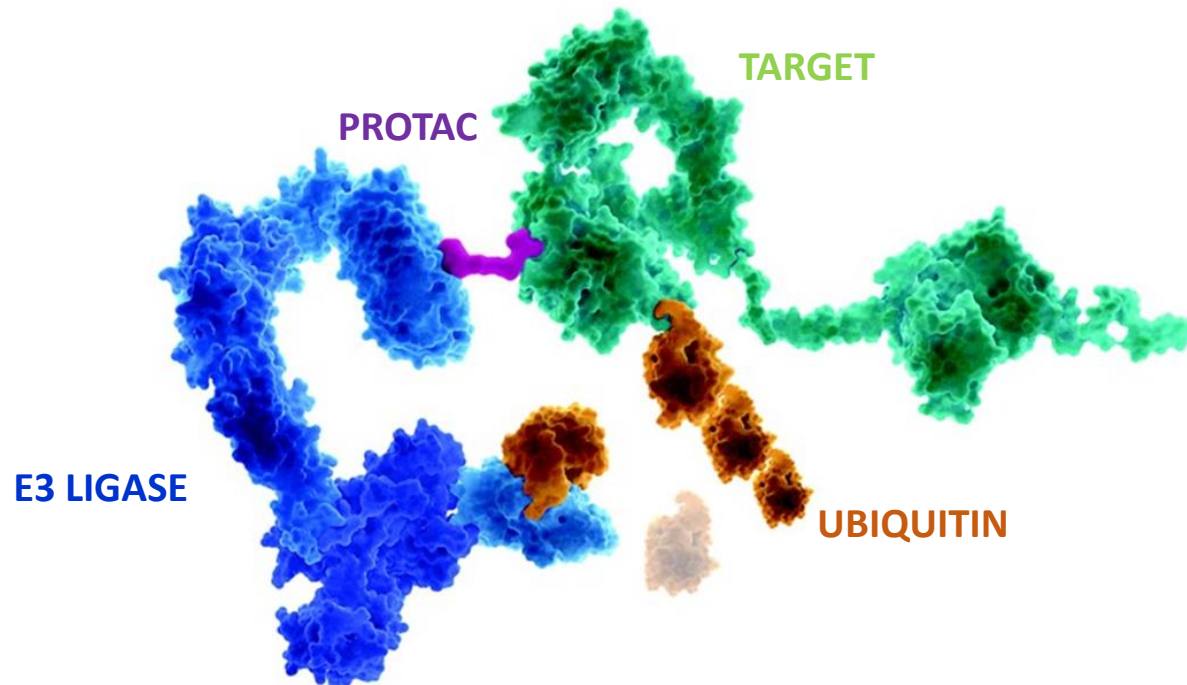


# PROTAC<sup>®</sup> Drugs: A Revolutionary Approach

Karteek Kadimisetty Ph. D  
Director, R&D



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Malvern PA 19355

Phone: 610-644-8845

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[www.lifesensors.com](http://www.lifesensors.com)

# LifeSensors Inc.

- Leading Biotech in PROTAC/UPS Drug Discovery and Diagnostic R & D
- ~500 Product lines, PROTAC assay plates, Ub ligases and DUBs, Affinity Reagents (TUBEs), Kits and SUMO Protein Expression Systems
- Drug Discovery, PROTAC/UPS in vitro and cell-based screening Services
- Profiling compounds against ubiquitin ligases and DUBs
- Custom Assay Development CRO Model
- Collaborative and Independent Research

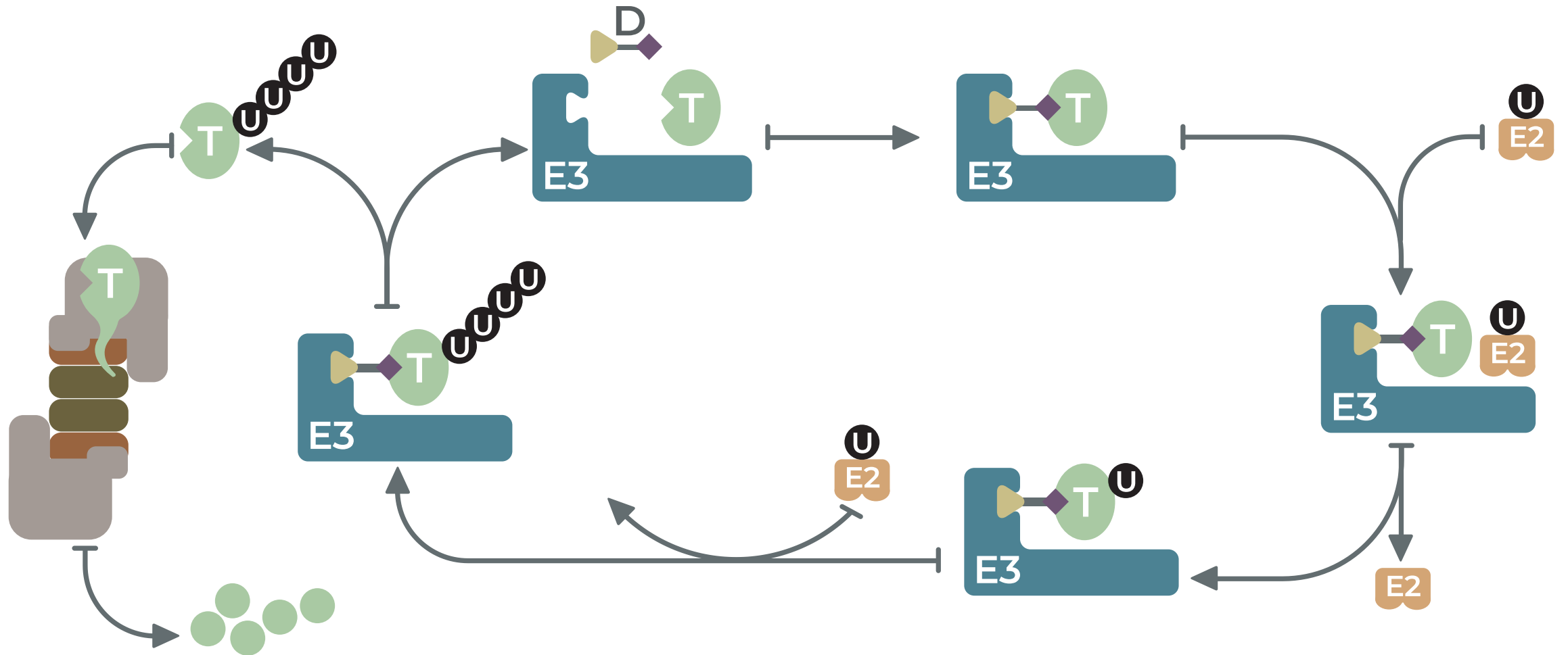
# Proteolysis-targeting Chimeric Molecules

## PROTACs

- Recruiting a Target Protein to an E3 ligase for ubiquitination and subsequent degradation
- Heterobifunctional molecule that recognizes an **E3 ubiquitin ligase** and a **Target Protein** and joined by a linker domain



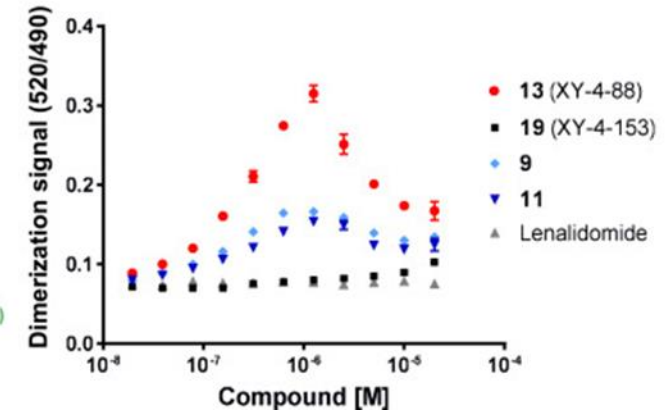
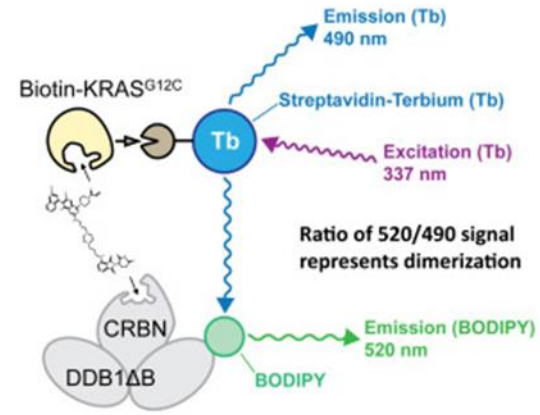
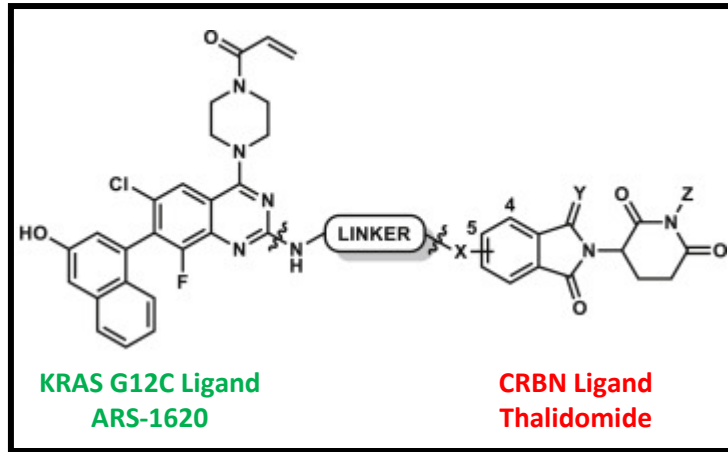
# PROTAC<sup>®</sup>s are Essential Catalytic Activators



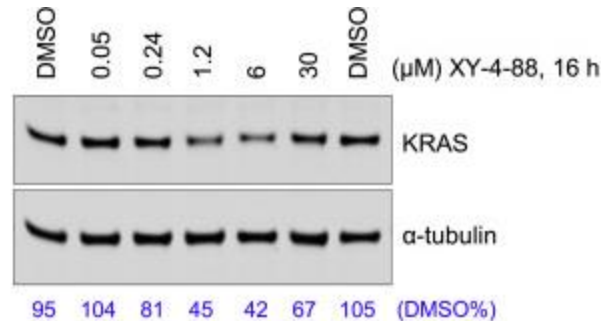
# Challenges with PROTAC<sup>®</sup> Discovery

- Lack of high-throughput assays to assess true efficacy of PROTACs
- Positive co-operativity (better  $K_D$ ) does not guarantee better degradation activity
- Ternary complex rigidity can be inhibitory to ubiquitination

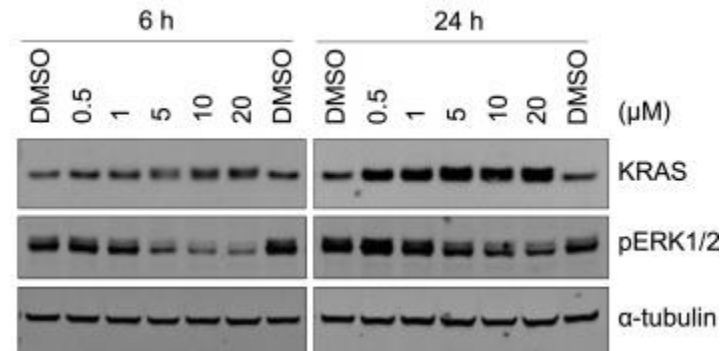
# Does All Ternary Binding Lead to Successful Degradation ?



## TR-FRET- based dimerization assay



GFP-KRAS G12C Degradation



Endogenous KRAS G12C Degradation





# LifeSensors PROTAC Discovery Approach

## Traditional Approaches

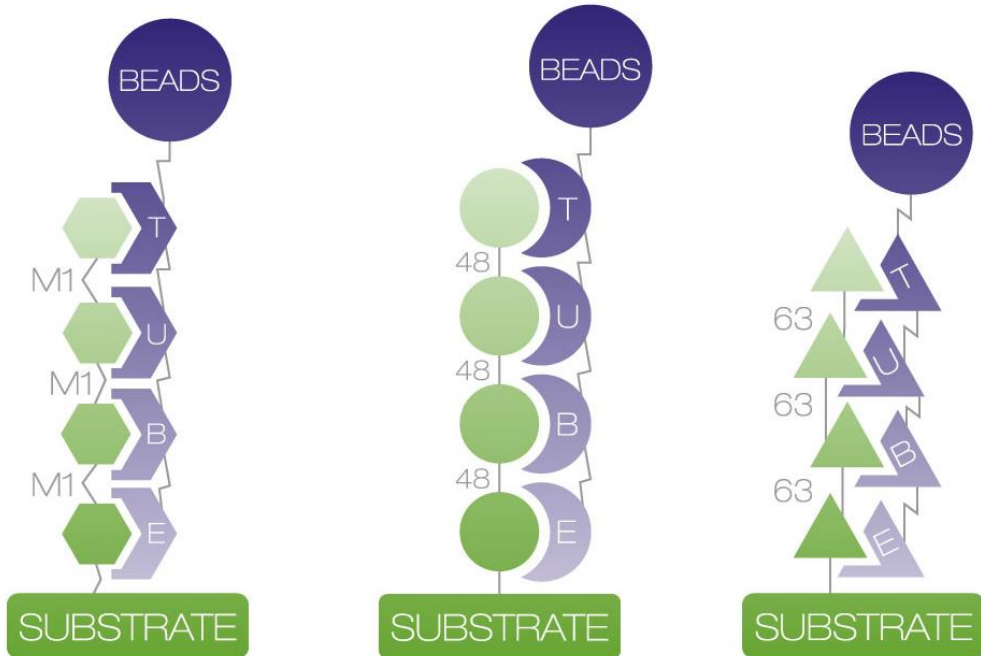
- **Reporter gene assays:** External tags with internal lysines lead to artifacts (Off target ubiquitination).
- **Proximity Ligand Assays:** Does not account for protein's intrinsic features crucial for ubiquitination.
- **Western Blotting:** Low throughput and Irreproducible.

## LifeSensors Approach

- No reporter tags needed, suitable for all targets and applicable to targets limited to primary cell lines.
- Measures **PROTAC mediated Ubiquitination** and degradation simultaneously – dependent on intrinsic lysine exposure.
- High-throughput, reproducible and compatible for multiplexing.



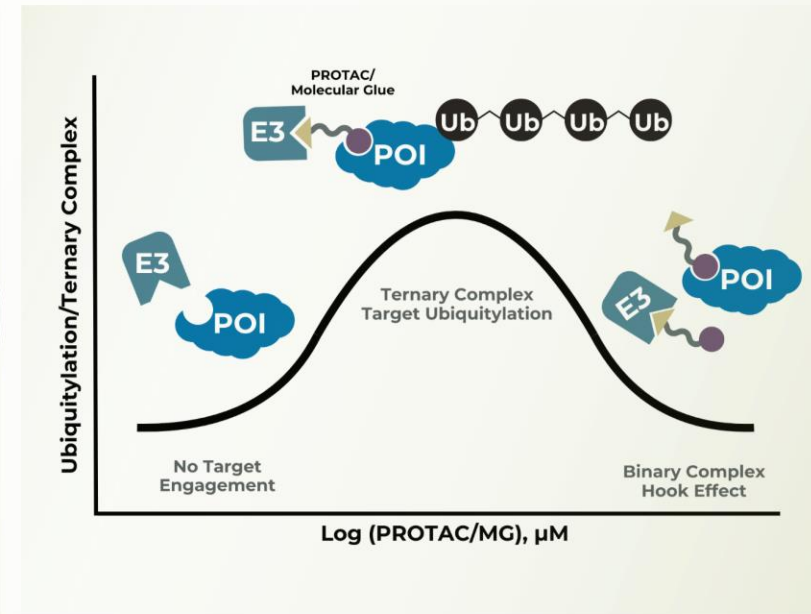
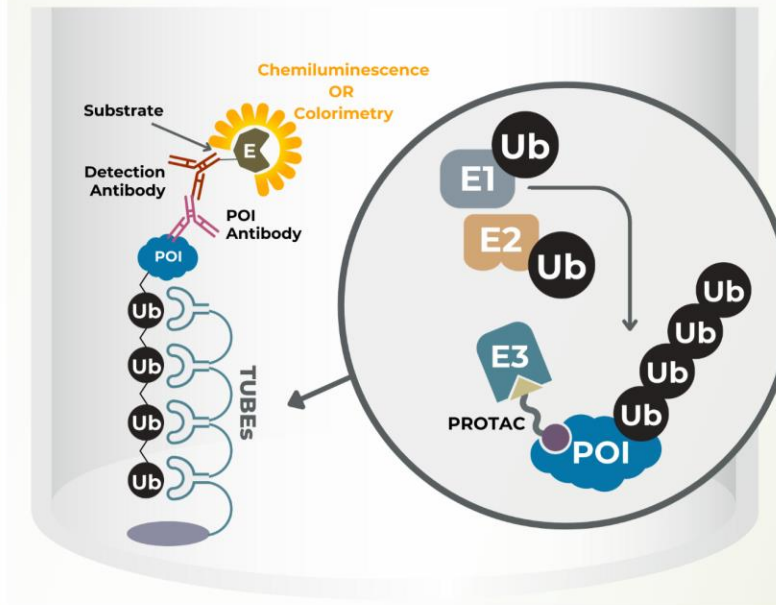
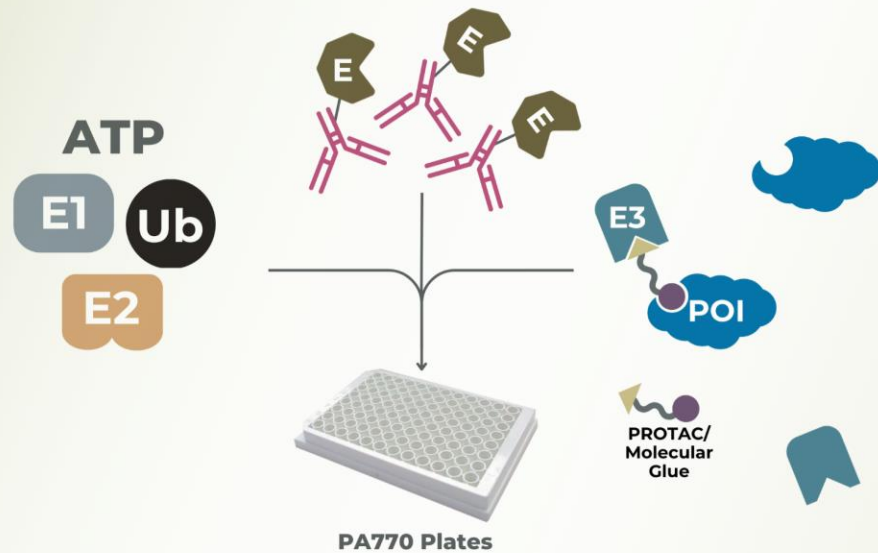
# TANDEM UBIQUITIN BINDING ENTITIES (TUBE<sub>s</sub>)



- [PROTAC Screening](#), PROTAC mediated of poly-ubiquitinated proteins from cells and tissue
- 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

Monitor PROTAC mediated ternary complex formation and ubiquitination

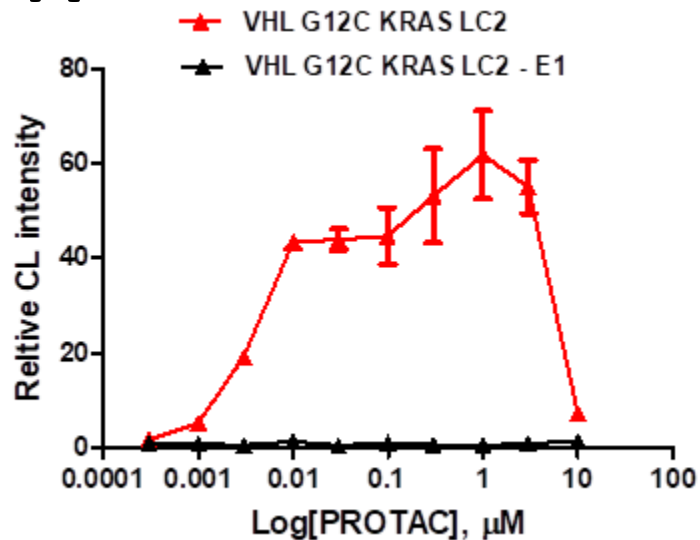


**TUBE capture & PROTAC/MG mediated ubiquitination of POI detection**

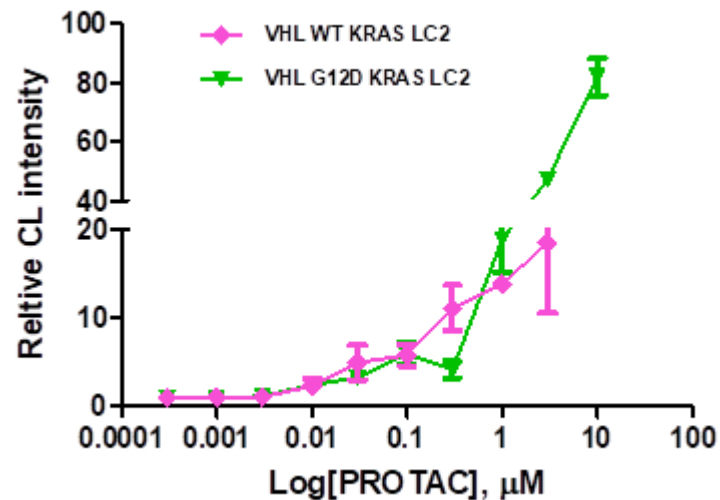
# VHL K-RAS PROTAC®

HTS – *In vitro* Ubiquitination Assays with KRAS G12C vs G12D vs WT

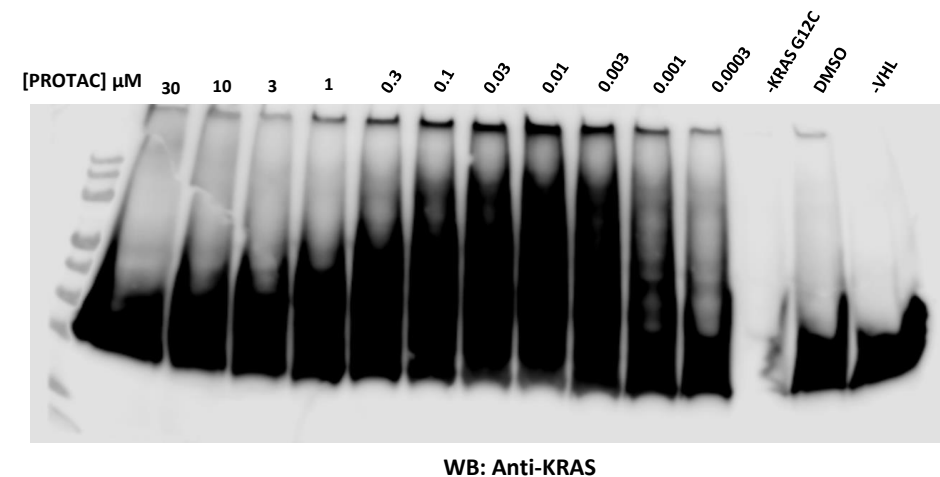
**A**



**B**



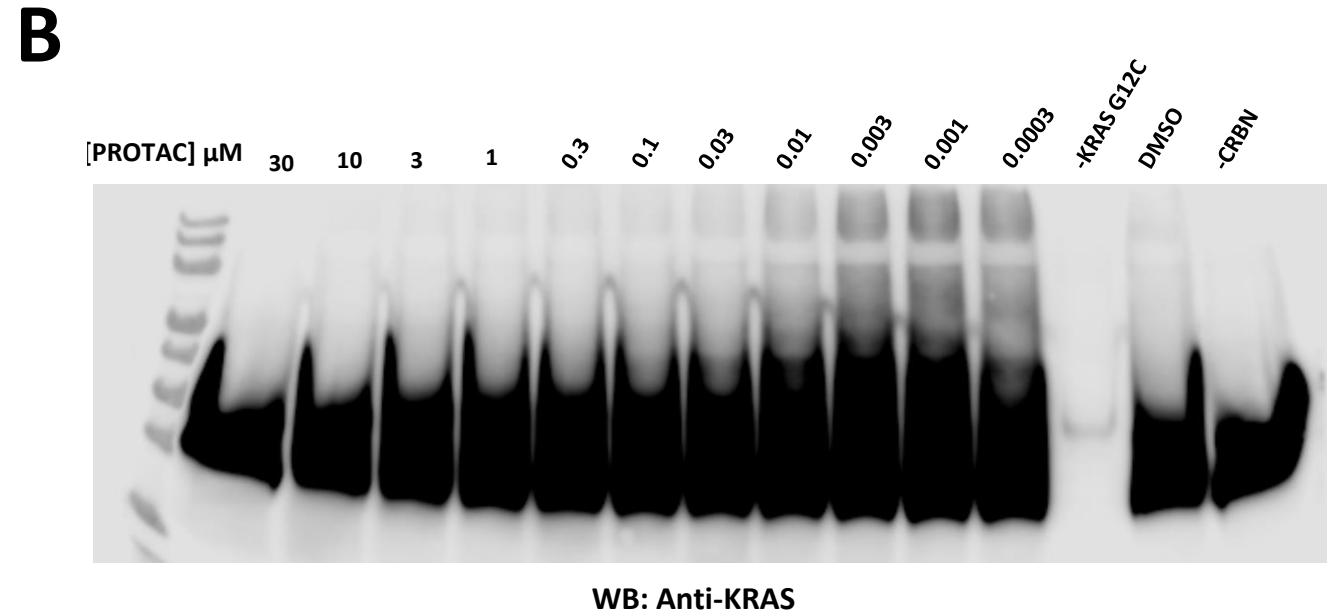
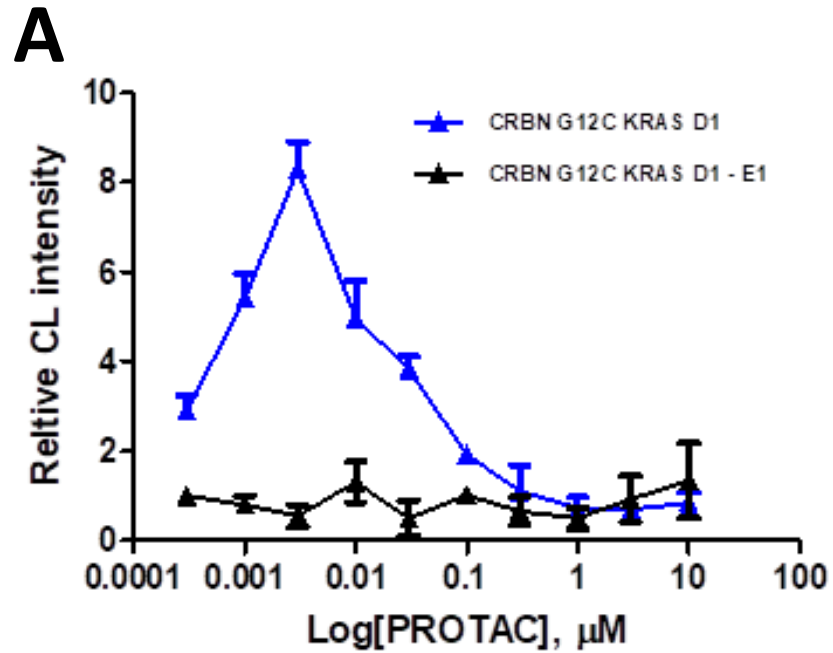
**C**



*In vitro* ubiquitination assay with KRAS G12C VHL based degraders: (A & B) VHL-based PROTAC LC2 in a dose response study to monitor PROTAC mediated ubiquitination of KRAS G12C, G12D and wildtype. CL intensities plotted in response to  $\frac{1}{2}$  log dose response demonstrates PROTAC mediated ubiquitination. (C) Western blot analysis to confirm PROTAC mediated ubiquitination via characteristic poly-ubiquitination smears with anti-KRAS antibody.

# Cereblon MRTX - K-RAS PROTAC<sup>®</sup>

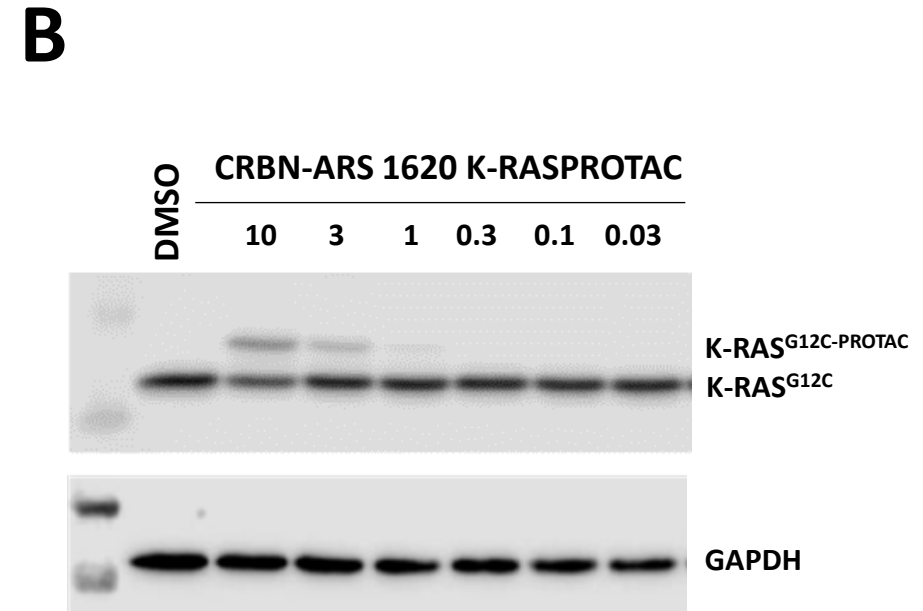
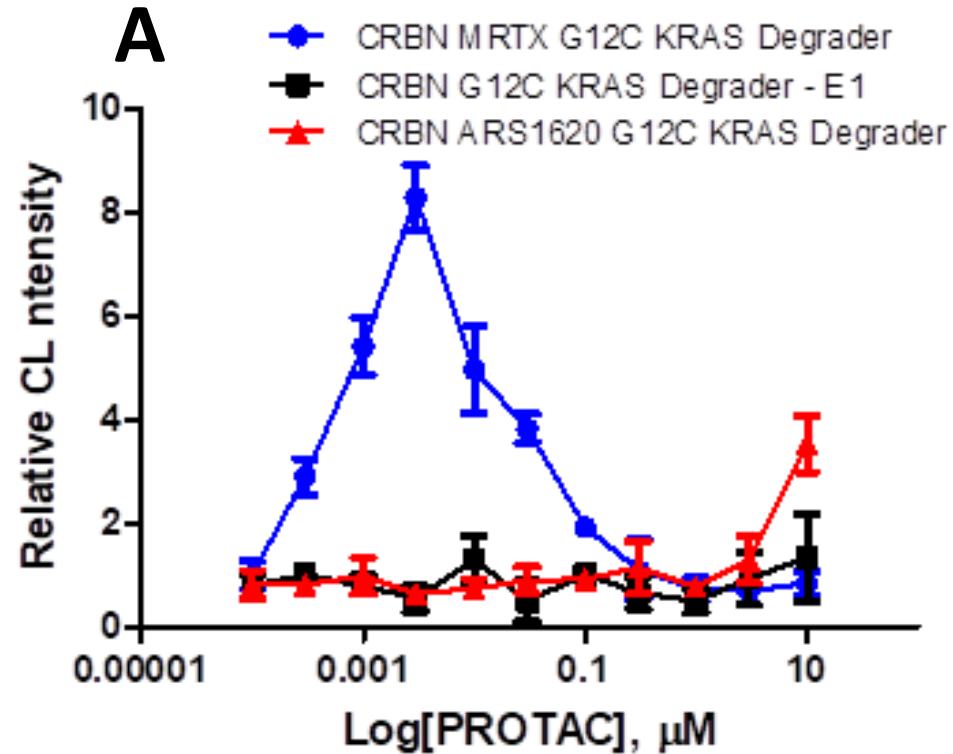
HTS – *In vitro* Ubiquitination Assays with KRAS G12C



*In vitro* ubiquitination assay with KRAS G12C CRBN based degraders: ((A) CRBN-based PROTAC degrader 1 (compound 518) in a dose response study to monitor PROTAC mediated ubiquitination of KRAS G12C. CL intensities plotted in response to  $\frac{1}{2}$  log dose response demonstrates PROTAC mediated ubiquitination. (B) Western blot analysis to confirm PROTAC mediated ubiquitination via characteristic poly-ubiquitination smears with anti-KRAS antibody.

# Cereblon ARS-1620 K-RAS PROTAC<sup>®</sup>

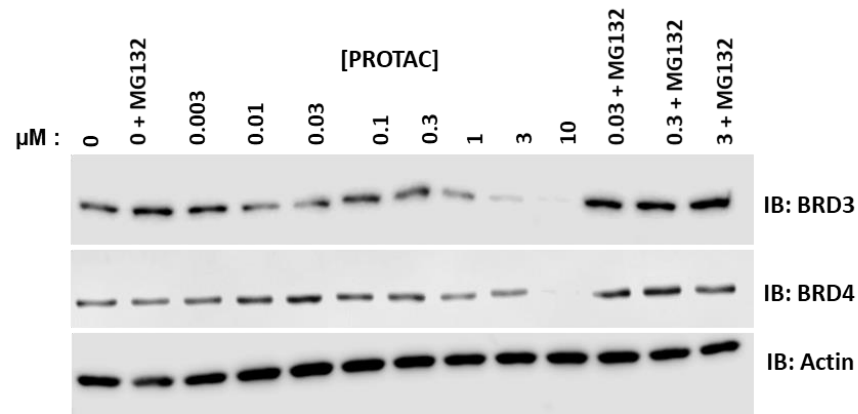
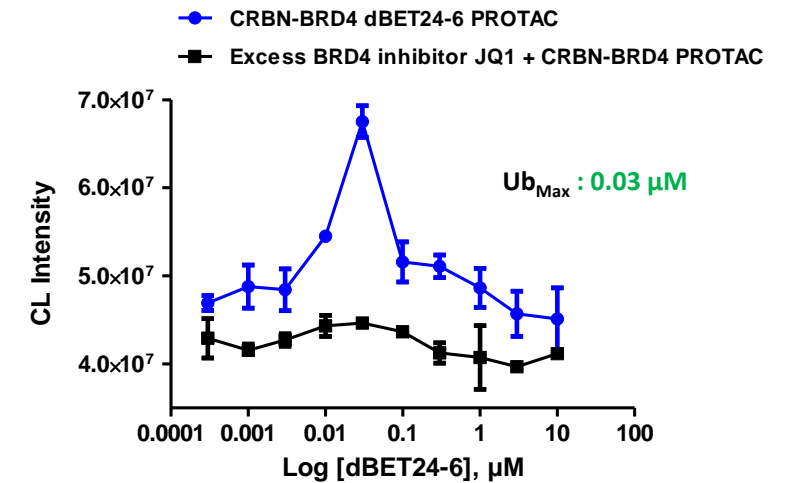
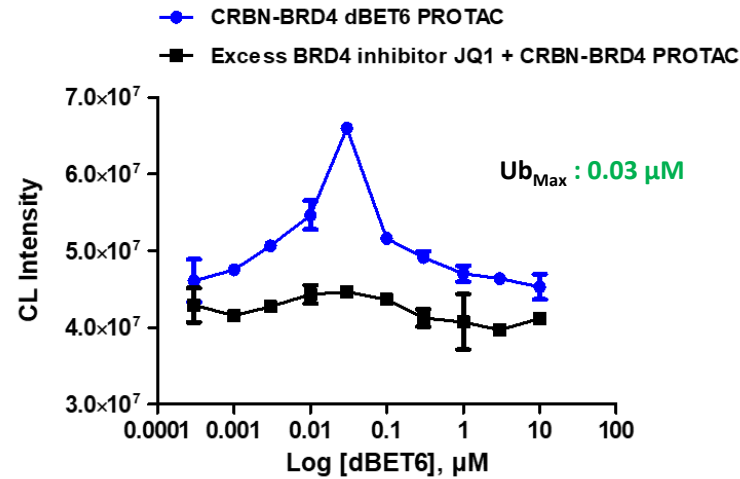
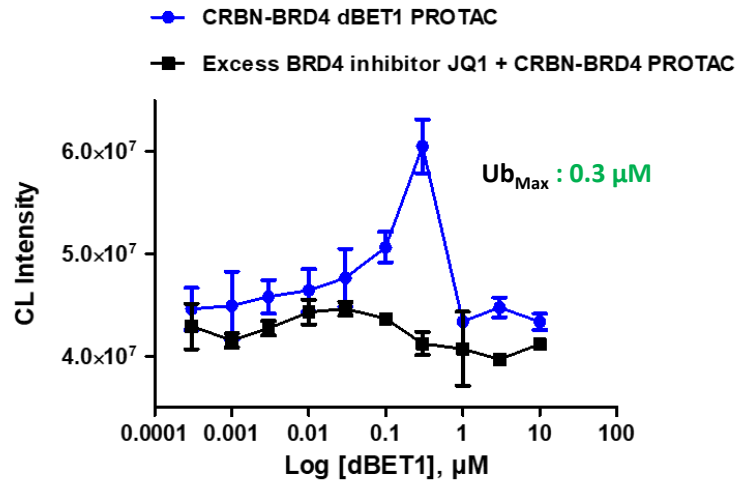
HTS – *In vitro* Ubiquitination Assays with KRAS G12C vs Cell based assay



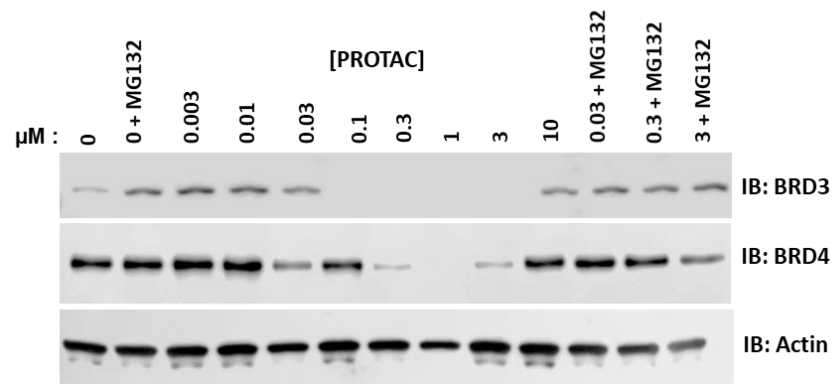
(A) *In vitro* ubiquitination assay with ARS 1620 KRAS G12C CRBN based degrader in a dose response study to monitor PROTAC mediated ubiquitination of KRAS G12C. CL intensities plotted in response to  $\frac{1}{2}$  log dose response demonstrates PROTAC mediated ubiquitination. (B) Cell based assay to monitor degradation of KRAS G12C in H358 cells - PROTAC mediated degradation in a dose response study.

# CRBN Bromodomain PROTAC<sup>®</sup>

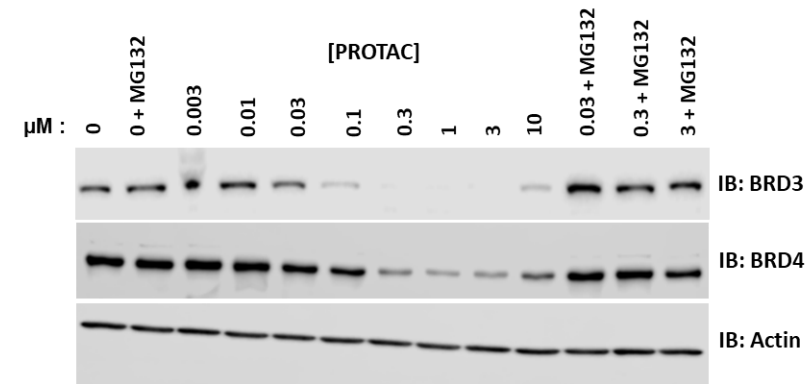
HTS – *In vitro* Ubiquitination Assays



CRBN-dBET1 PROTAC –   
 $DC_{50}$  : 1.0  $\mu\text{M}$



CRBN-dBET6 PROTAC   
 $DC_{50}$  : 0.03 – 0.1  $\mu\text{M}$

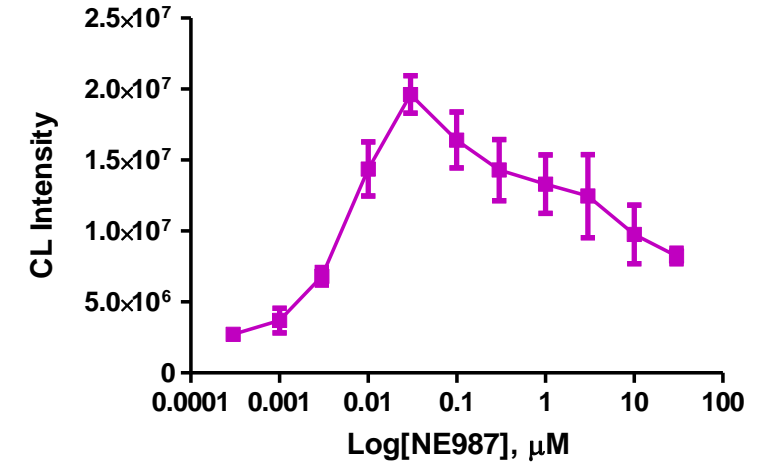
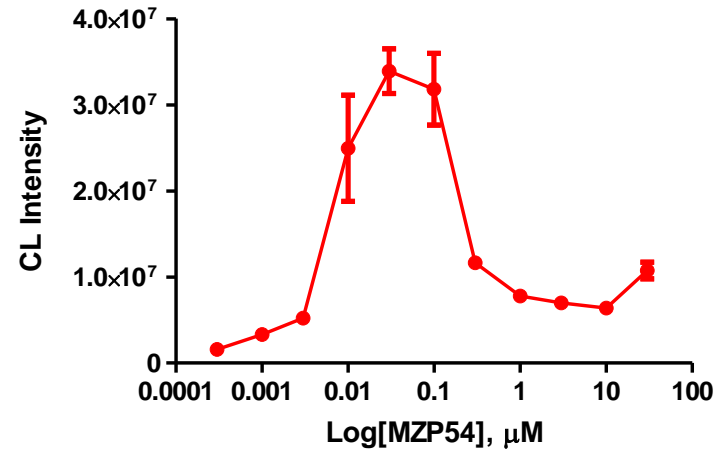
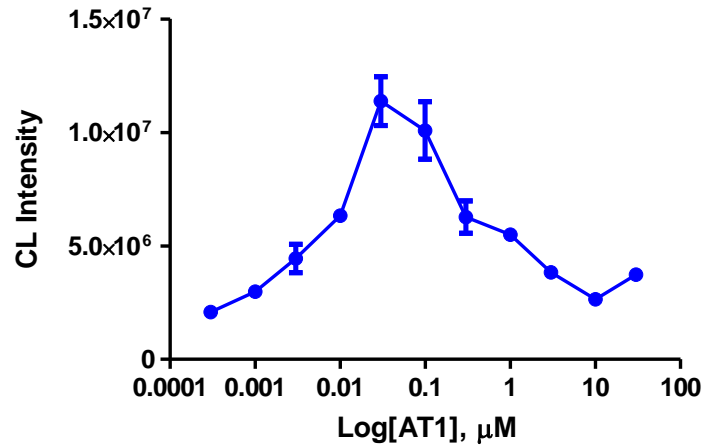


CRBN-dBET24-6 PROTAC   
 $DC_{50}$  : 0.1– 0.3  $\mu\text{M}$



# VHL Bromodomain PROTAC<sup>®</sup>

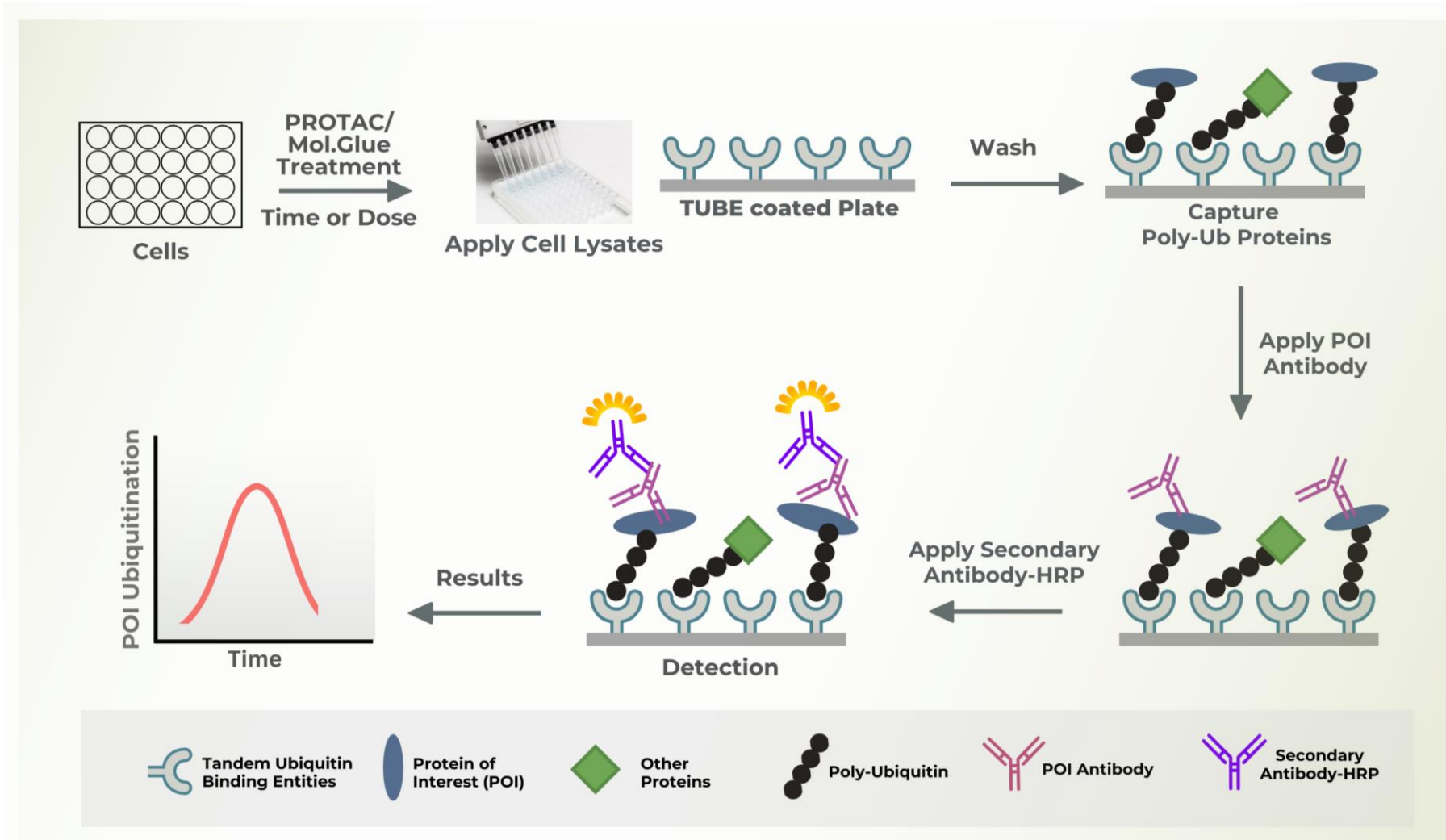
HTS – *In vitro* Ubiquitination Assays



PROTAC	<i>In vitro</i> Ubiquitination vs Binding Affinity		
	Ub <sub>Max</sub> ( <i>In vitro</i> ), μM	Kd, 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

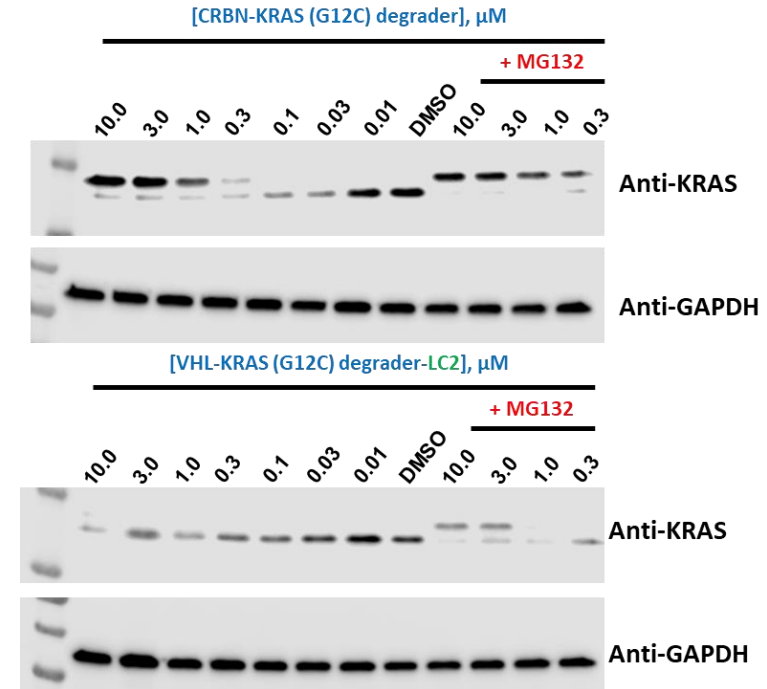
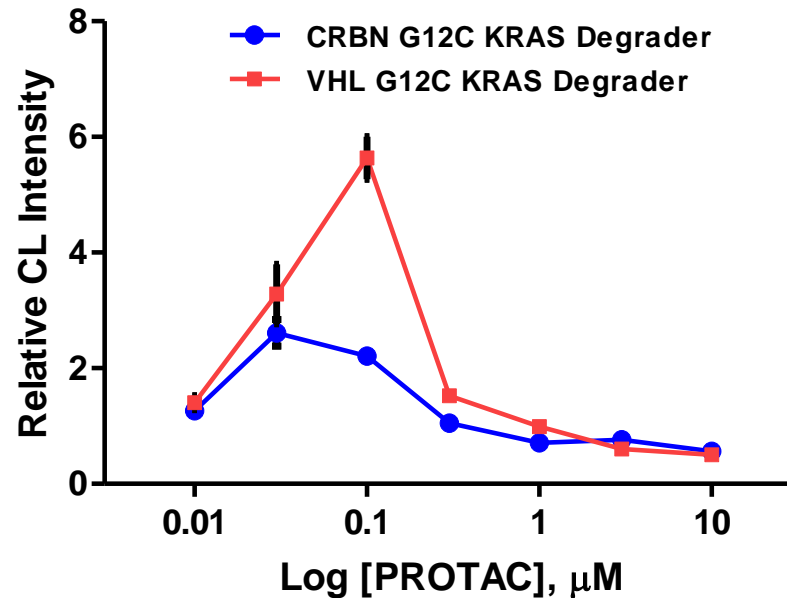


# PROTAC<sup>®</sup> Assay Plate – Cell Based Assays



# CRBN & VHL – K-RAS G12C degraders

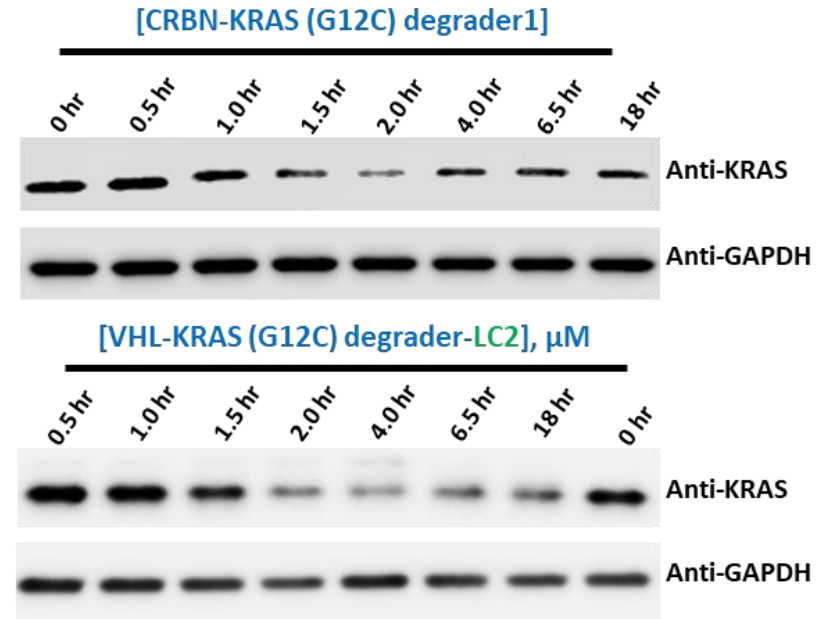
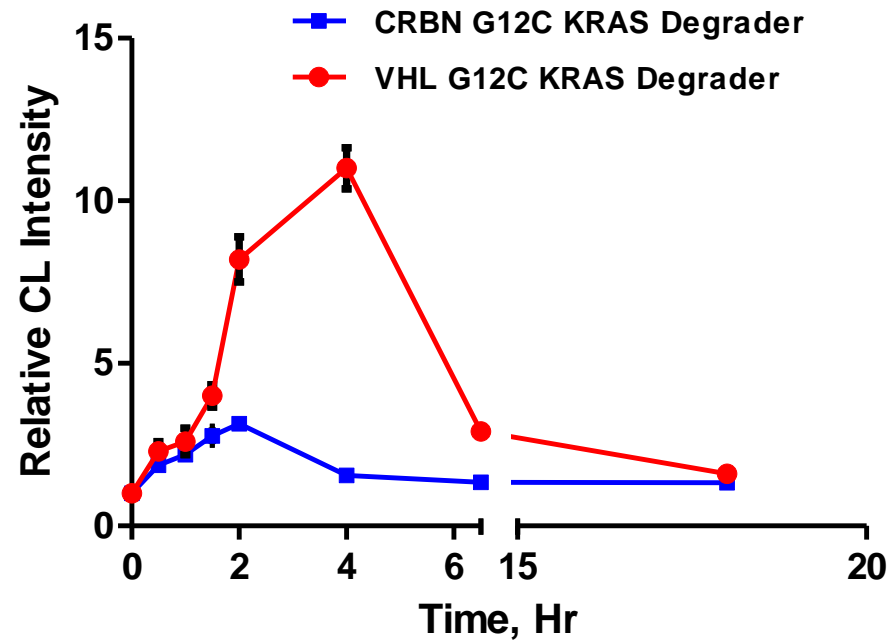
Cellular ubiquitination and degradation profiles – Dose Response Study



**Monitoring Ubiquitination – Dose Response** : changes in ubiquitination profiles of endogenous KRAS and subsequent degradation in H358 cells with changes in dose of both VHL and CRBN KRAS degraders. VHL and CRBN PROTACs designed with covalent ligands to engage KRAS G12C and successfully ubiquitinate and degrade with 3hrs of treatment between 30-100 nm.

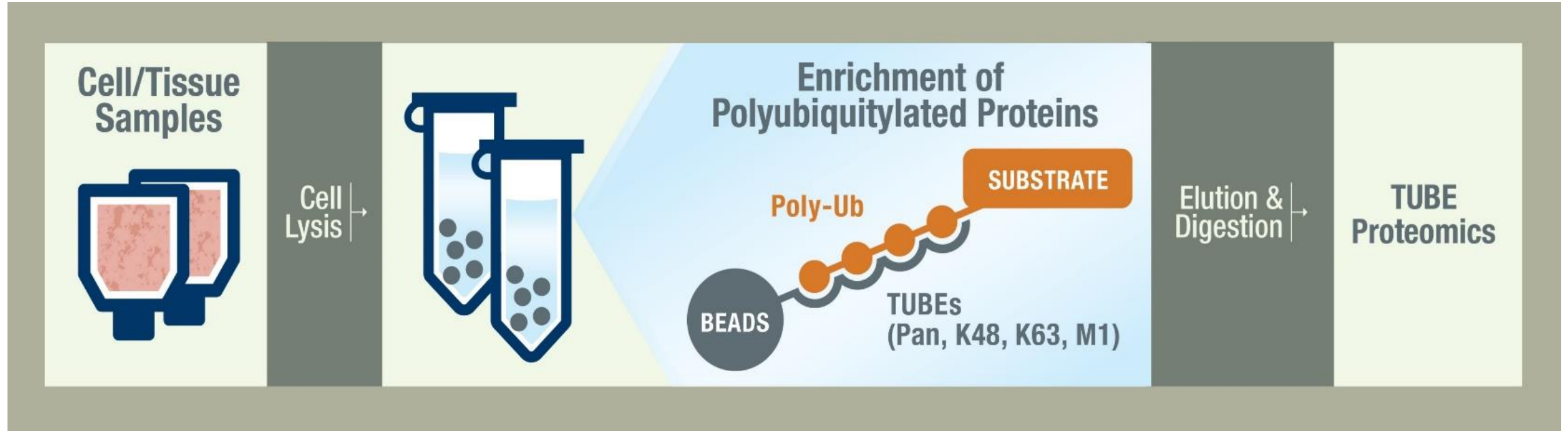
# CRBN & VHL – K-RAS G12C degraders

Cellular ubiquitination and degradation profiles – Time Course Study



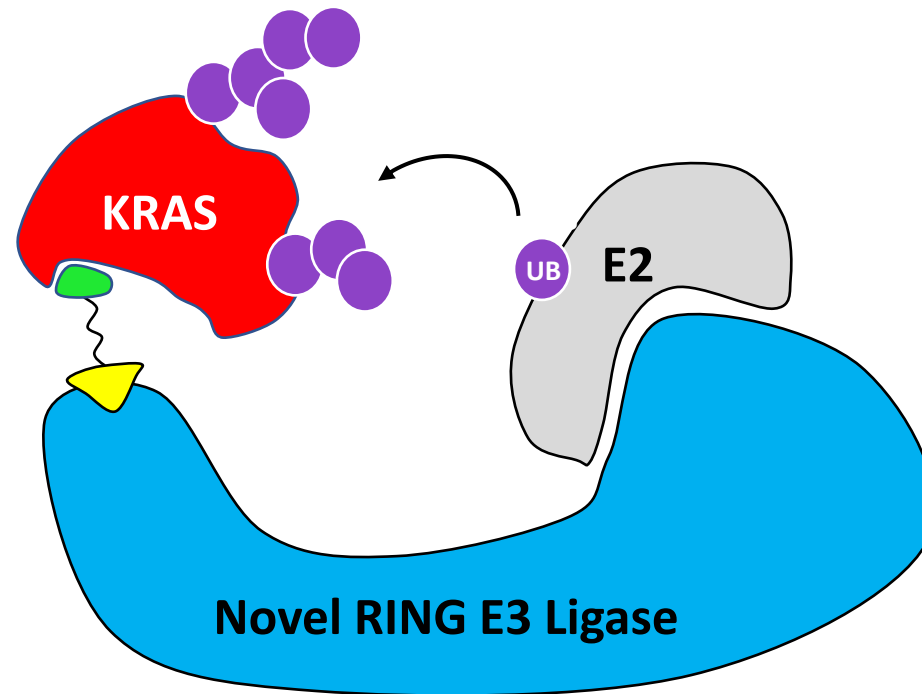
**Monitoring Ubiquitination Kinetics** – changes in PROTAC mediated ubiquitination profiles of endogenous KRAS and subsequent degradation in H358 cells. VHL and CRBN PROTACs designed with covalent ligands to engage KRAS G12C and successfully ubiquitinate and degrade within 2-4hrs of treatment.

# TUBE-based Proteomics



- Rapid, Inexpensive and Simple method for identifying total Ubiquitome changes
- Quantitative method for examining drug effect of PROTACs in cells

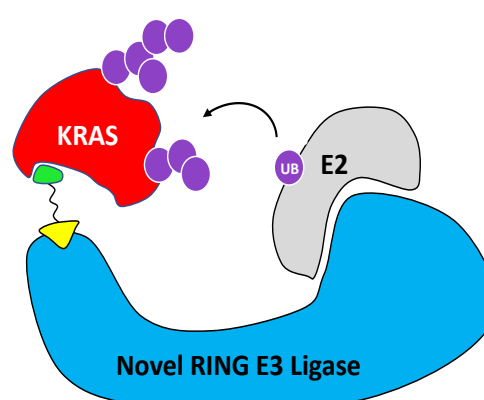
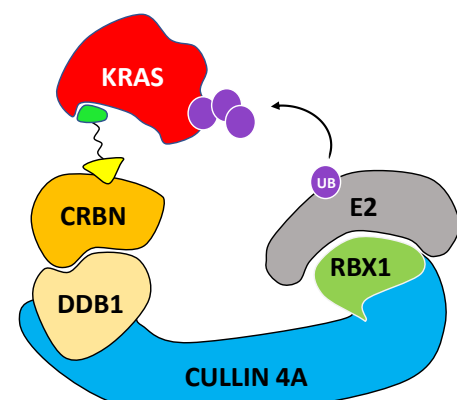
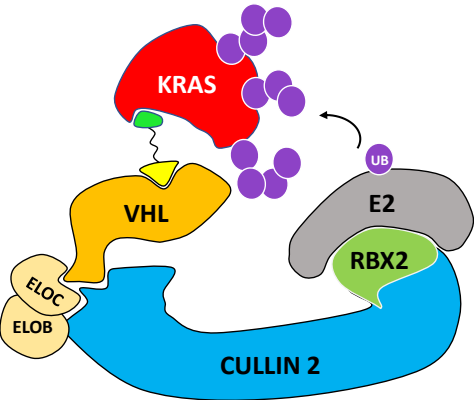
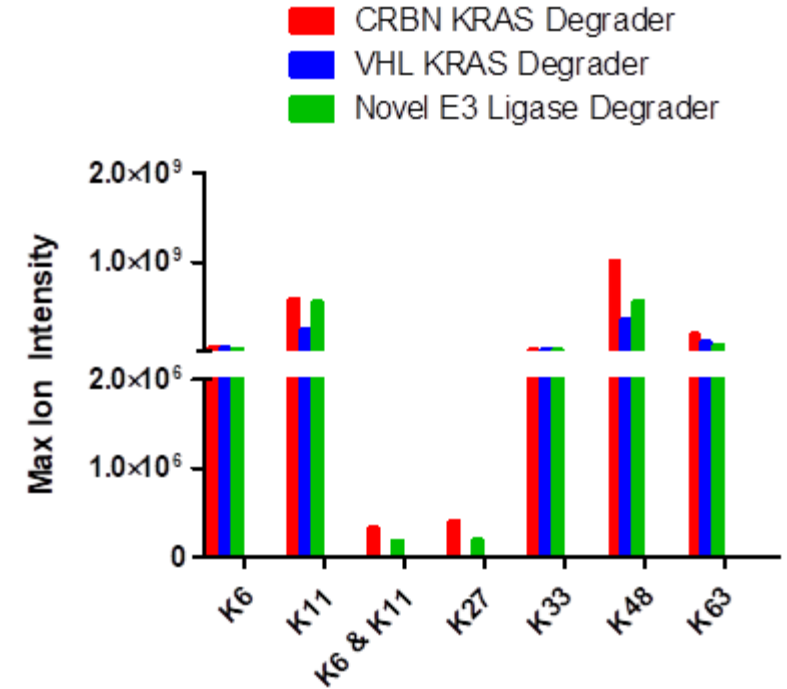
# Novel E3 Ligase K-RAS PROTAC<sup>®</sup>



# MS Evaluation of K-RAS PROTAC<sup>®</sup>

Comparative E3 Ligase Study – In vitro Ubiquitination Mass Spectrometry Analysis

K-RAS Degradator / Ubiquitinated Peptide	K86	K103	K127	K146	K183
VHL based Degradator	High	High	High	High	High
CRBN based Degradator	High	Low	High	Low	Low
Novel E3 based Degradator	Confidential				



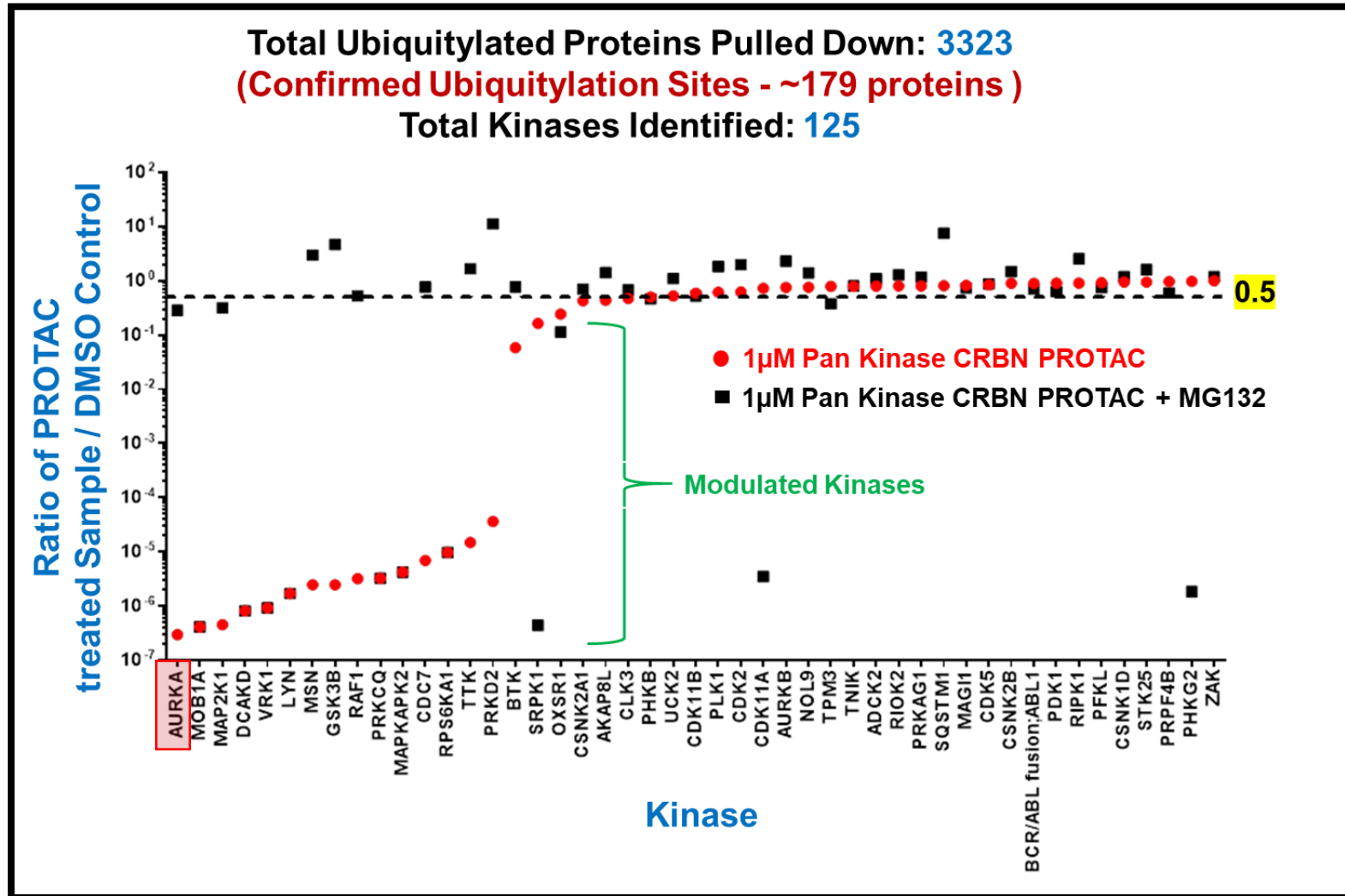
VHL E3 Ligase

Cereblon E3 Ligase

Novel E3 Ligase

# TUBE-based Proteomics-PROTAC®

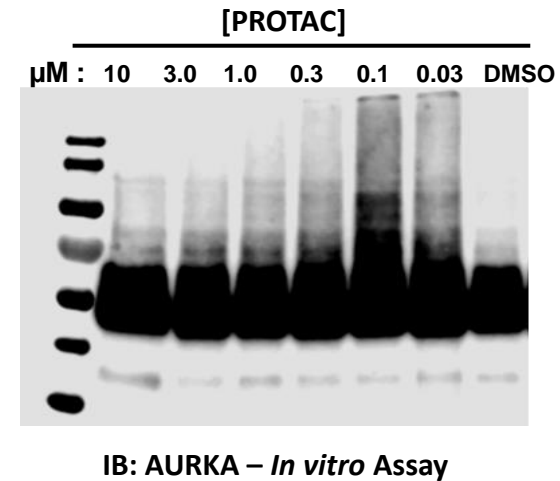
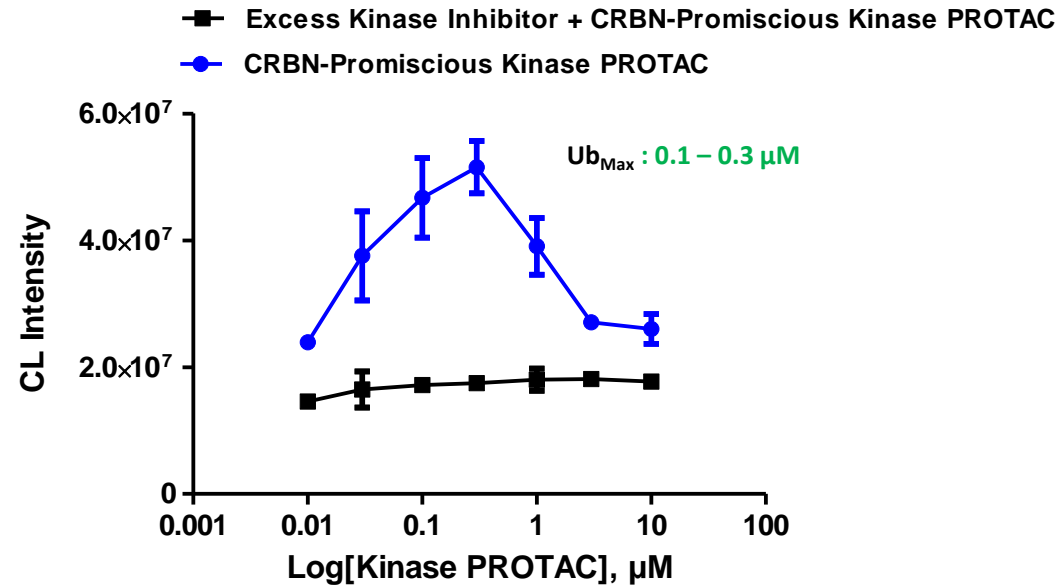
Multi-kinase CRBN-PROTAC ubiquitinated ~15 kinases that were rescued by proteasome inhibitor





# CRBN Multi-Kinase degraders

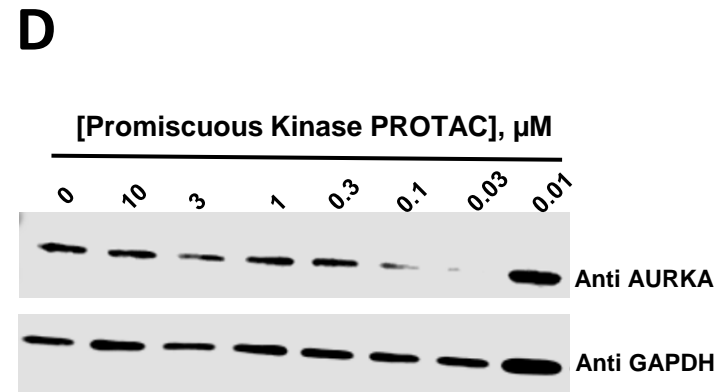
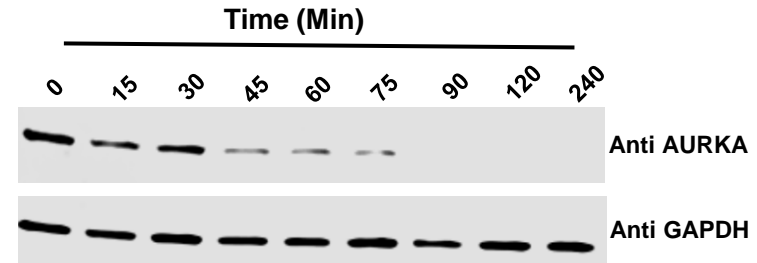
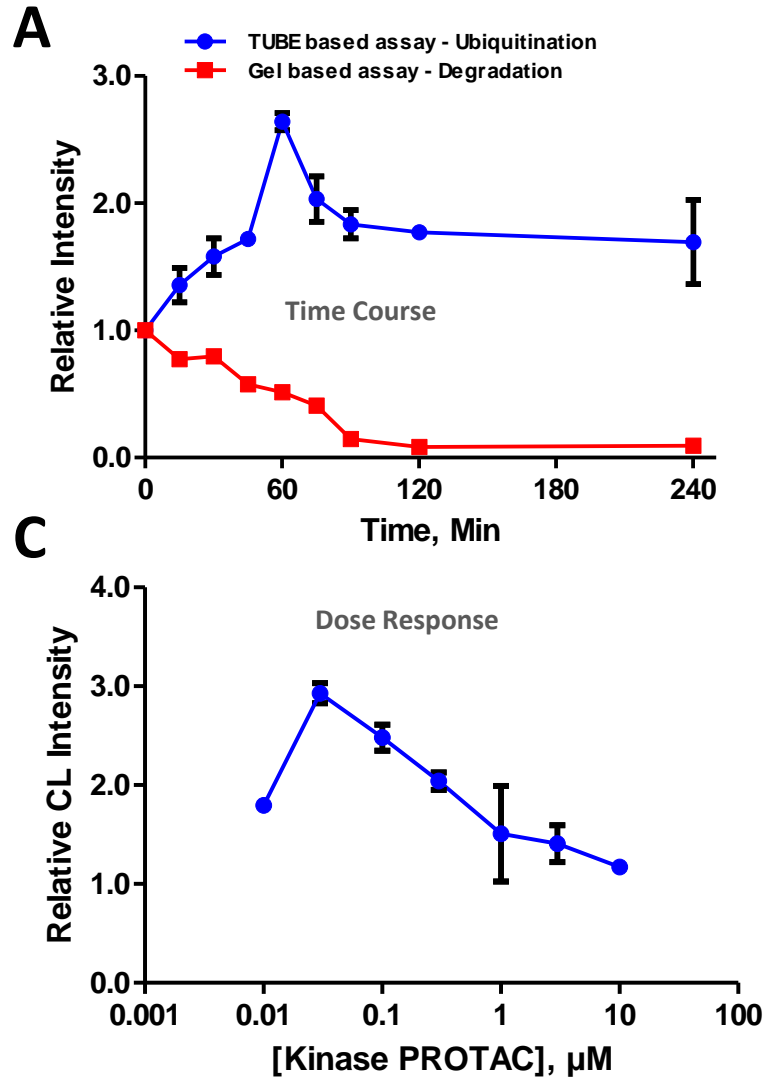
## HTS PROTAC mediated Ubiquitination of AURKA



**AURKA *in vitro* ubiquitination assays:** recombinant AURKA ubiquitination was monitored as function of cereblon PROTAC dose response. Excess kinase inhibitor competes with PROTAC interaction compromising ubiquitination of the target. A complementary gel-based assay to represent changes in poly-ubiquitination profiles mediated by PROTACs.

# CRBN Multi-Kinase degraders

Cellular ubiquitination HTS In vitro Screening



- A time course study to evaluate intracellular ubiquitination and degradation to establish the cooperative binding between CRBN and AURKA.
- Monitor degradation kinetics to compare  $Ub_{max}$  with degradation establish the ubiquitination as precursor step of degradation.
- Evaluate  $DC_{50}$  of the promiscuous kinase PROTAC in K562 cells using  $Ub_{max}$

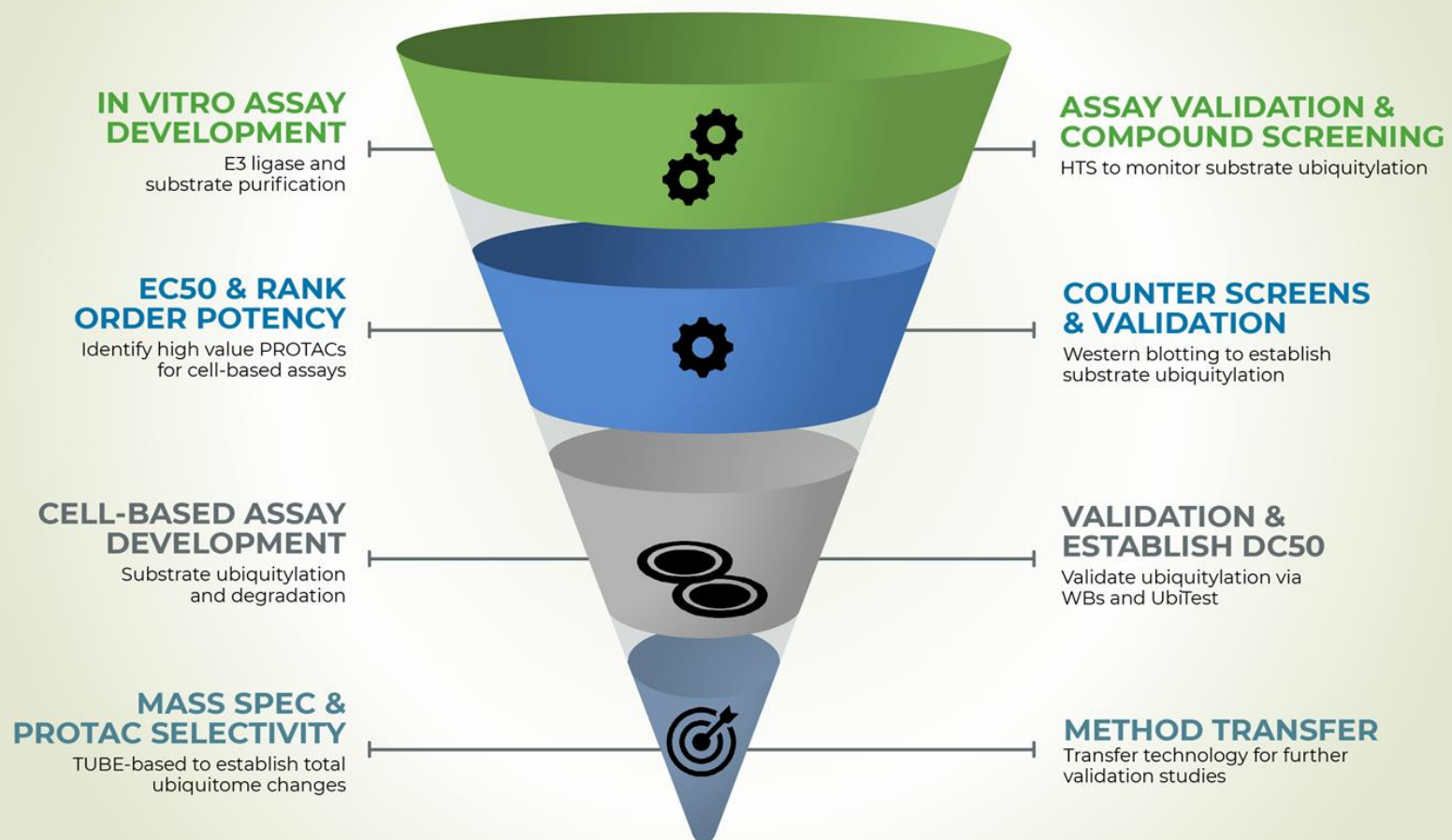
# TUBE based PROTAC<sup>®</sup> Assays

- Establish rank-order potencies of PROTAC variants in a HT fashion
- Rapid ubiquitination and degradation kinetics of **native** targets
- Guiding Med Chem to establish rapid SAR

“Ub<sub>Max</sub>” A better way to measure potency of PROTACs

**TUBEs based PROTAC<sup>®</sup> Assays provides a link between ubiquitination and degradation**

# Pathway to PROTAC Drug Discovery



# Thank You

We are your partner for PROTAC Drug Discovery

Contact Us!

## Contact Information

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Custom Service & Assays	BD	<a href="mailto:bd@lifesensors.com">bd@lifesensors.com</a>	610-644-8845 (ext 310)