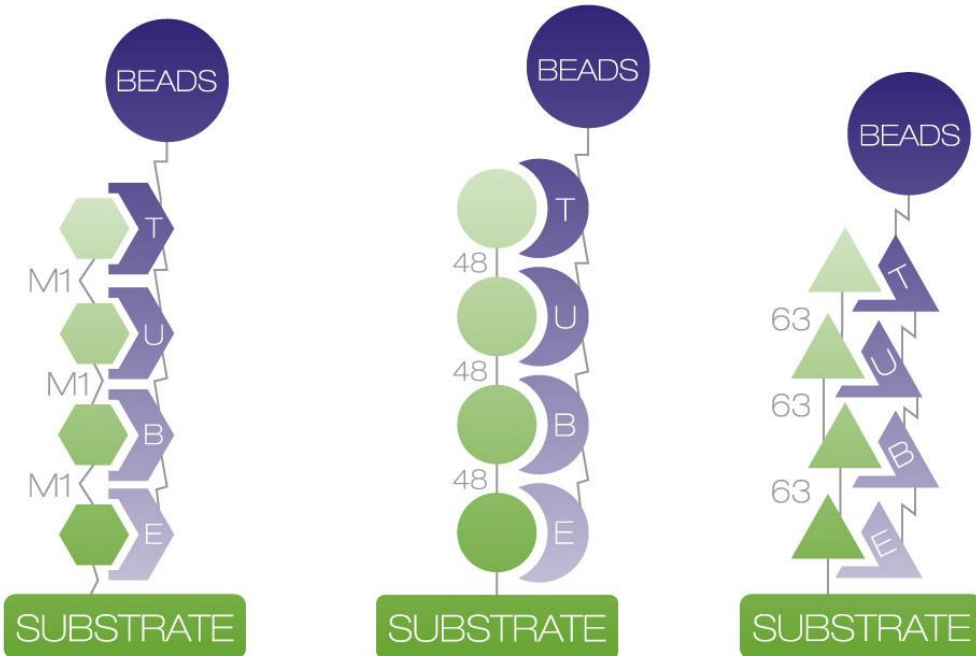


Accelerating PROTAC Drug Discovery: Relationship Between Ubiquitination and Degradation of Target Proteins



TANDEM_UBIQUITIN_BINDING_ENTITIES (TUBE_s)

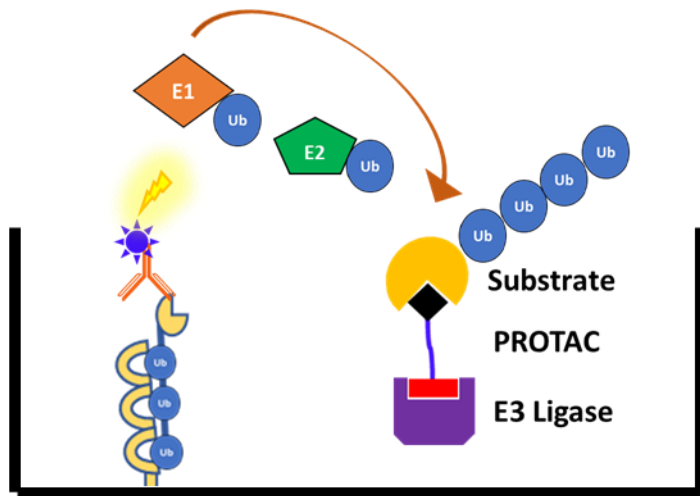
LifeSensors
www.lifesensors.com



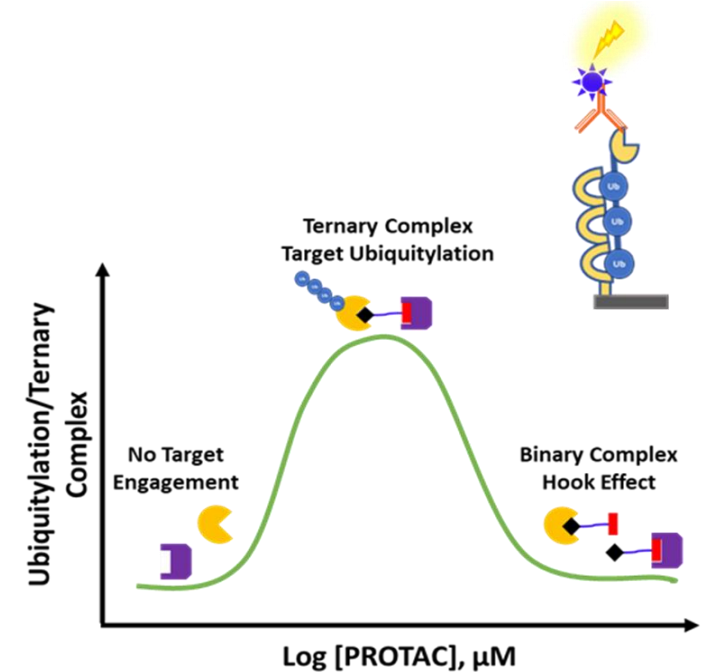
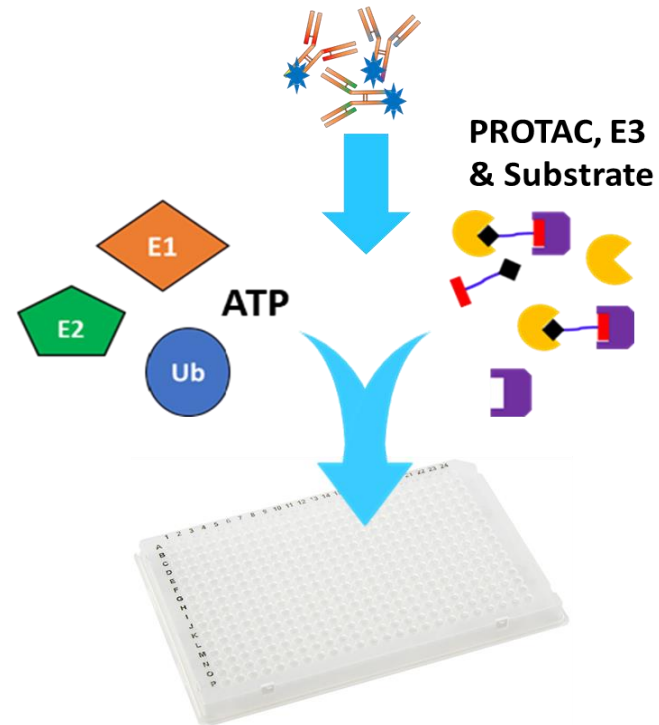
- Isolation of poly-ubiquitinated substrates from cell lysates
- Superior to antibodies, detection by Western blot
- [E3 ligase](#) and [DUB](#) assays
- *In situ* detection with fluorescence
- [Ubiquitin mass spec proteomics](#) bypassing SILAC

HTS-*in vitro* PROTAC Screening Platform

Efficient Large-Scale Screening Tool



TUBE Capture and Substrate Detection



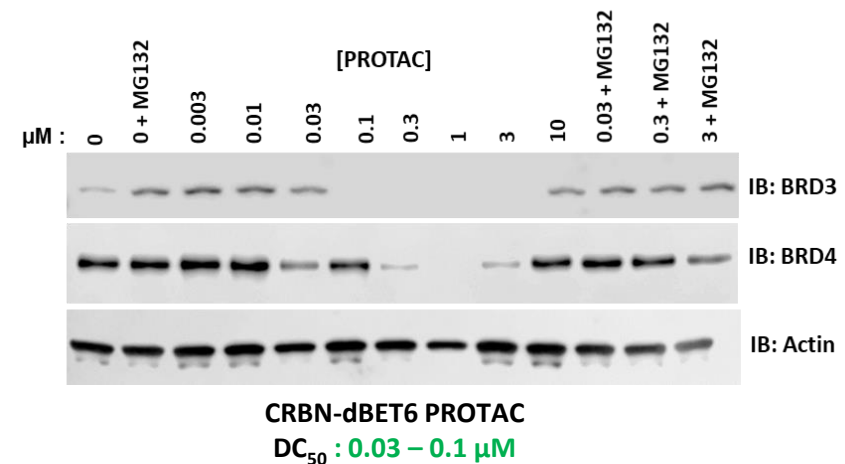
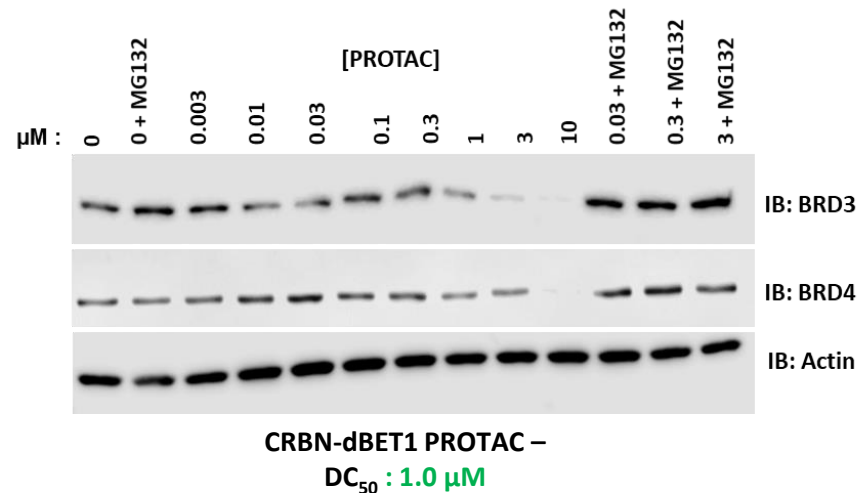
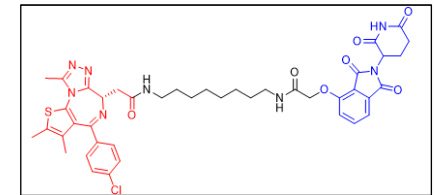
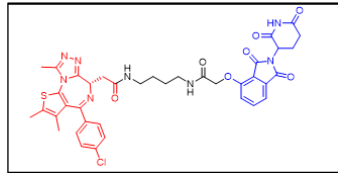
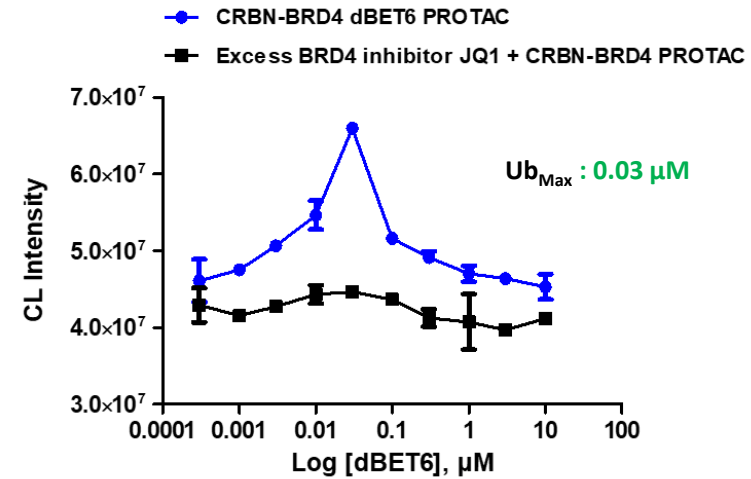
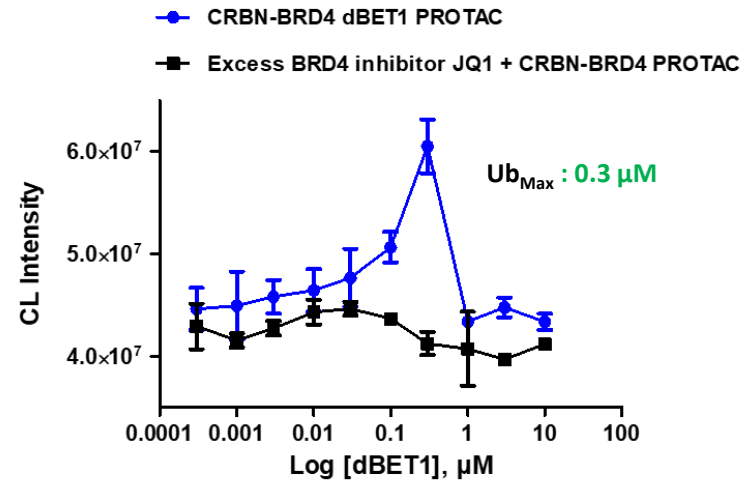
HTS-*in vitro* PROTAC Screening Platform

- How to differentiate multiple PROTAC variants in a HT fashion
- Rapid ubiquitination kinetics and dose response of **native** targets
- Guiding Med Chem to establish rapid SAR

“Ub_{Max}” A better way to measure potency of PROTACs

CRBN based Bromodomain PROTAC®

Effect of Linker Length on ubiquitination of BET proteins



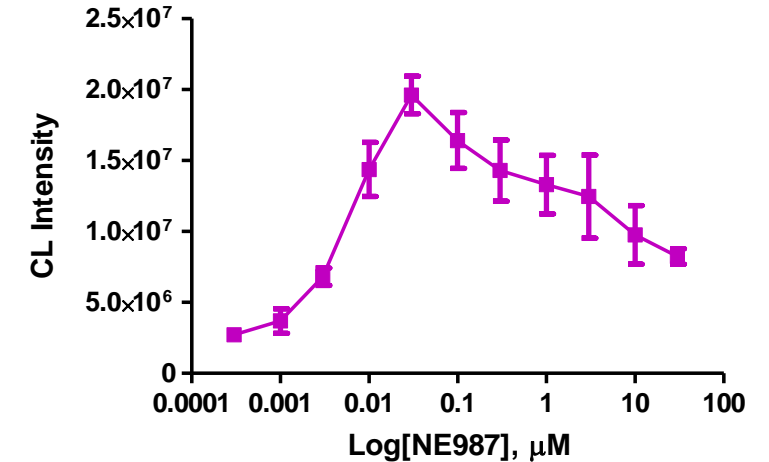
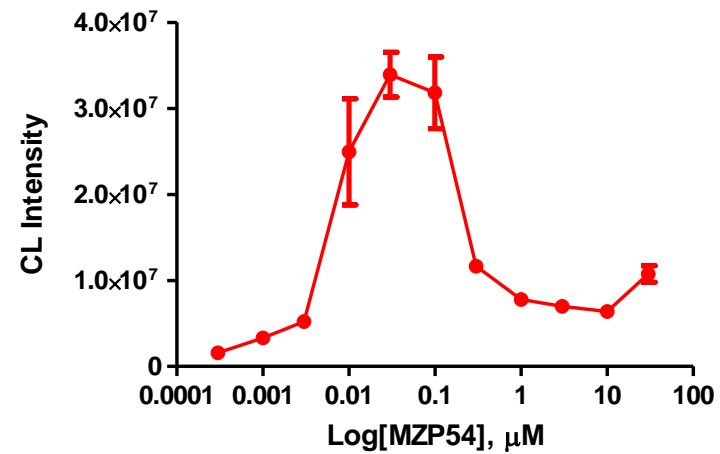
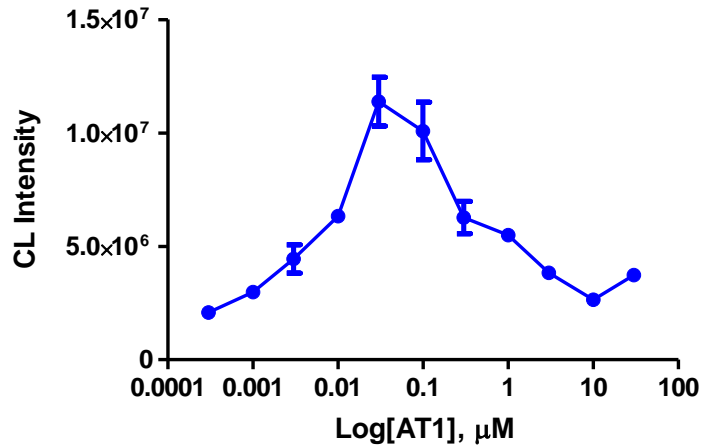
Summary – *In vitro* vs Cellular assays

PROTAC	<i>In vitro</i> vs Cellular degradation		<i>In vitro</i> vs Cellular degradation	
	Ub _{Max} (<i>In vitro</i>), μM	DC ₅₀ (Cell), μM	Hook effect (<i>In vitro</i>), μM	Hook effect (Cell), μM
Multi-Kinase PROTAC	0.1-0.3	N/A	3.0	1.0
dBET1	0.3	1.0	1.0	N/A
dBET6	0.03	0.03	3.0	3.0
BETd-24-6	0.03	0.1	3.0	10.0

Table . Comparison and correlation of Ub_{Max} and Hook effect from *In vitro* assays to cellular degradation assays.

VHL based Bromodomain PROTAC®

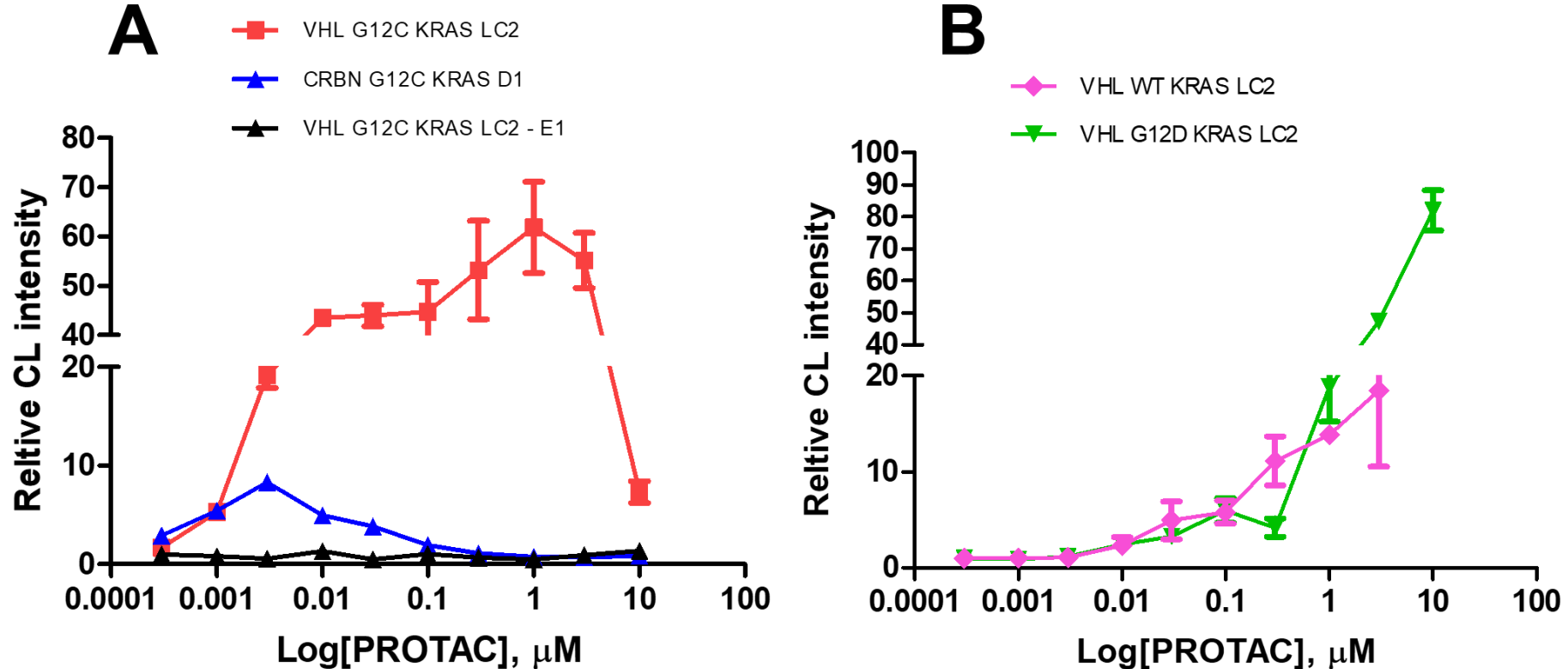
HTS In vitro Screening with BET proteins



PROTAC	<i>In vitro Ubiquitination vs Binding Affinity</i>		
	Ub_{Max} (<i>In vitro</i>), μM	K_d , μM	Hook effect (<i>In vitro</i>), μM
AT1	0.1-0.03 (3-fold Peak)	0.04	0.1
MZP54	0.03 (10-fold Peak)	0.004	0.3
NE987	0.03 (8-fold Peak)	0.004	0.1

PROTAC® mediated ubiquitination of KRAS G12C

HTS In vitro Screening

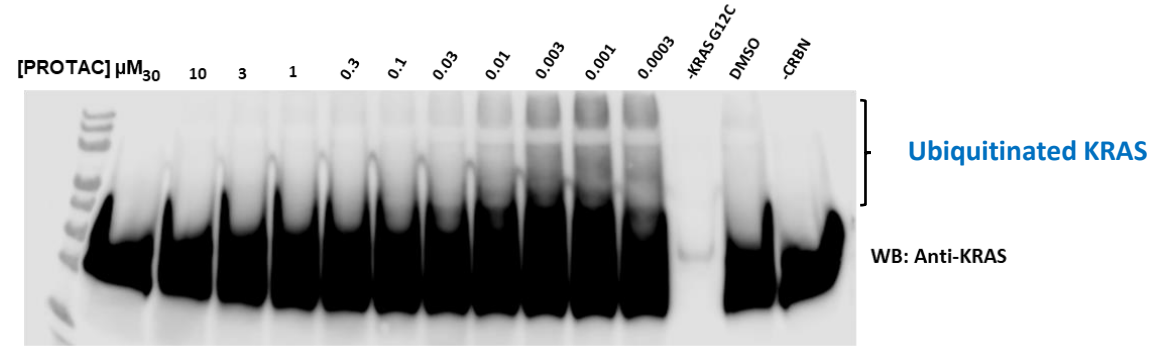


KRAS in vitro ubiquitination assays: recombinant G12C KRAS ubiquitination was monitored as function of cereblon/VHL PROTAC dose response (A) comparison of KRAS G12C ubiquitination using VHL and Cereblon PROTACs. (B) KRAS selectivity assays for G12D and WT using G12C VHL PROTACs.

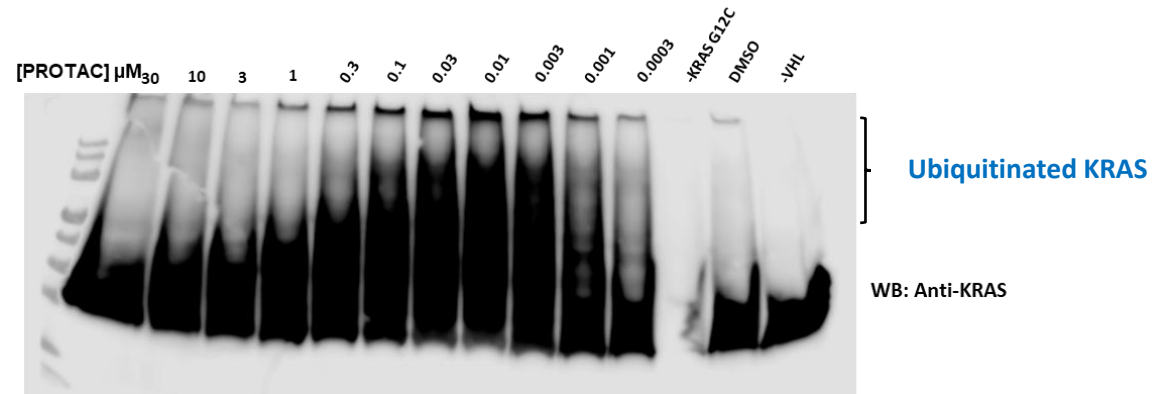
PROTAC® mediated ubiquitination of KRAS G12C

Gel based Assay

CRBN KRAS G12C
Degradar



VHL KRAS G12C
Degradar (LC2)



KRAS in vitro ubiquitination assays: Western blot based KRAS ubiquitination-validation to demonstrate PROTAC mediated ubiquitination.

Pathway to PROTAC Drug Discovery

