Accelerating PROTAC and Mol Glue Drug Discovery

Unleashing the Potential of TUBE Embedded Microtiter Plate Technology

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LifeSensors Leadership in UPS Drug Discovery

Leading Biotech in PROTAC and Mol Glue Drug Discovery

500 Products supporting UPS research

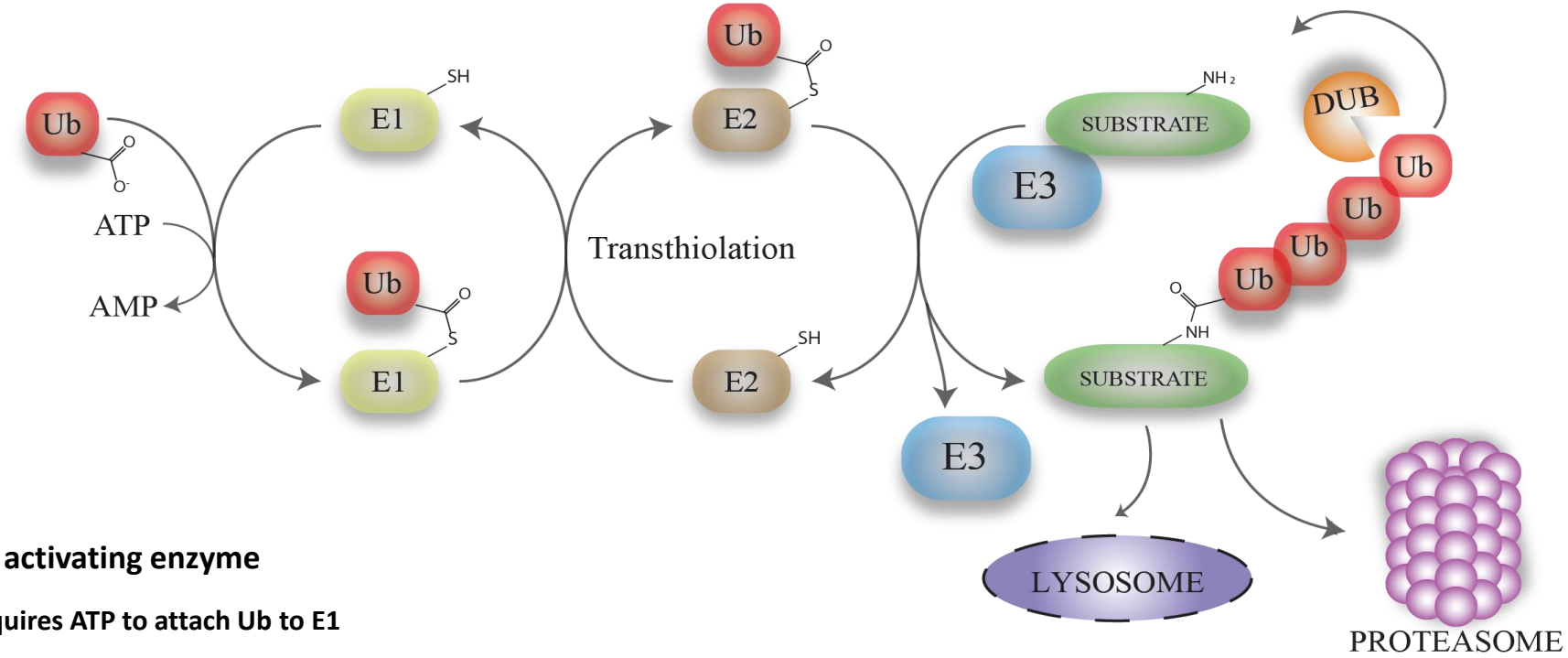
E3 ligase and DUB compounds profiling

Discovery services for new E3 ligands, PROTACs and Mol Glues

Collaborative and Independent Research

Malvern, Pennsylvania, USA

Understanding Ubiquitin Proteasome System in Drug Discovery



E1 – Ubiquitin activating enzyme

Requires ATP to attach Ub to E1

E2 – Ubiquitin conjugating enzyme

Transfers Ub from E1 to E3

E3 – Ubiquitin ligases

Transfers Ub to self or substrate

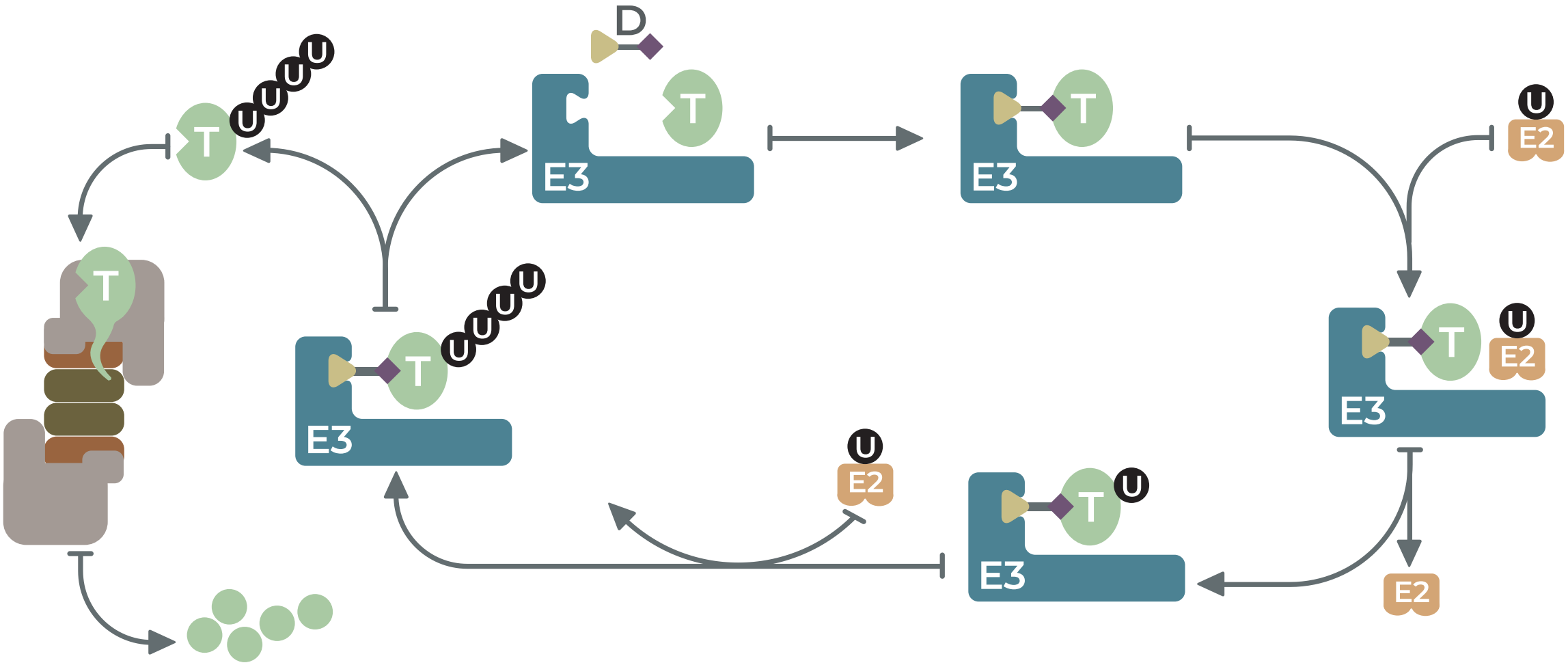
Forms mono-Ub or poly-Ub chains

DUB – Deubiquitinase

Removes mono-Ub or poly-Ub chains

Proteasome – Degrades ubiquitylated proteins

PROTAC®s - Essential Catalytic Activators



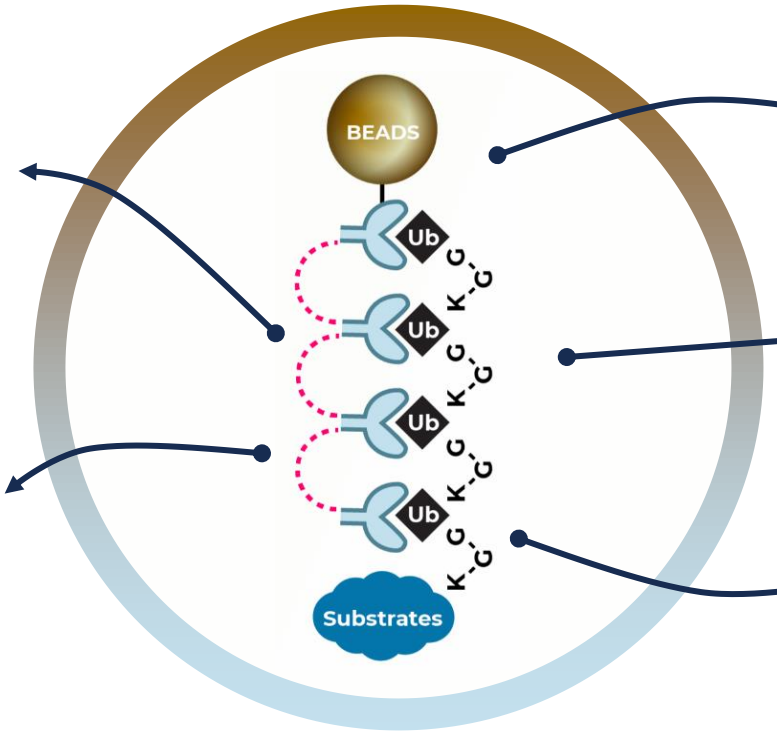
" LifeSensors makes it easy to study Ubiquitination "

TANDEM UBIQUITIN BINDING ENTITIES

TUBEs: A Versatile Tool in E3 Ligase Assays

Ubiquitin Binding Domains

Engineered for Chain Selectivity

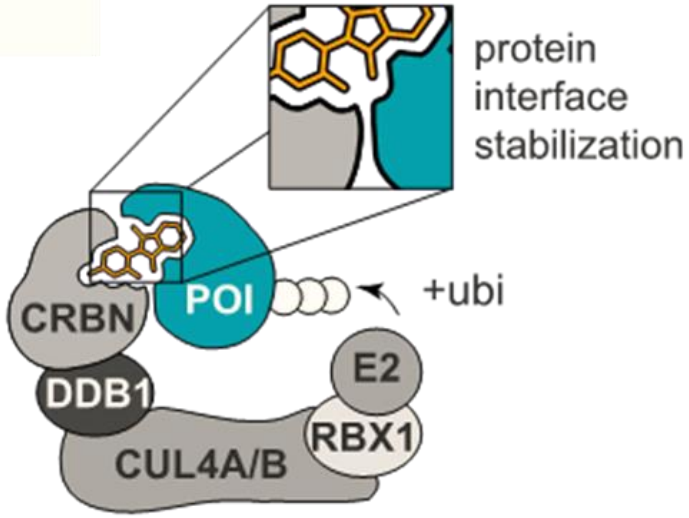


Versatile applications

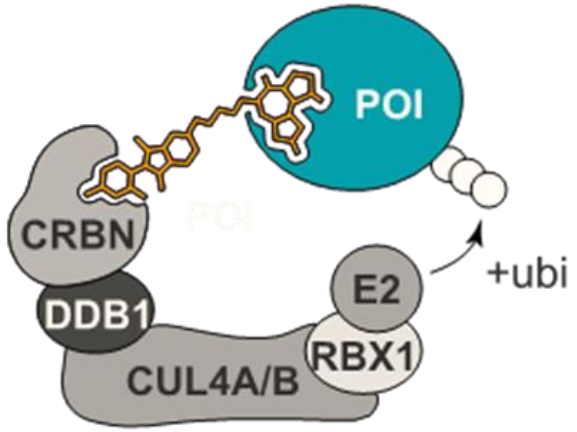
Sub-nanomolar affinity binding

Protection against intrinsic DUBs

Targeted Protein Degradation



Molecular Glue



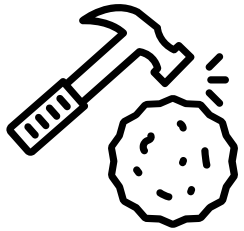
PROTAC

Immense promise for molecular glues challenged by lack of reliable approaches to rationally design them

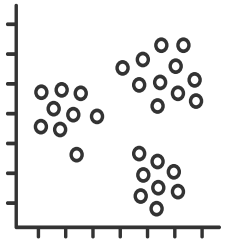
Expectations for PROTACs are very high, but recent clinical data suggest developmental challenges

TUBEs address critical TPD challenges

Strong binders / better K_D does not guarantee better degradation



Ternary complex rigidity



Ligand dependent ubiquitination, true functional HTS

LifeSensors Approaches Standout

Traditional Approaches

- **Reporter gene assays**:

  External tags (Off target ubiquitination)

- **Proximity Ligand Assays**

  protein's intrinsic factors - overlooked

- **Western Blotting**

  Low throughput and Irreproducible

LifeSensors Approaches

- **Invitro Ubiquitination assays :**

  Ligand mediated ubiquitination, fast and functional approach

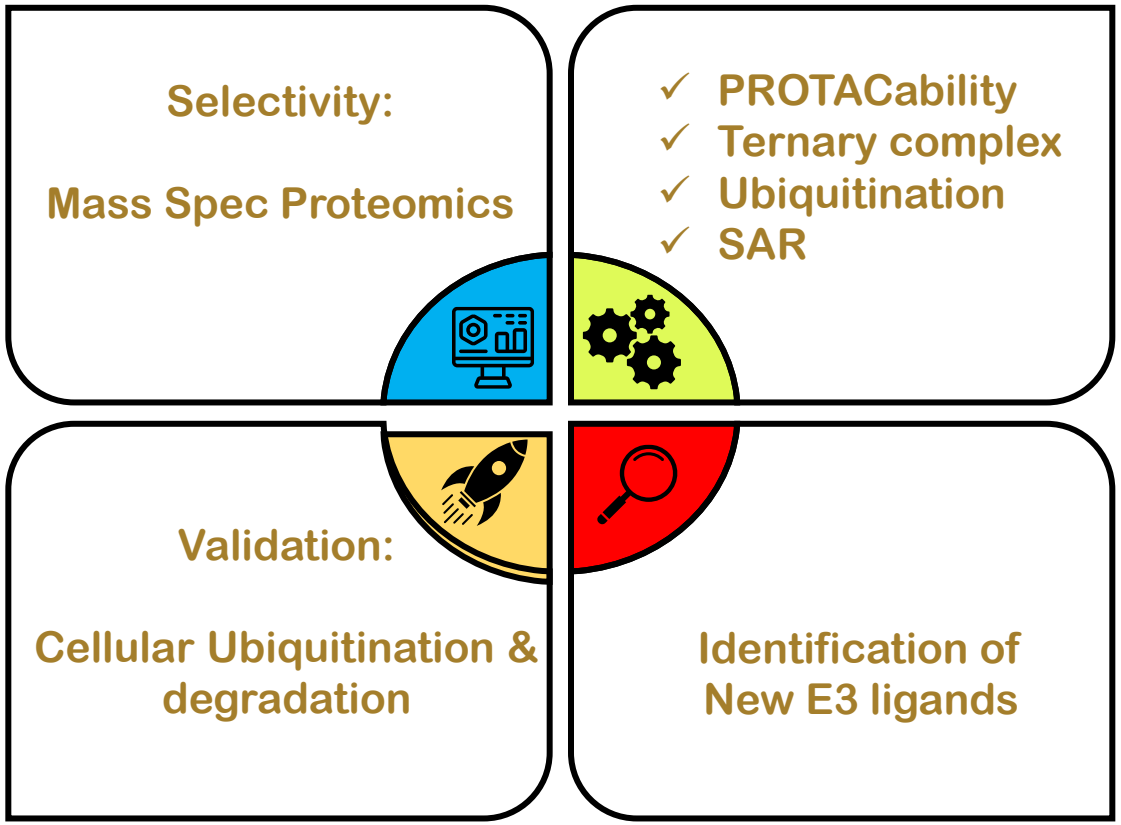
- **Cellular degradation assays:**

  No reporter tags required, applicable to clinical and preclinical studies "Biomarker"

- **HTS screening**

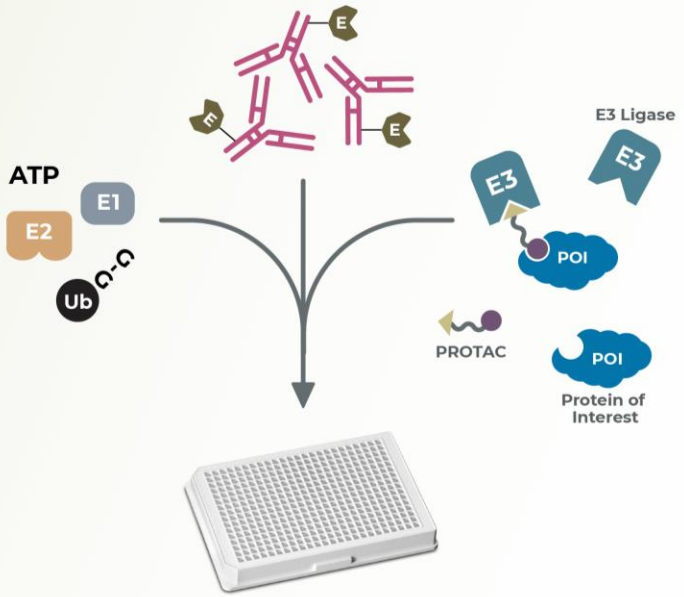
  Native lysines playing a role

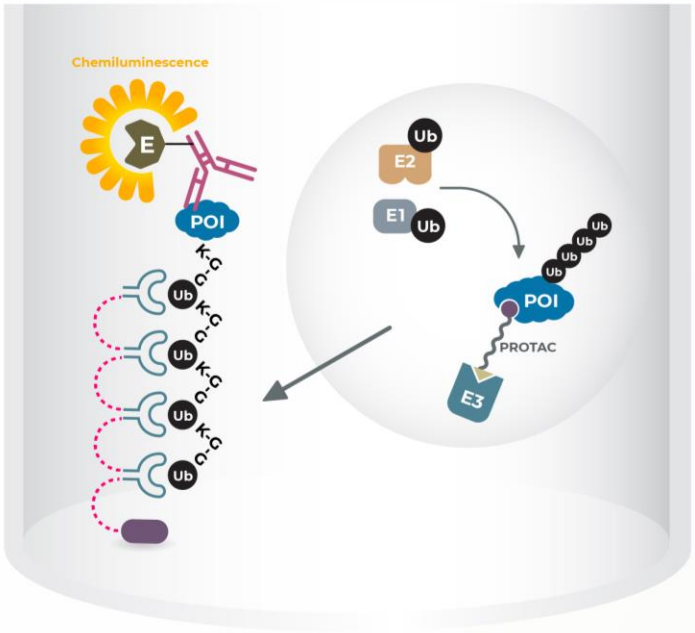
LifeSensors Approaches for Targeted Protein Degradation

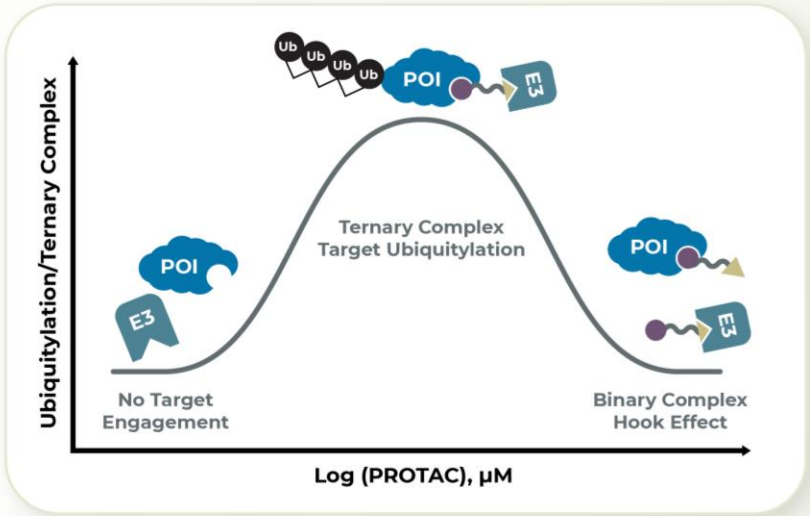


HTS- In Vitro Biochemical Assay

To study functional ternary complex and PROTACability

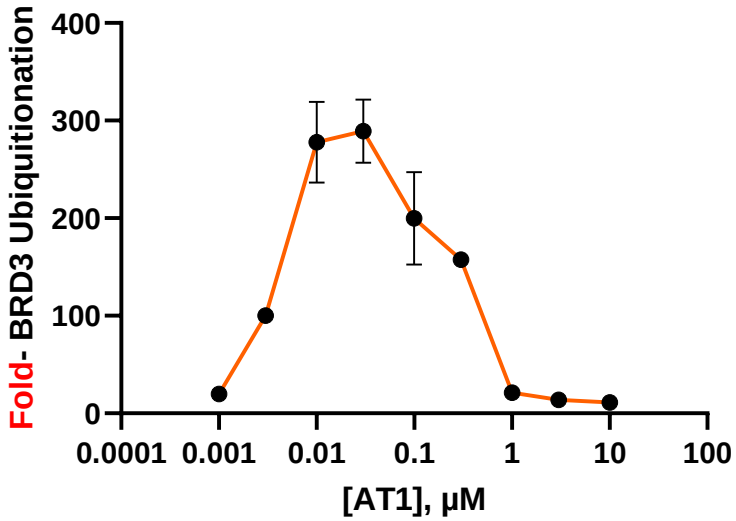


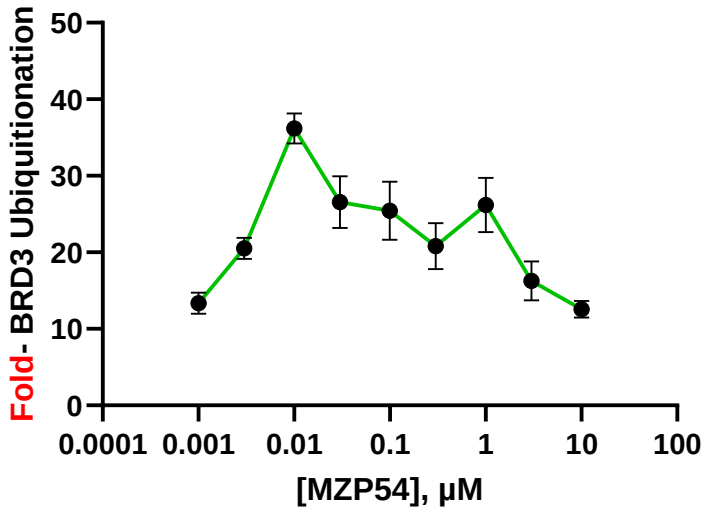


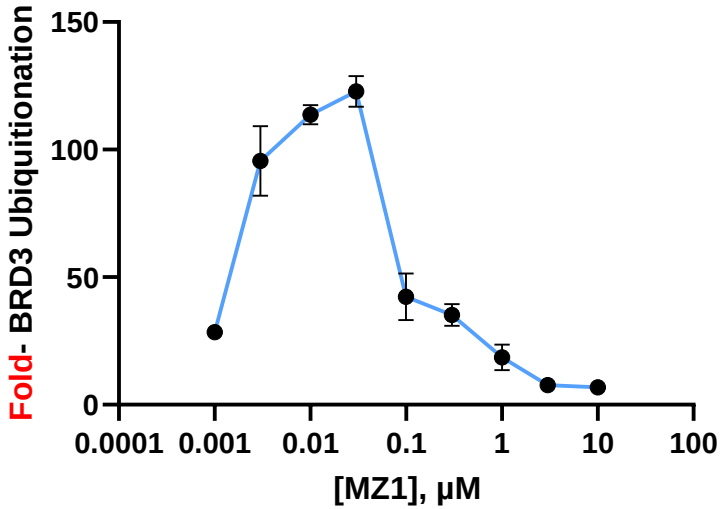


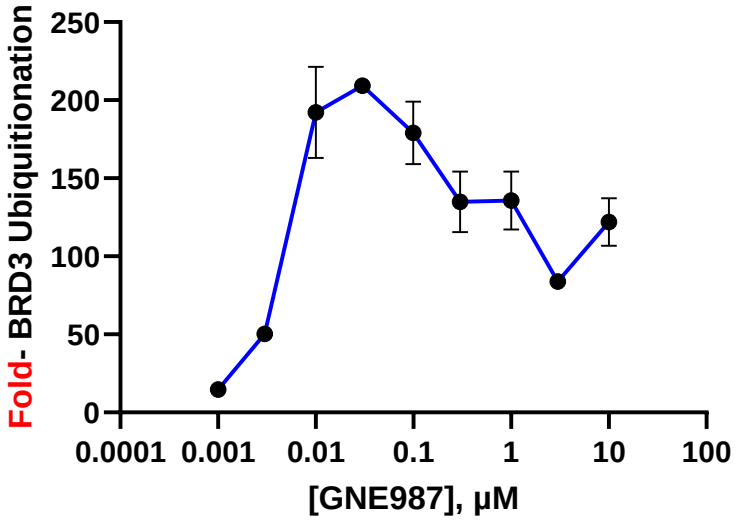
TUBE Capture & PROTAC Mediated Ubiquitination of POI Detection

VHL Bromodomain PROTACs

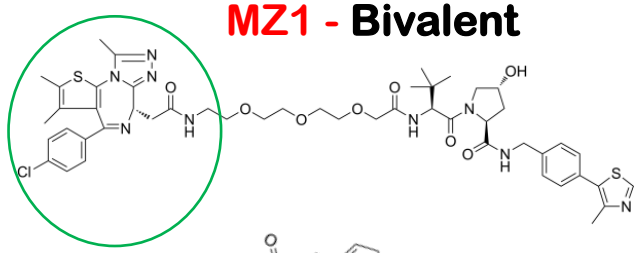


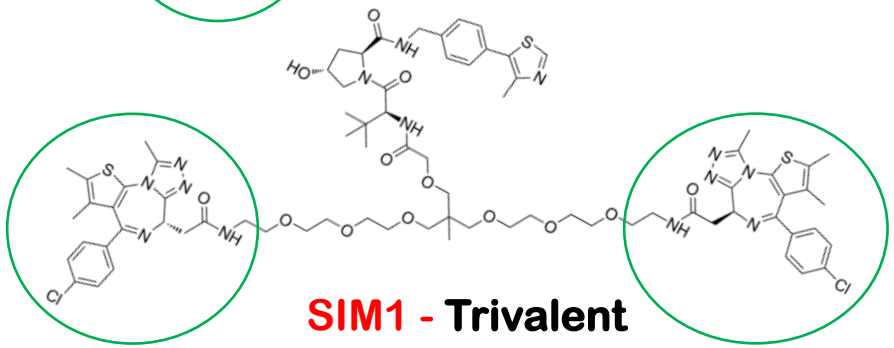




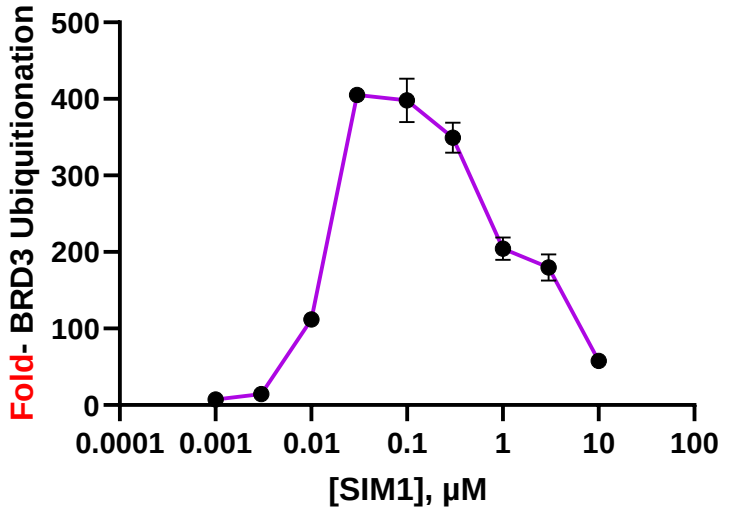


MZ1 - Bivalent

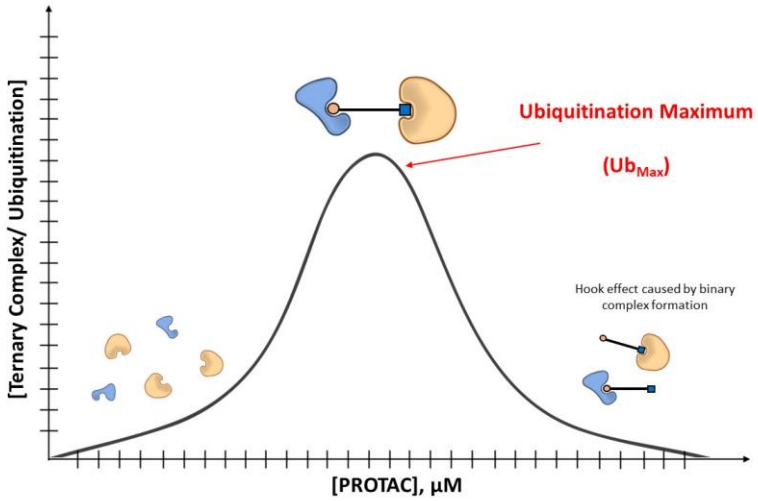


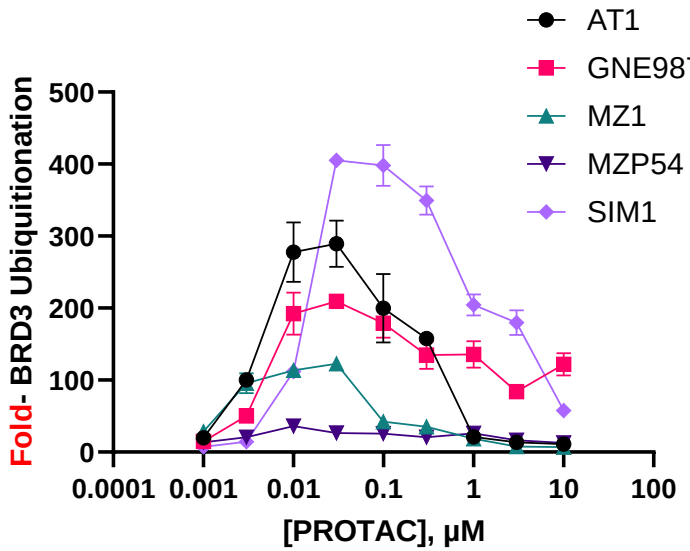


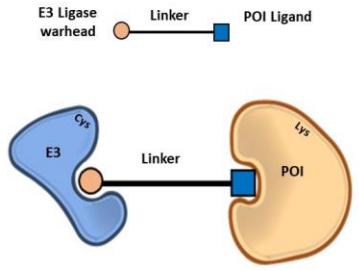
SIM1 - Trivalent



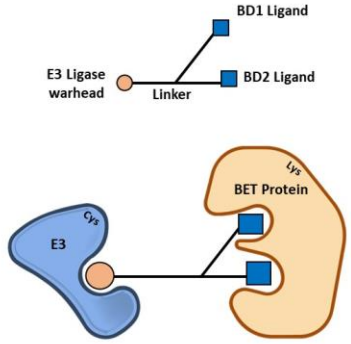
VHL Bromodomain degraders







MZ1 - Bivalent



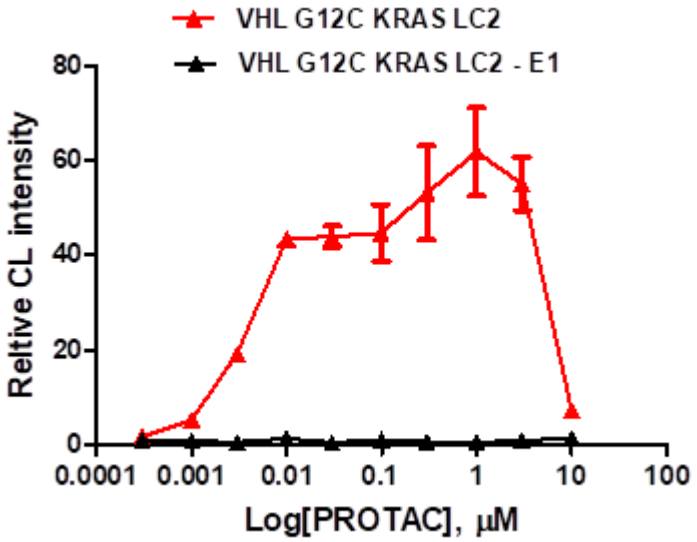
SIM1 - Trivalent

PROTAC	Cellular EC_{50}	Area Under the curve
AT1	~100.0 nM	244.4
GNE987	9.9 nM	1084.0
MZ1	189.9 nM	112.4
MZP54	~10.0 nM	167.3
SIM1	1-10 nM	1519.0

K-RAS PROTACs

VHL PROTAC mediated in vitro ubiquitination

A



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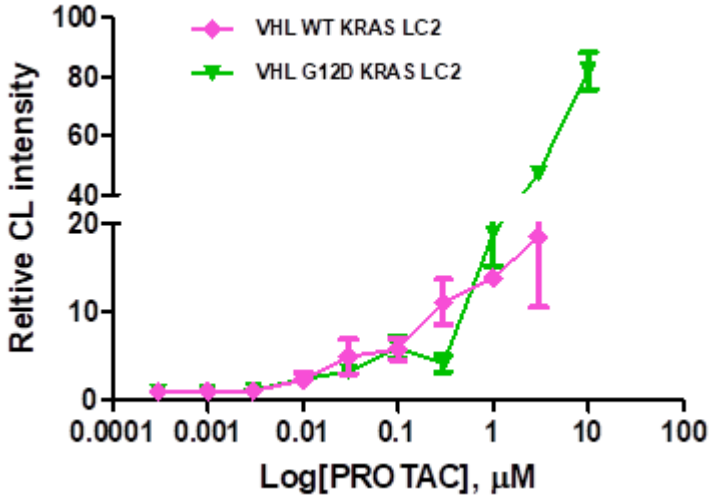

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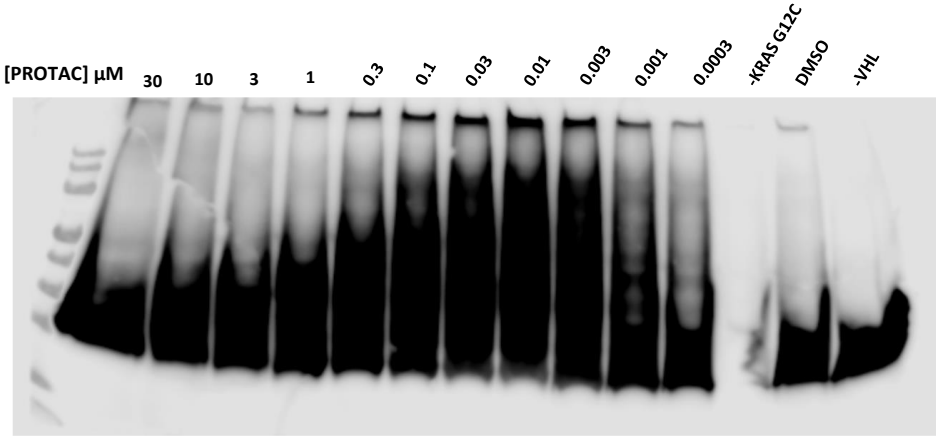


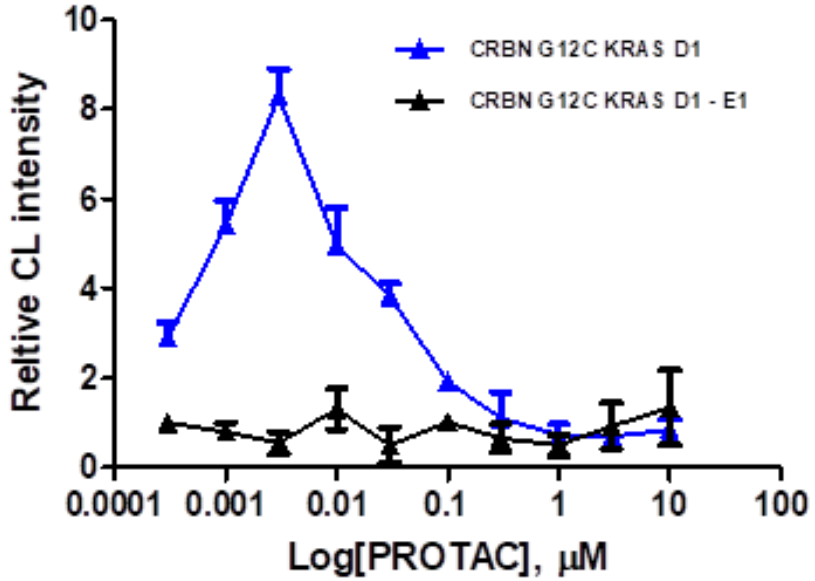
WB: Anti-KRAS

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 ½ 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.

K-RAS PROTACs

CRBN PROTAC mediated in vitro ubiquitination

A



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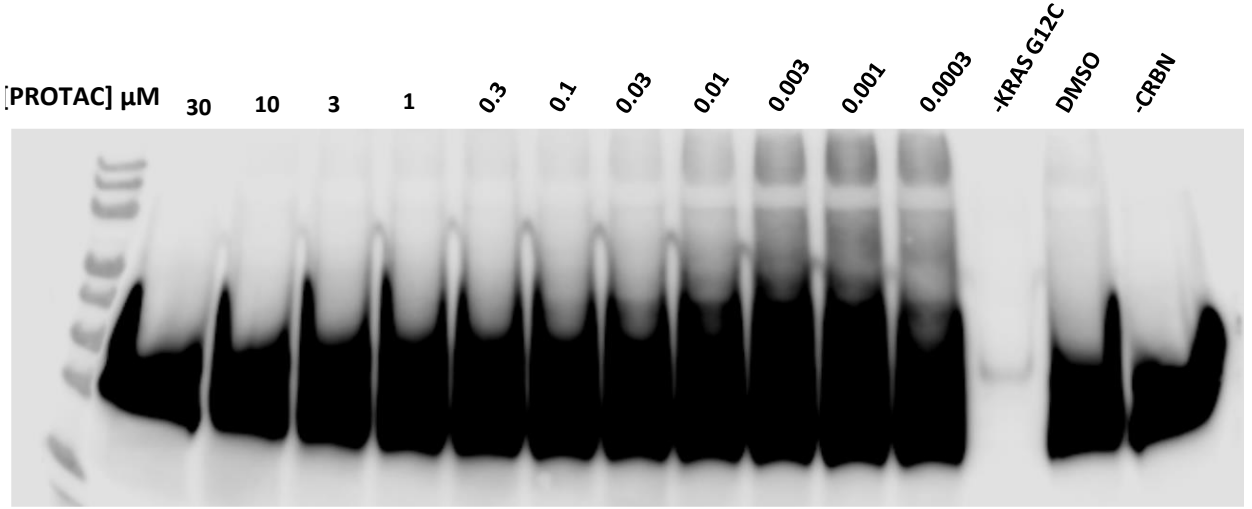
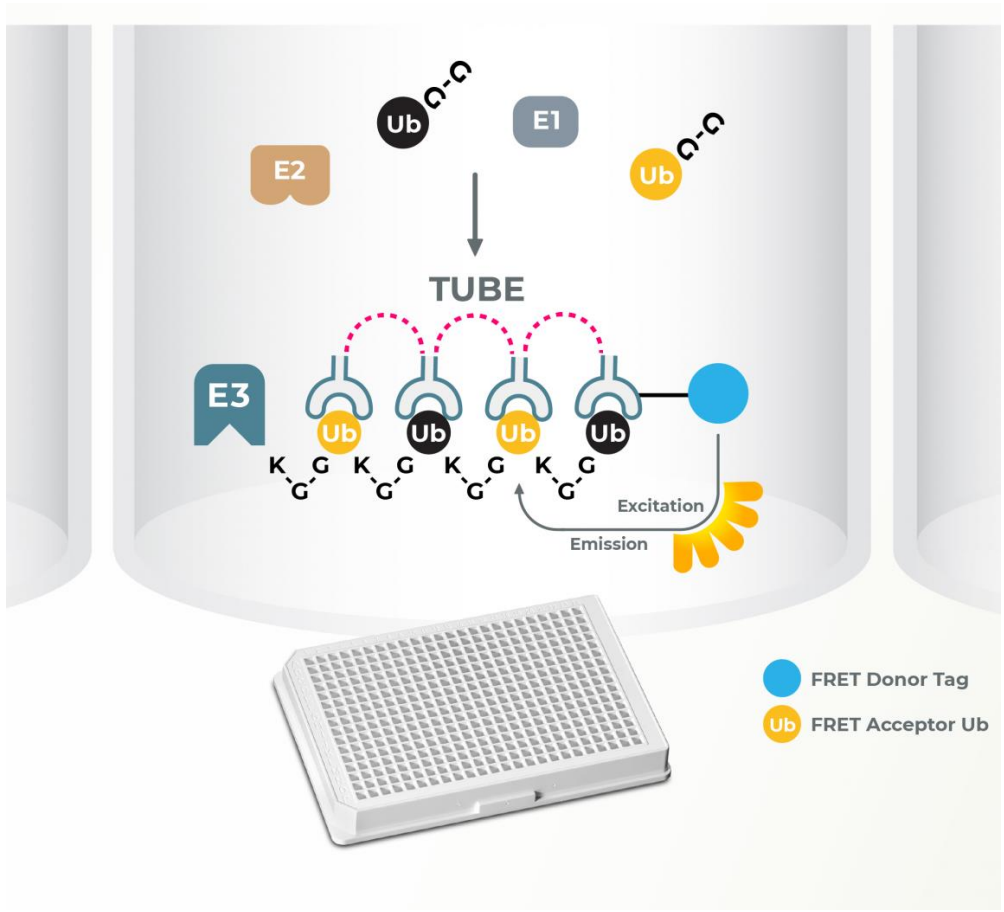


WB: Anti-KRAS

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 ½ 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.

Novel E3 Ligase for KRAS

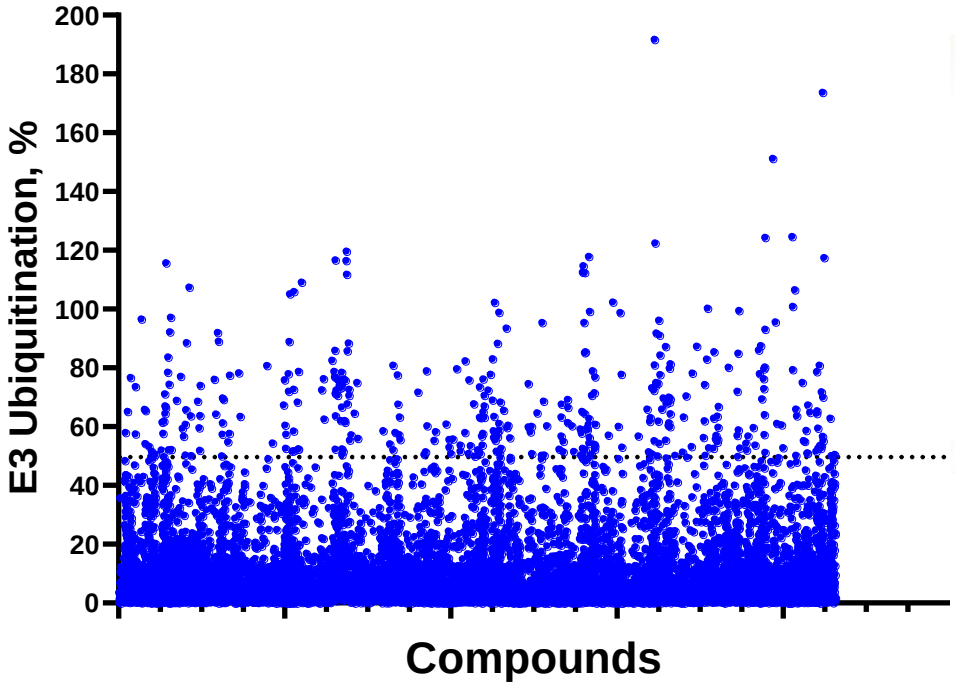


- ✓ High throughput screening for E3 ligase activators
- ✓ Homogenous assay for library screening
- ✓ Identification of novel E3 ligands
- ✓ SPR / TSA based confirmation and PROTACability

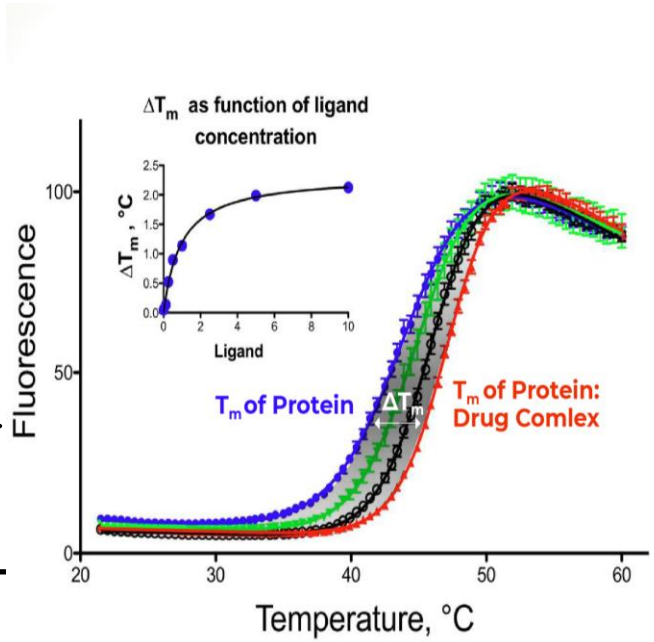
Discovery of Novel E3 Ligase for KRAS

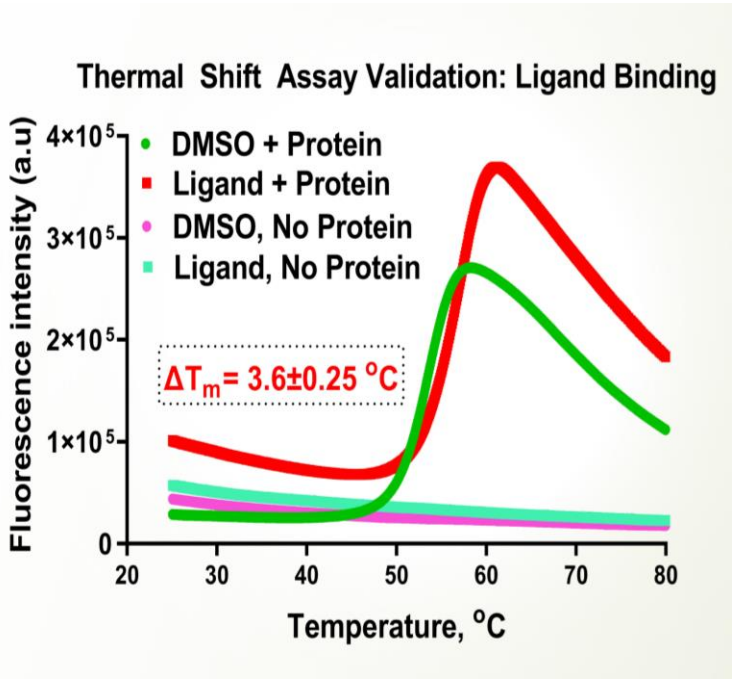
Superior Properties as compared to VHL and CBRN

50K compound library screening

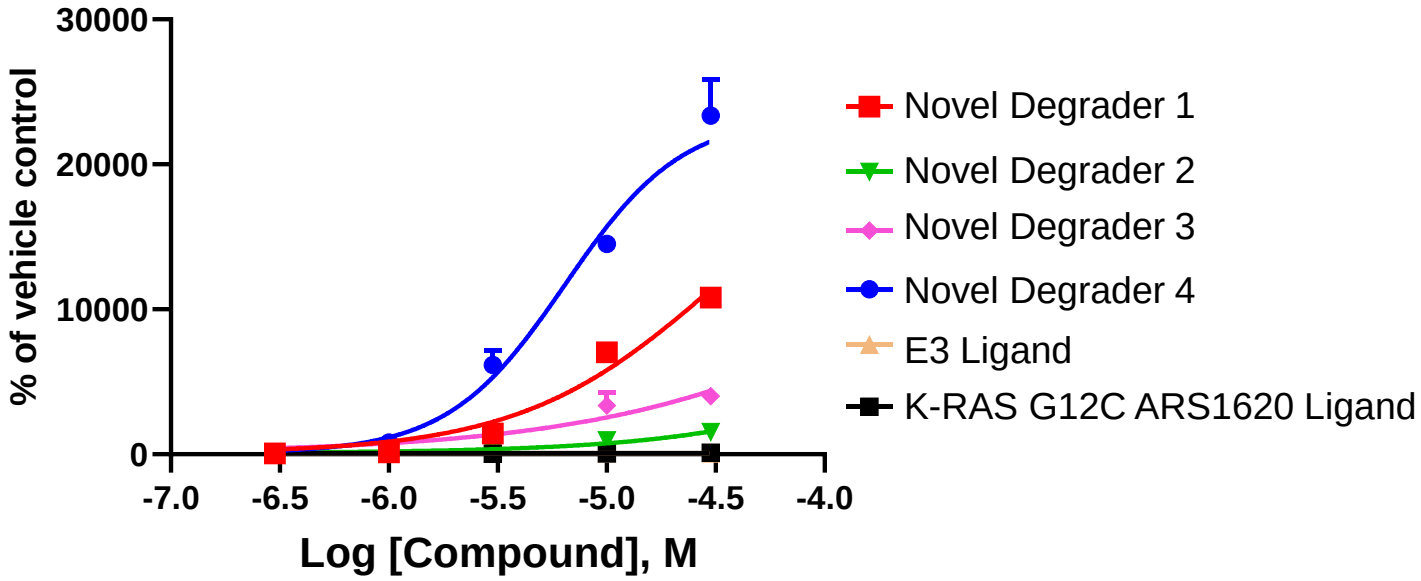


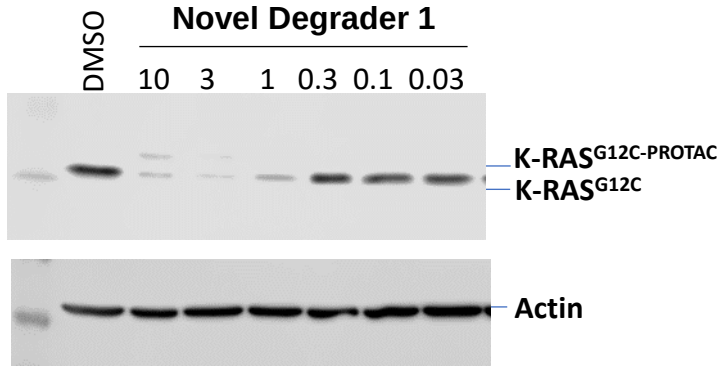
Thermal Shift Assay Validation

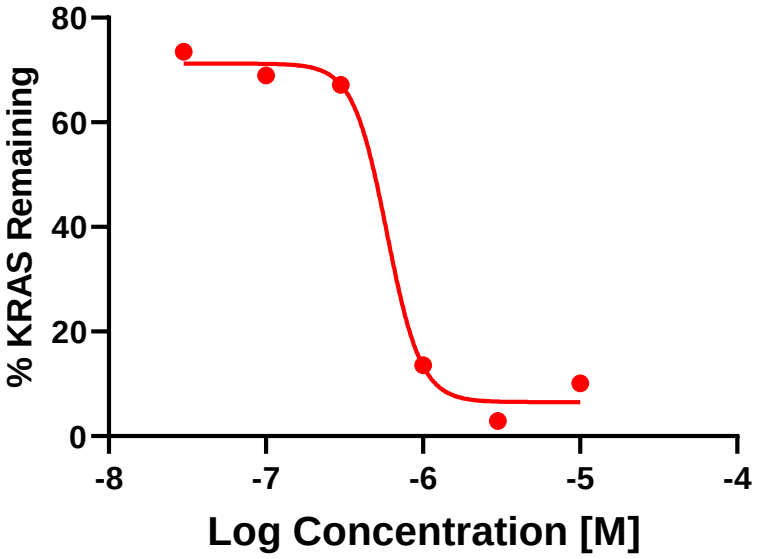




Novel E3 Ligand with ARS-1620 for KRAS Degradation

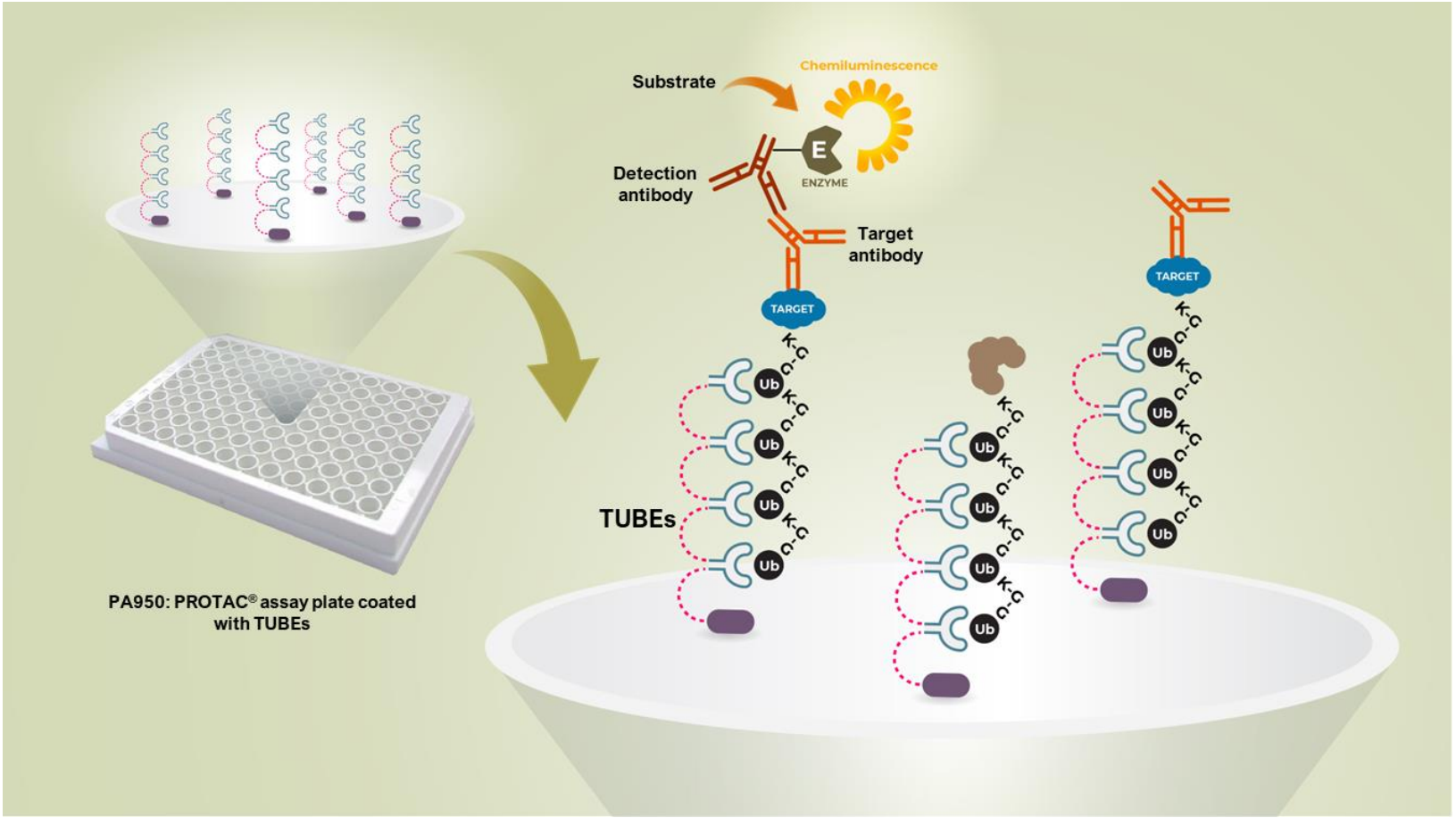




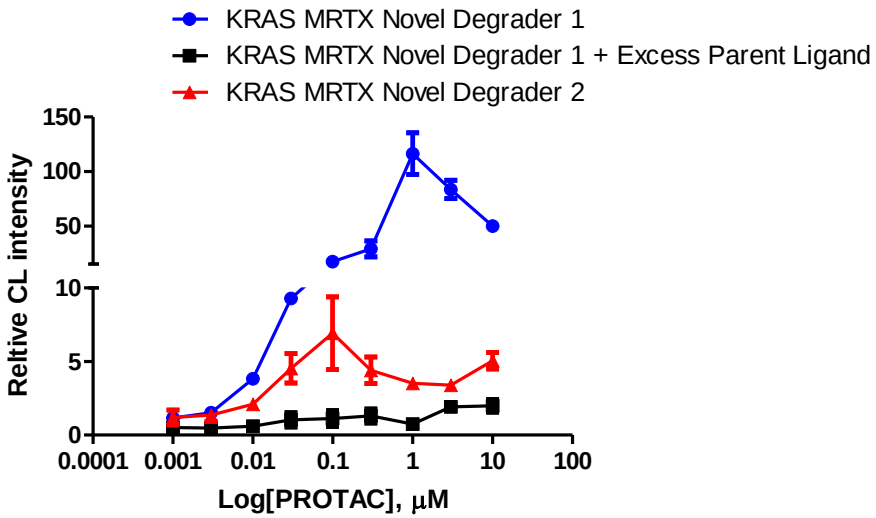


(A) *In vitro* ubiquitination assay with ARS 1620 KRAS G12C Novel E3 based degraders : dose response study to monitor PROTAC mediated ubiquitination of KRAS G12C. CL intensities plotted in response to ½ log dose response demonstrates PROTAC mediated ubiquitination. (B) Cell based assay to monitor degradation of KRAS G12C in H358 cells - PROTAC mediated degradation in a dose response study. (C) Representation of D_{max} as dose dependent degradation.

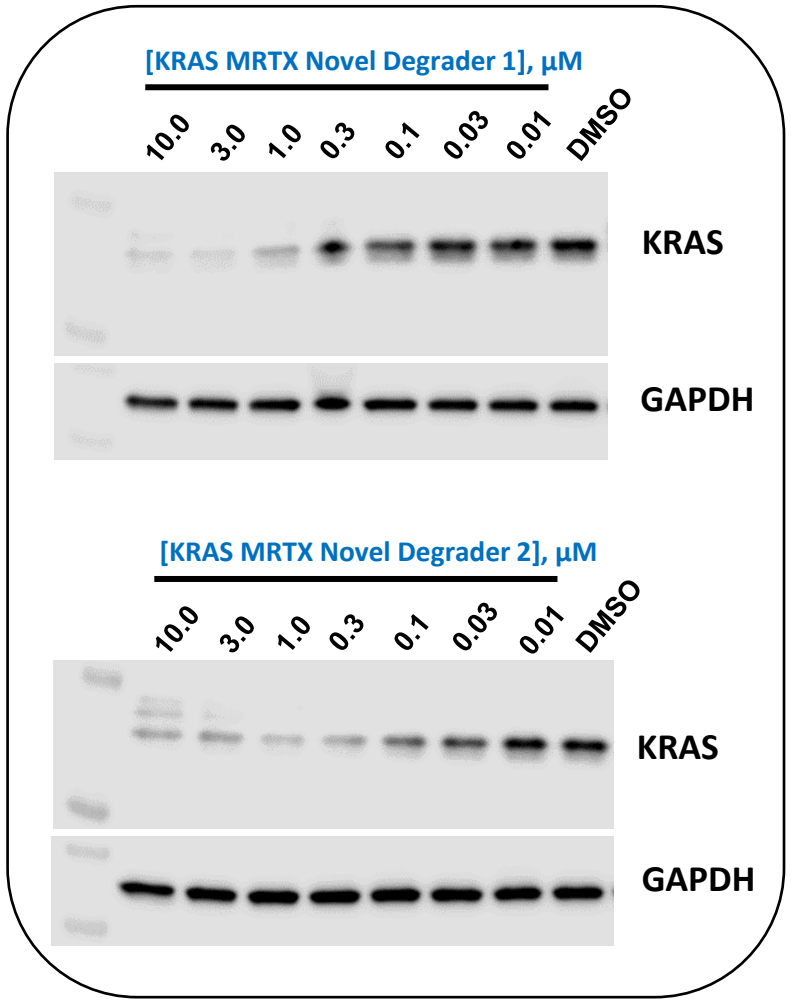
K-RAS Cellular Ubiquitination and Degradation

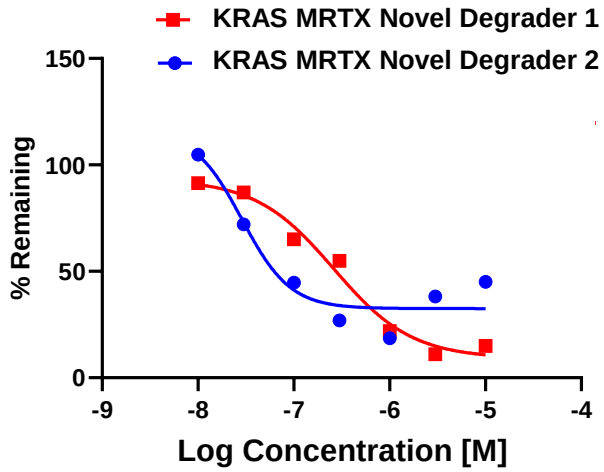


Novel E3 Ligand with MRTX-849 for KRAS Degradation



In vitro Ubiquitination



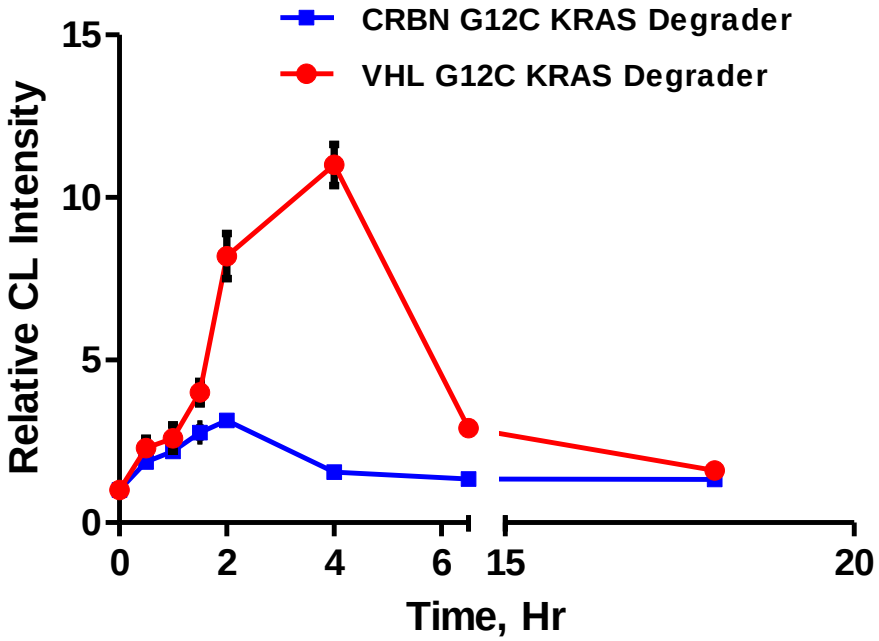


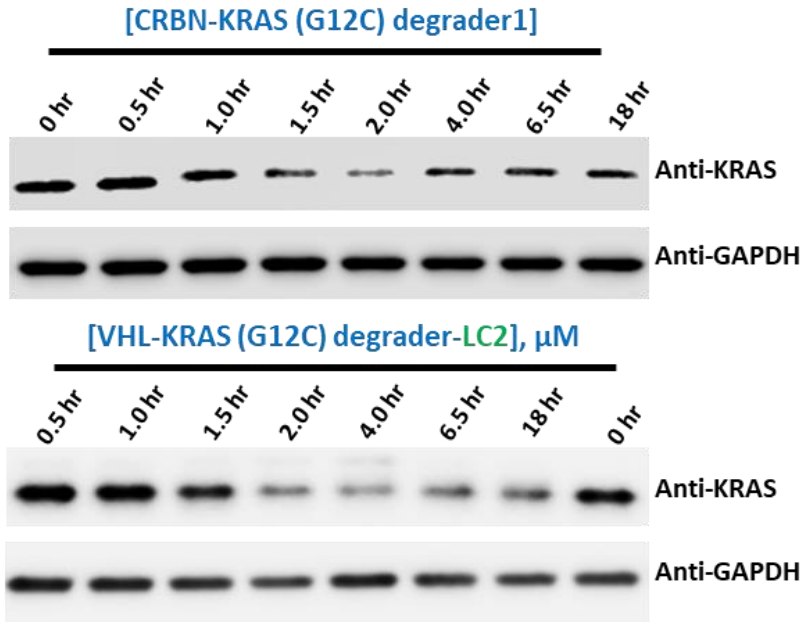
Cellular Degradation

CRBN & VHL KRAS Degraders

Monitor PROTAC mediated Cellular Ubiquitylation and Degradation

Time Course Study - HTS



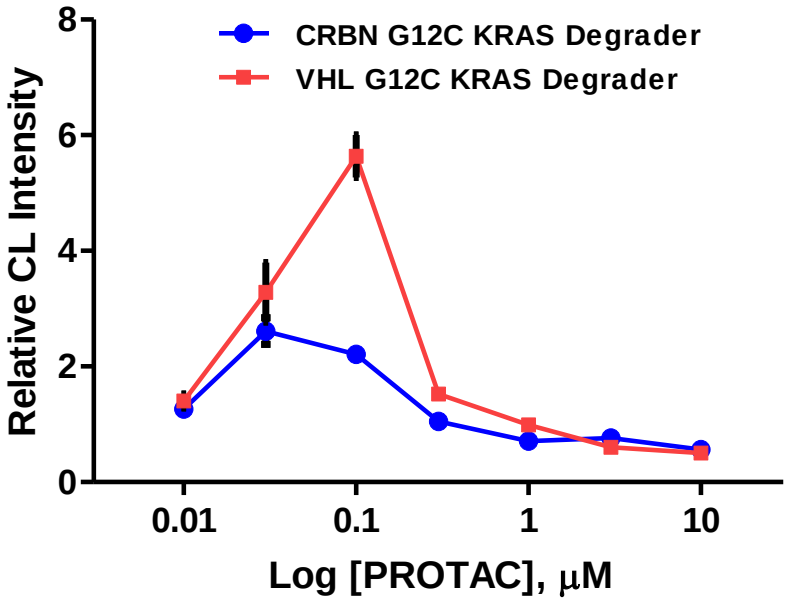


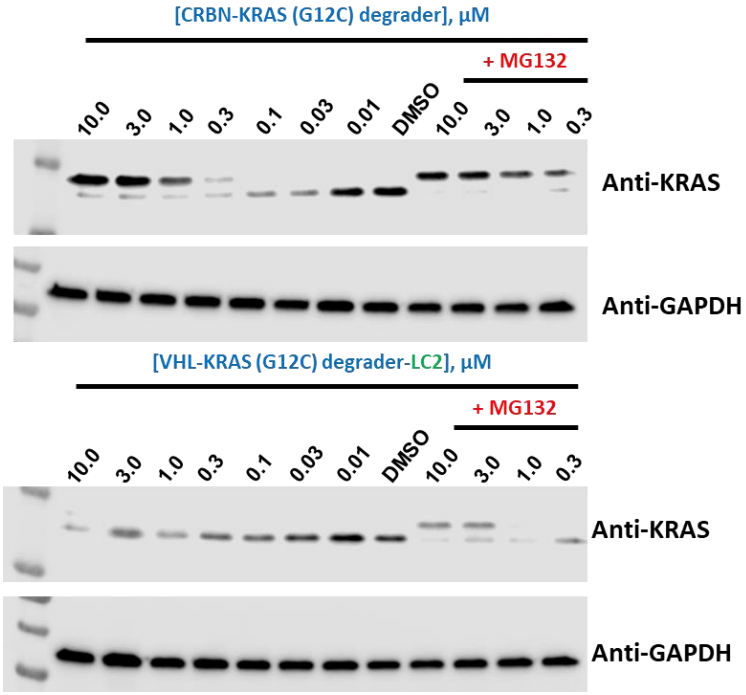
Monitoring Ubiquitination Kinetics – changes in PROTAC mediated ubiquitination profiles of endogenous KRAS and subsequent degradation in H358 cells. VHL and CRBN PROTACs designed with covalent ligands to engage KRAS G12C and successfully ubiquitinate and degrade within 2-4hrs of treatment.

CRBN & VHL KRAS Degraders

Monitor PROTAC mediated Cellular Ubiquitylation and Degradation

Dose Response Study - HTS

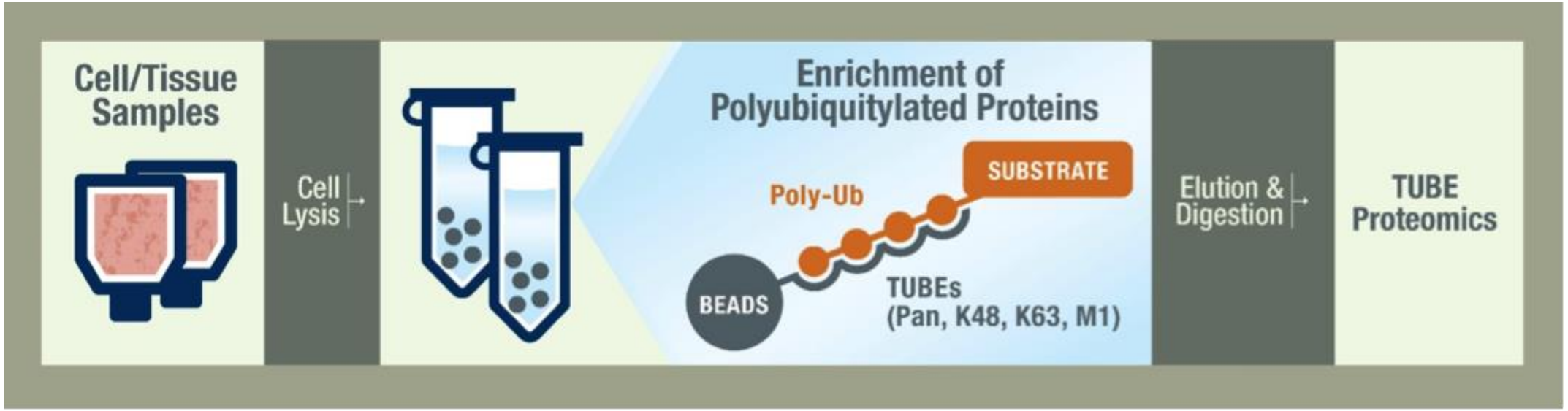




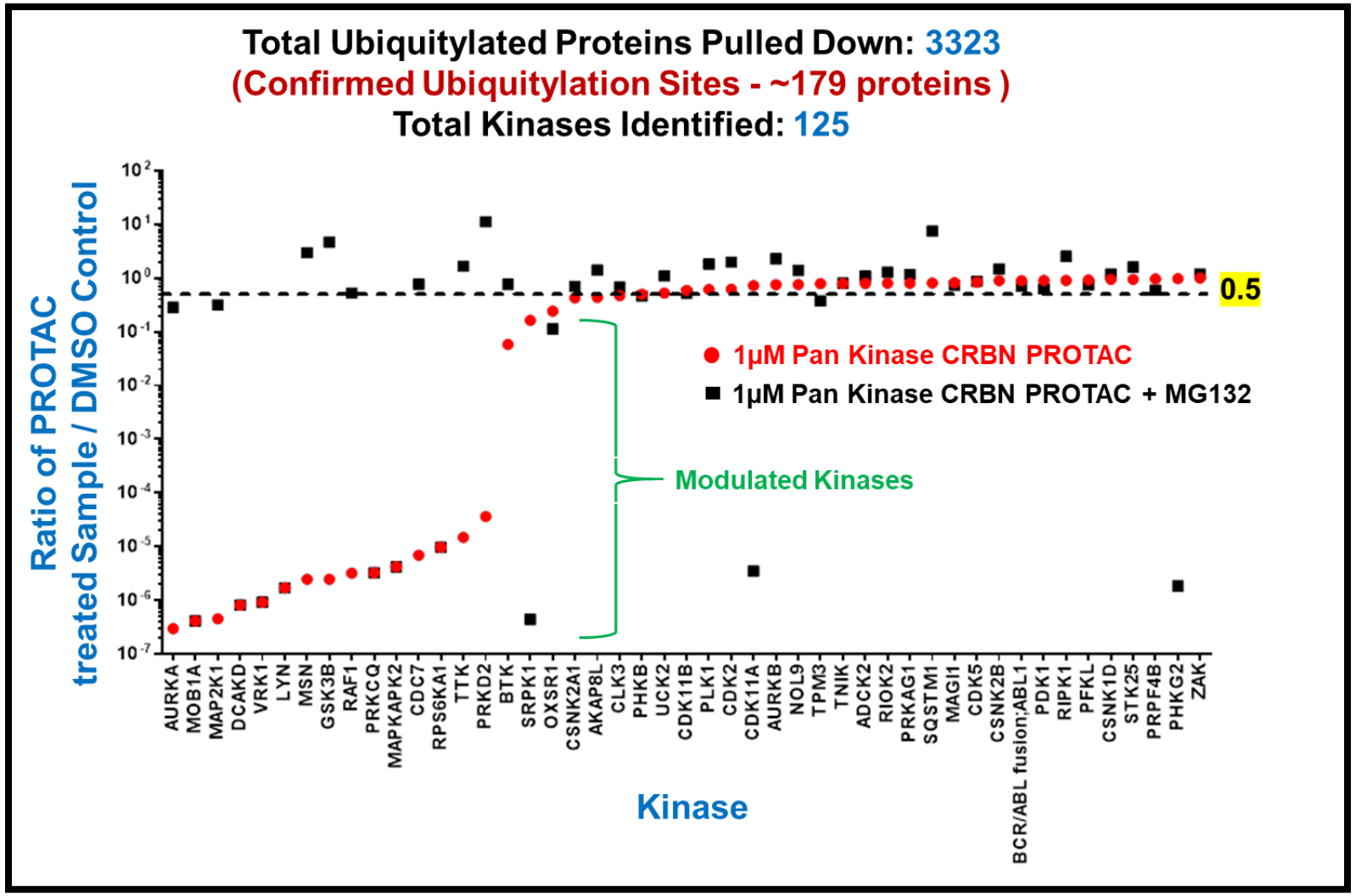
Monitoring Ubiquitination – Dose Response : changes in ubiquitination profiles of endogenous KRAS and subsequent degradation in H358 cells with changes in dose of both VHL and CRBN KRAS degraders. VHL and CRBN PROTACs designed with covalent ligands to engage KRAS G12C and successfully ubiquitinate and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

TUBE Based Ubiquitin Mass spec Proteomics

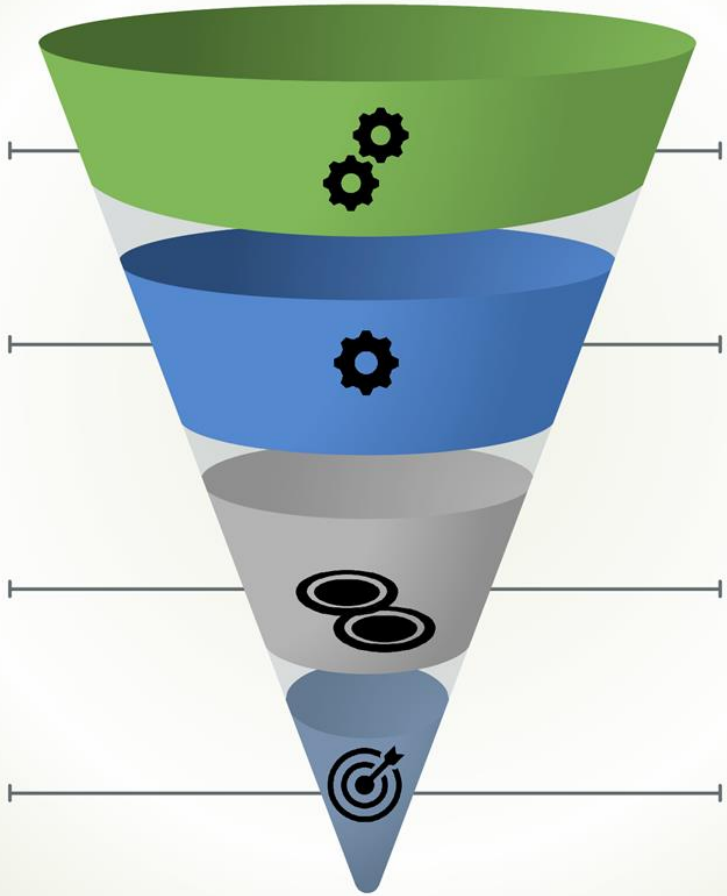
To Validate PROTAC Mediated Ubiquitination and Degradation



Mass Spectrometry Studies with Pan Kinase Degrader



Pathway to PROTAC Drug Discovery



IN VITRO ASSAY DEVELOPMENT
E3 ligase and substrate purification

EC50 & RANK ORDER POTENCY
Identify high value PROTACs for cell-based assays

CELL-BASED ASSAY DEVELOPMENT
Substrate ubiquitylation and degradation

MASS SPEC & PROTAC SELECTIVITY
TUBE-based to establish total ubiquitome changes

ASSAY VALIDATION & COMPOUND SCREENING
HTS to monitor substrate ubiquitylation

COUNTER SCREENS & VALIDATION
Western blotting to establish substrate ubiquitylation

VALIDATION & ESTABLISH DC50
Validate ubiquitylation via WBs and UbiTest

METHOD TRANSFER
Transfer technology for further validation studies

- ✓ **TUBEs based PROTAC® Assays provides a link between ubiquitination and degradation**
- ✓ **Robust SAR with true function of ubiquitination for development of potent degraders**

Contact Us!

We are your partner for PROTAC drug discovery

Contact Information

Research & Product Inquiries	**R&D**	**info@lifesensors.com**	**610-644-8845 (ext 339)**
Custom Service & Assays	**BD**	**bd@lifesensors.com**	**610-644-8845 (ext 354)**