

Rapid Assays for Accelerating PROTAC[®] & Molecular Glue Drug Discovery

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Director, R&D



Leading Biotech in UPS based Drug Discovery



500 Products supporting UPS research



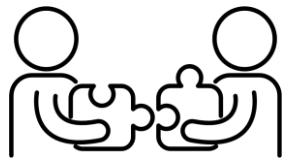
E3 ligase and DUB compounds profiling



Discovery services for new E3 ligands

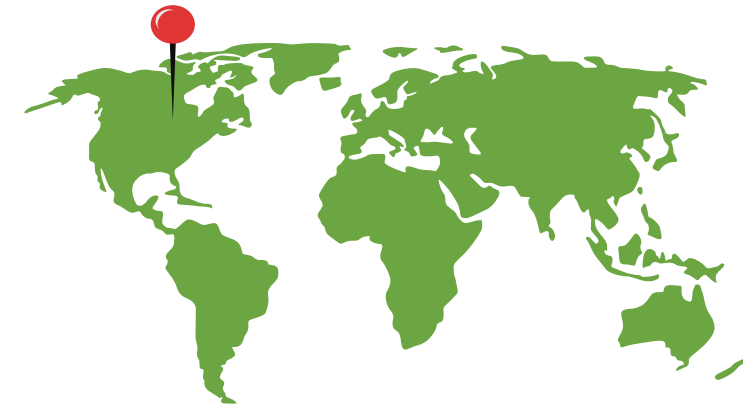


Custom PROTAC and mol glue assay development

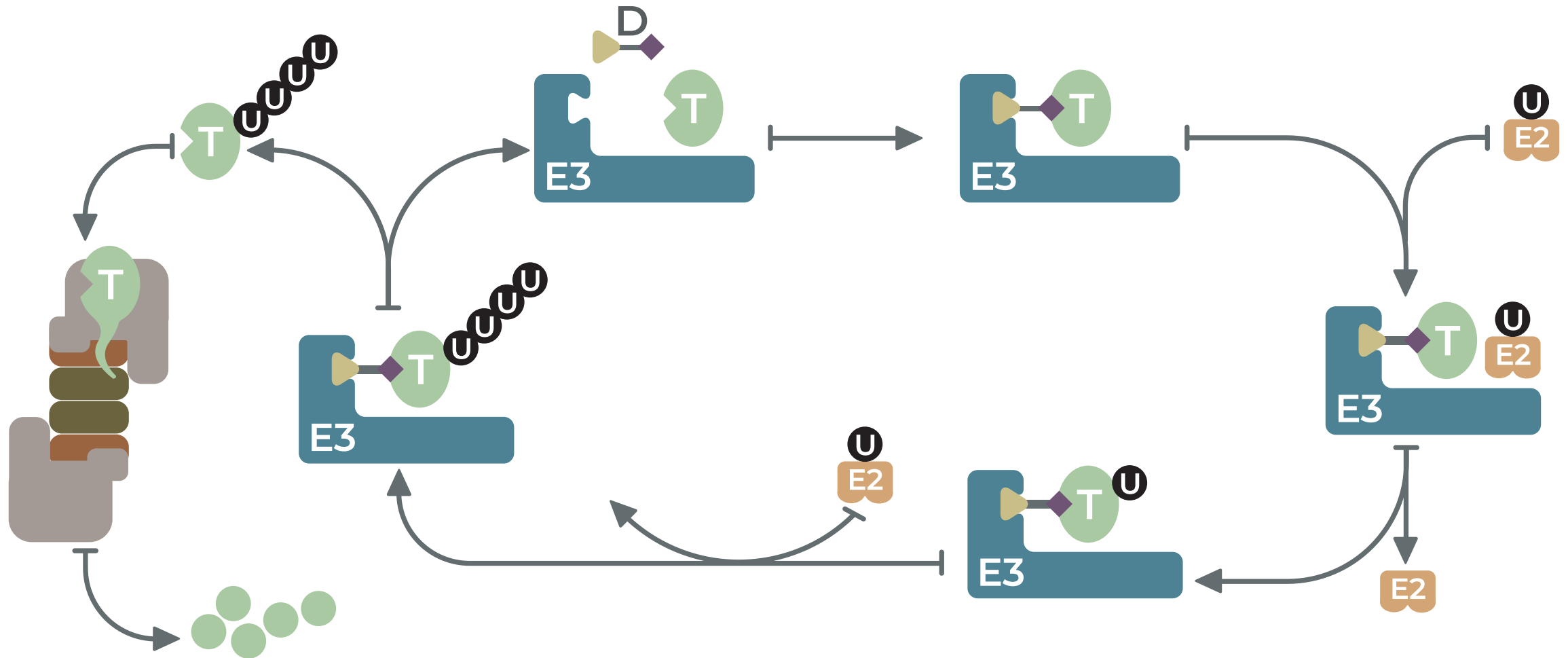


Collaborative and Independent Research

Malvern, Pennsylvania, USA

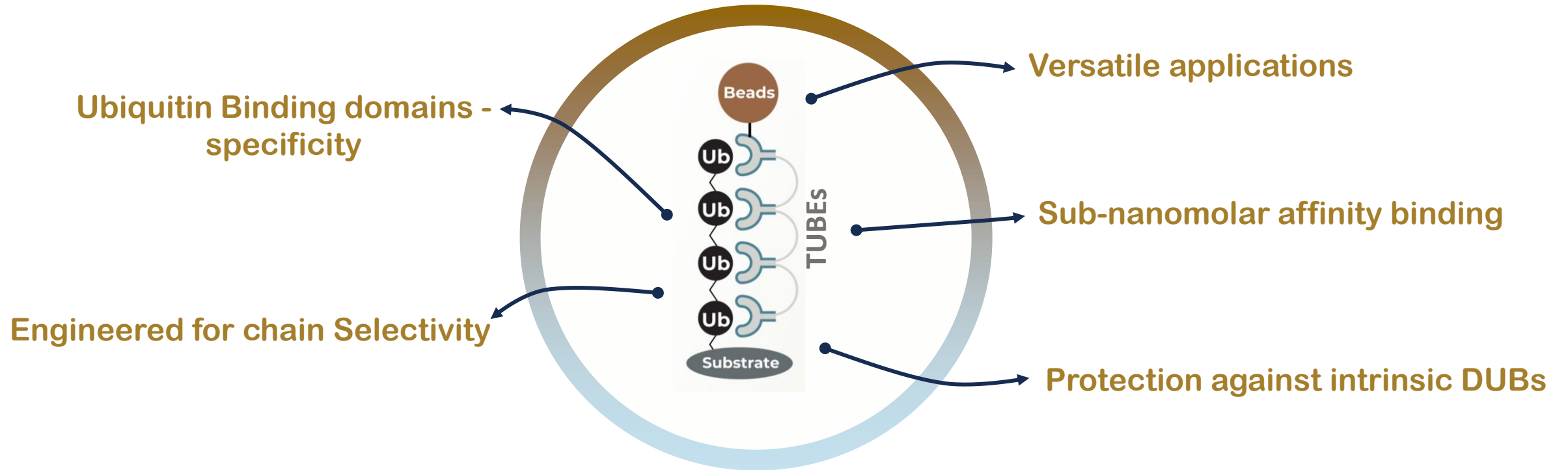


PROTAC[®]s & MGs - Essential Catalytic Activators

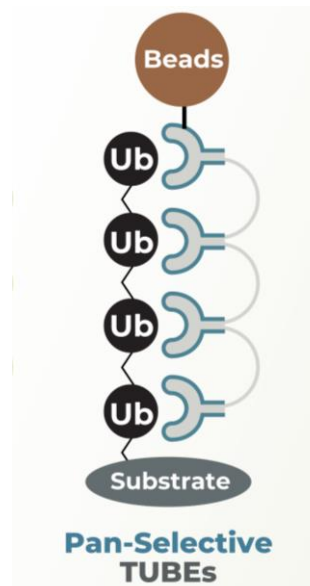
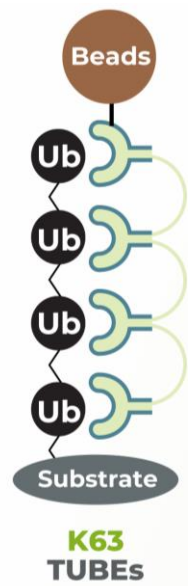
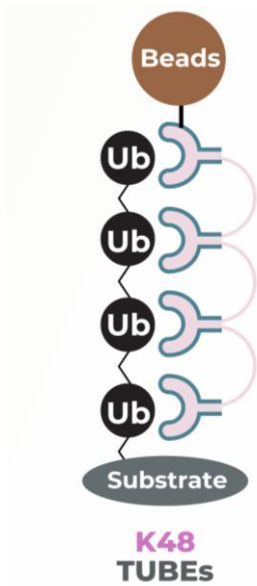
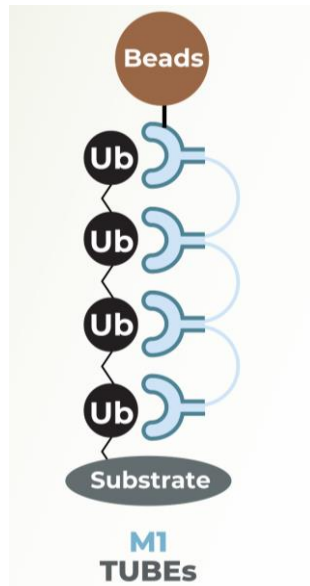


“ LifeSensors makes it easy to study Ubiquitination ”

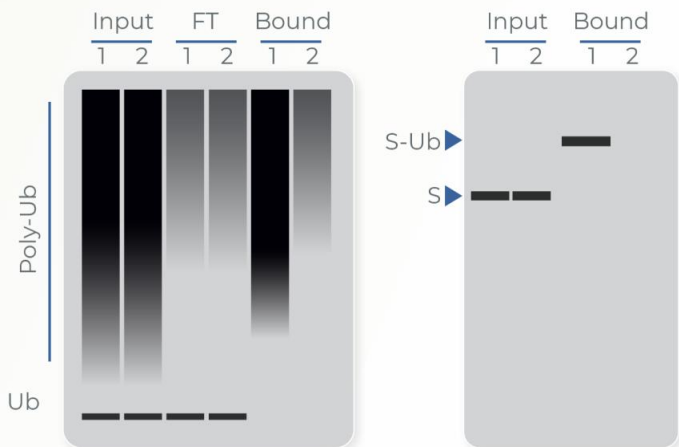
TANDEM_UBIQUITIN_BINDING_ENTITIES (TUBES)



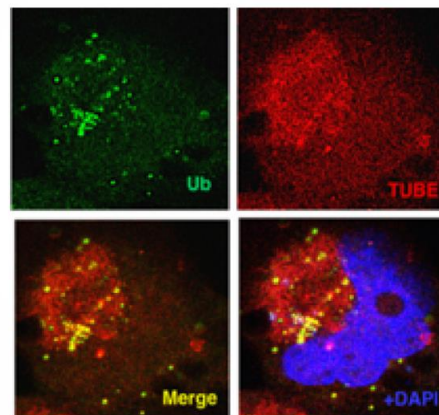
TANDEM UBIQUITIN BINDING ENTITIES (TUBE_s)



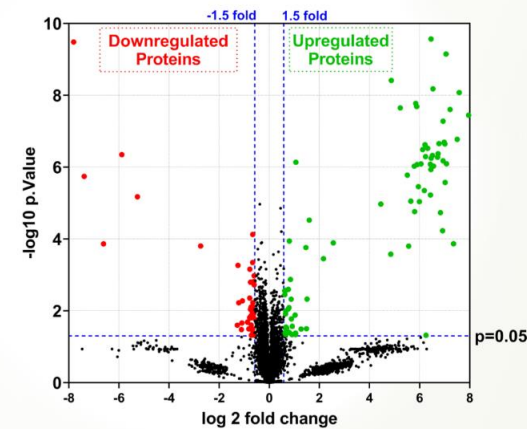
Poly-Ubiquitin Substrate Enrichment & Western Blotting



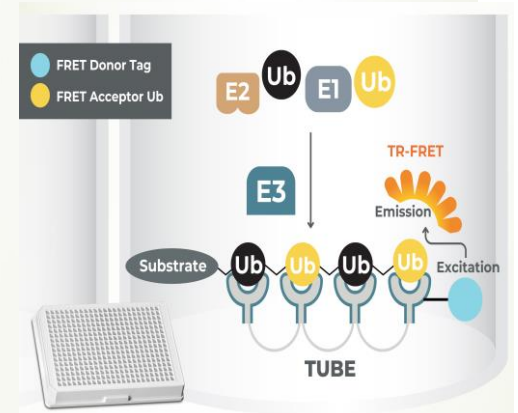
Fluorescent Microscopy



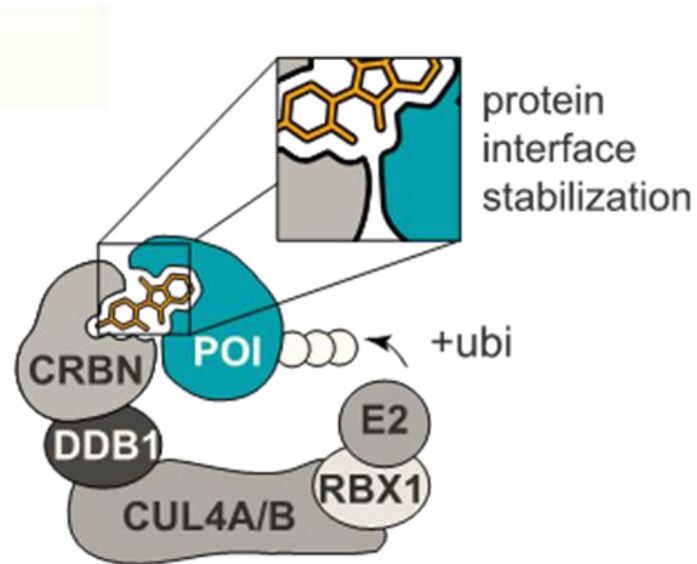
Mass Spectrometry Ubiquitomics



High-Throughput Screening

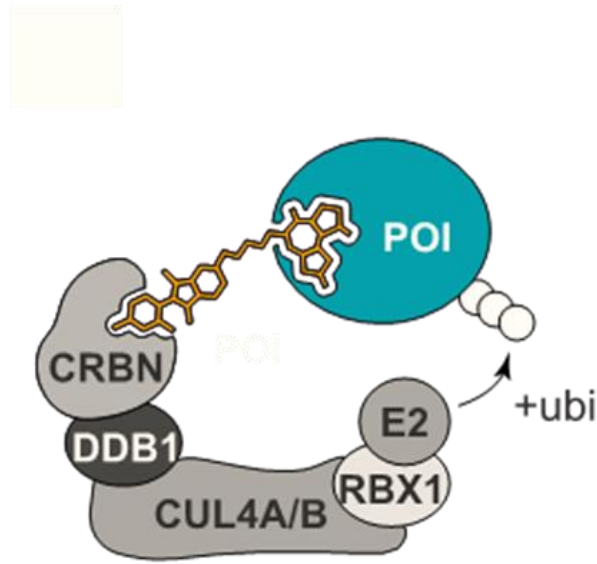


Targeted Protein Degradation



Molecular Glue

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



PROTAC

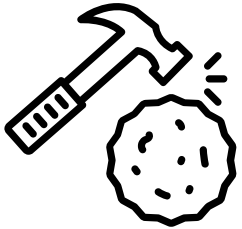
Expectations for PROTACs are very high, but there are developmental challenges

Image credit: <https://www.protein-degradation.org/groups/winter/>

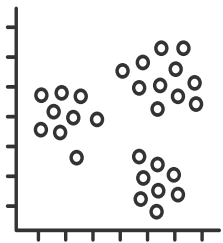
TUBEs address critical TPD challenges



Strong binders / better K_D does not guarantee better degradation



Ternary complex rigidity



Ligand dependent ubiquitination, true functional HTS

LifeSensors approaches standout

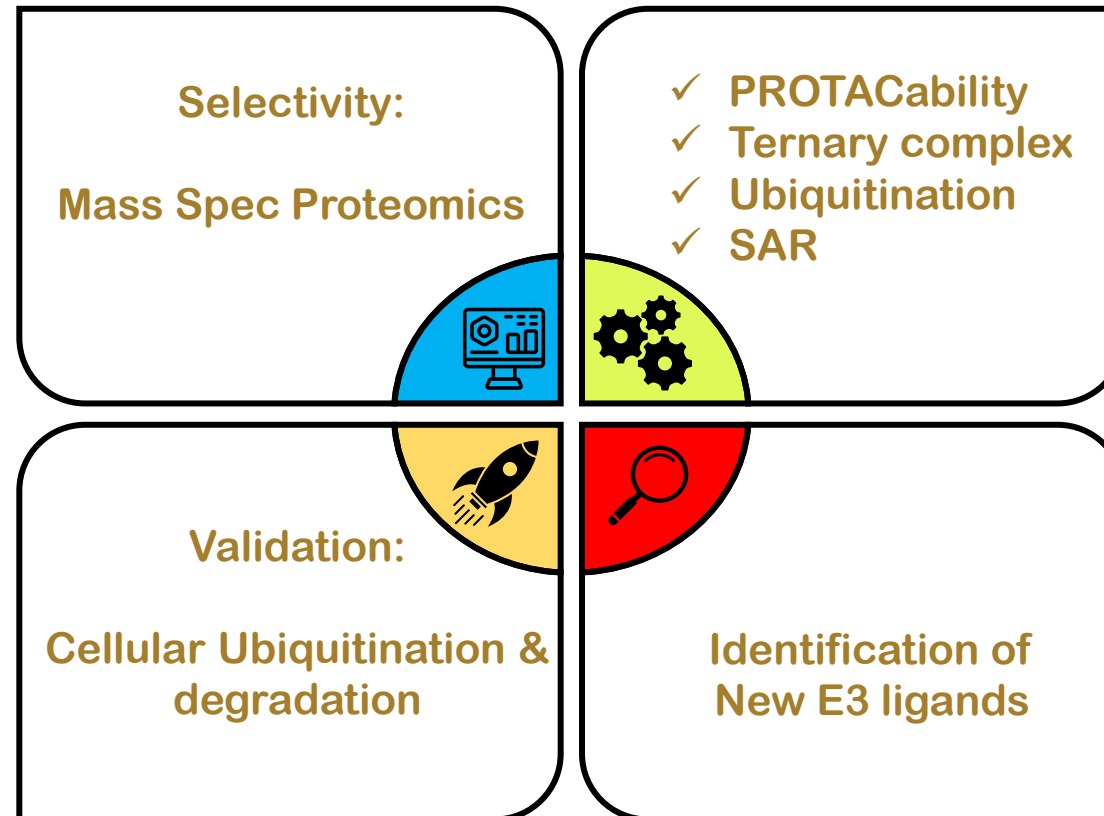
Traditional Approaches

- **Reporter gene assays:**
External tags (Off target ubiquitination)
- **Proximity Ligand Assays**
protein's intrinsic factors - overlooked
- **Western Blotting**
Low throughput and Irreproducible

LifeSensors Approaches

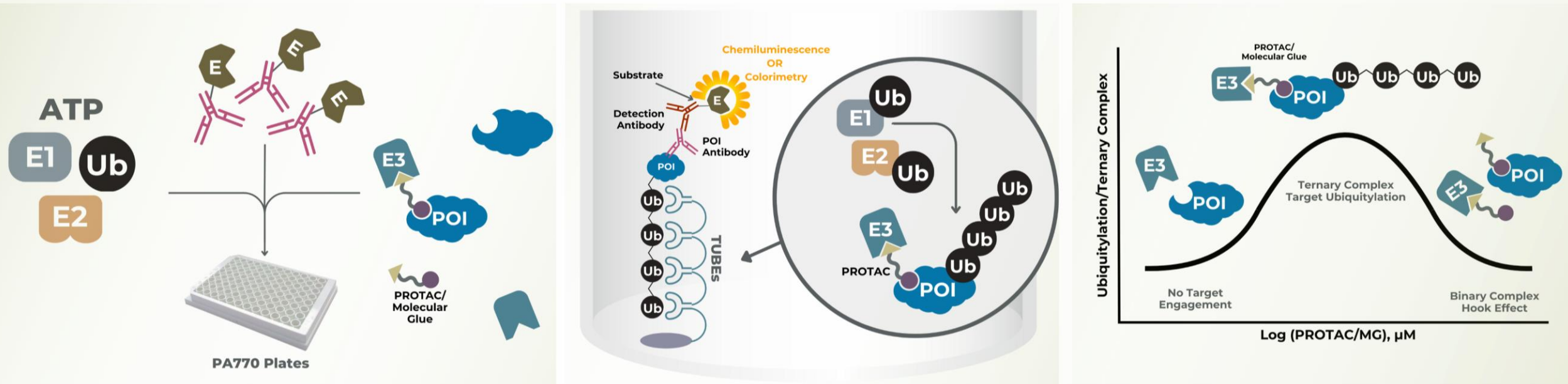
- **Invitro Ubiquitination assays :**
Ligand mediated ubiquitination, fast and functional approach
- **Cellular degradation assays:**
No reporter tags required, applicable to clinical and preclinical studies "Biomarker"
- **HTS screening**
Native lysines playing a role

LifeSensors Approaches for Targeted Protein Degradation



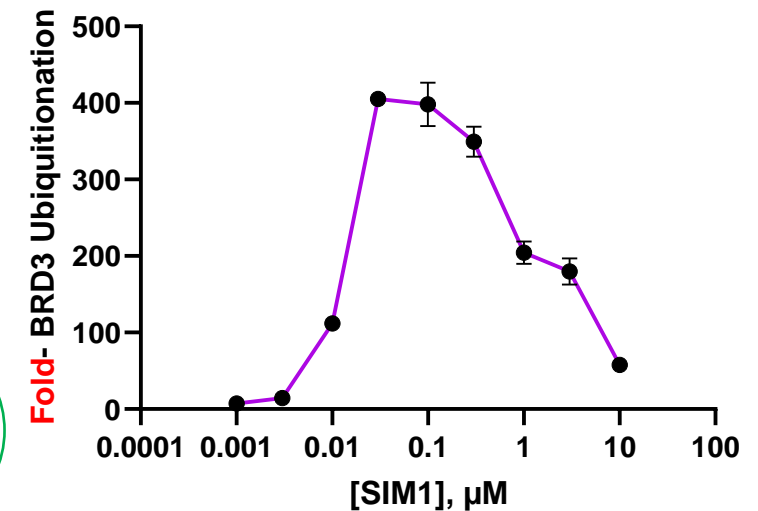
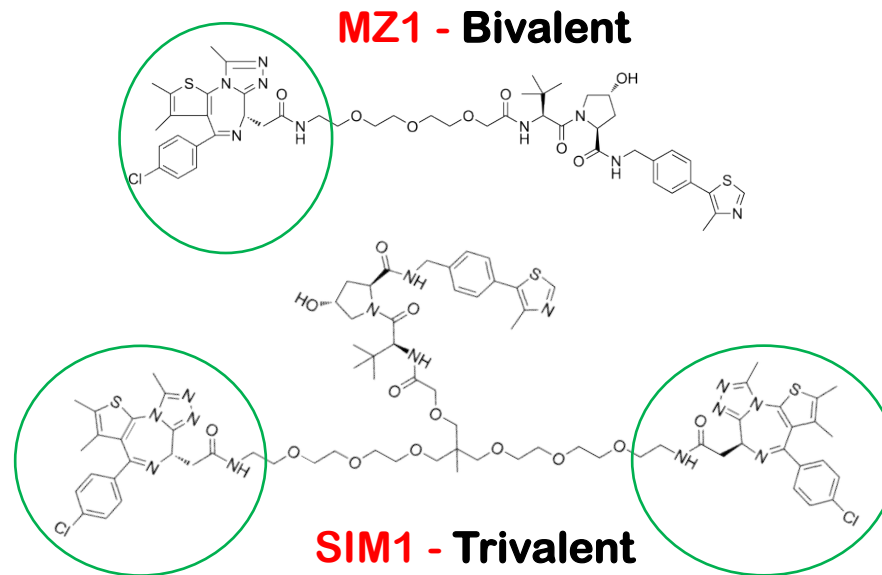
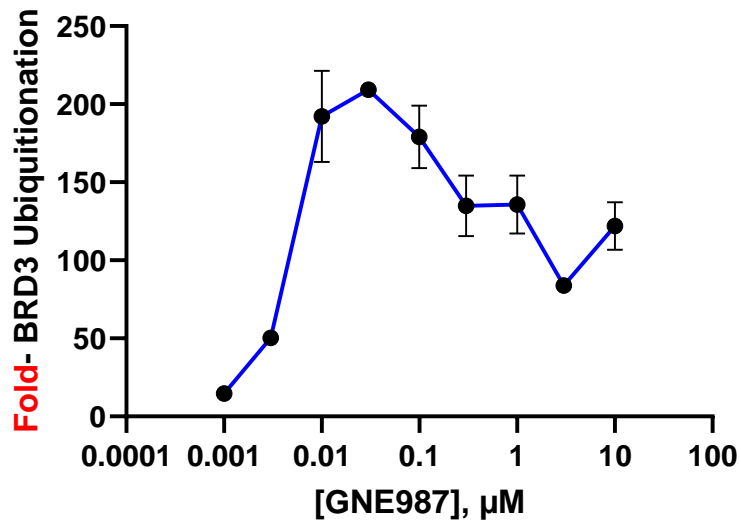
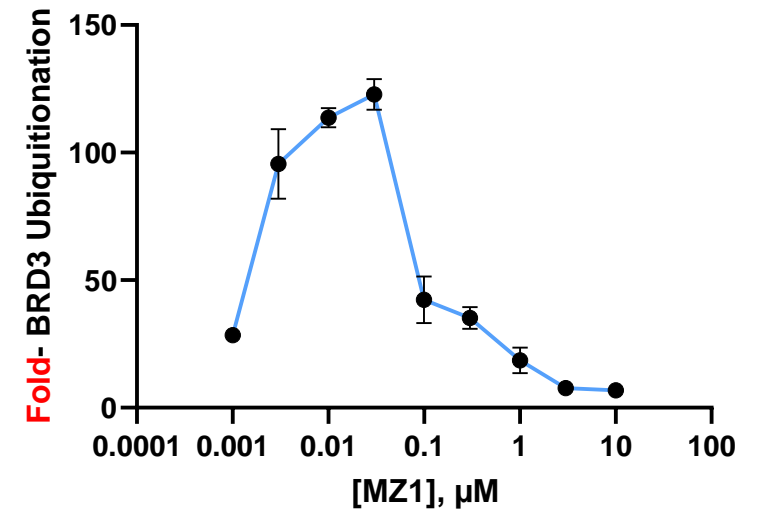
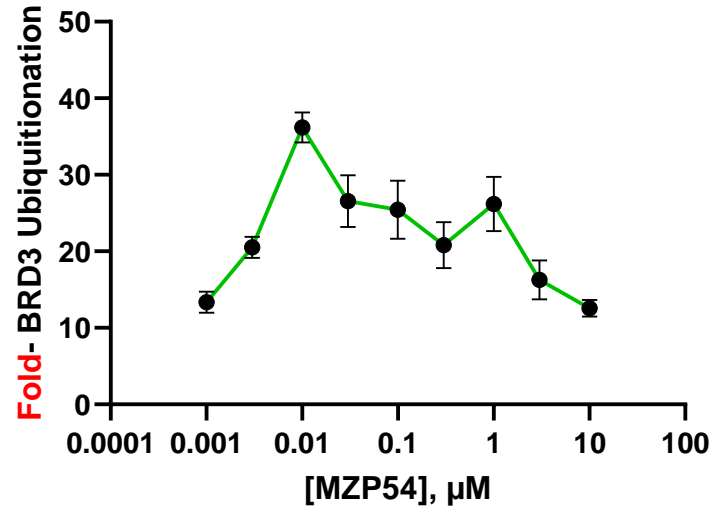
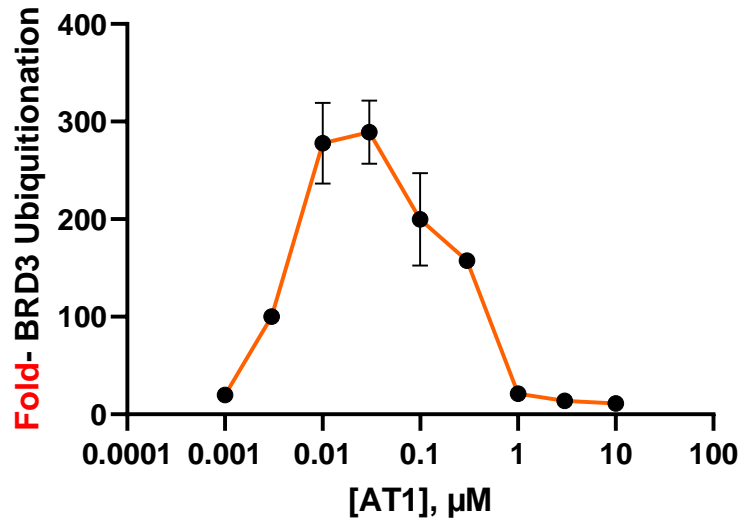
HTS- In Vitro Biochemical Assay

To study functional ternary complex and PROTACability

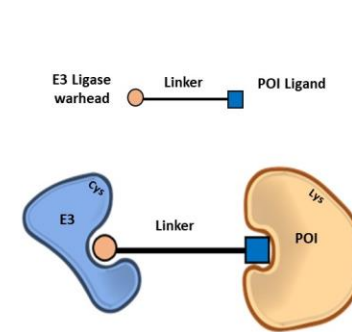
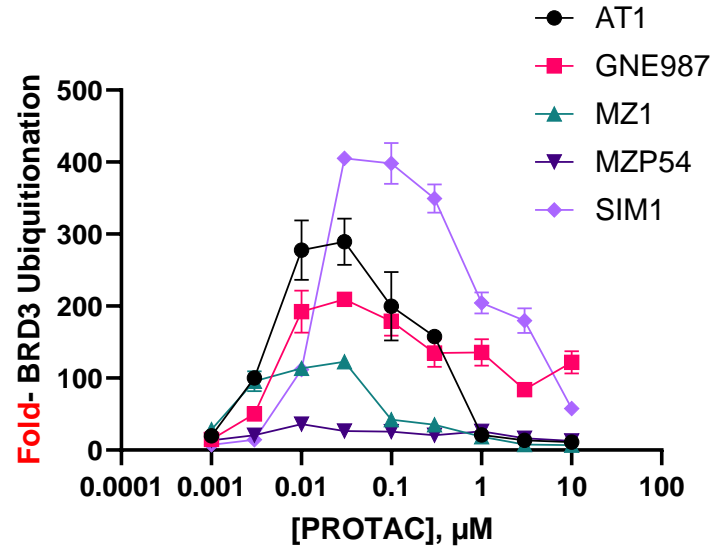
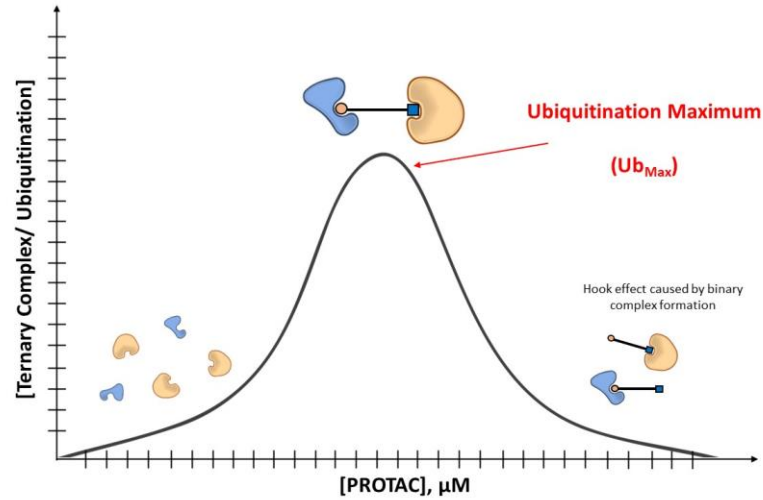


TUBE capture & PROTAC/MG mediated ubiquitination of POI detection

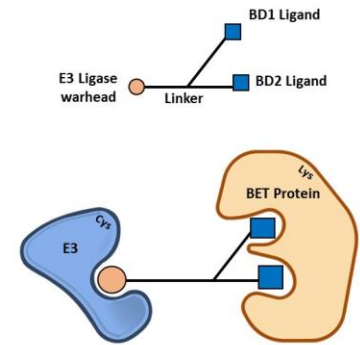
VHL Bromodomain PROTACs



VHL Bromodomain degraders



MZ1 - Bivalent

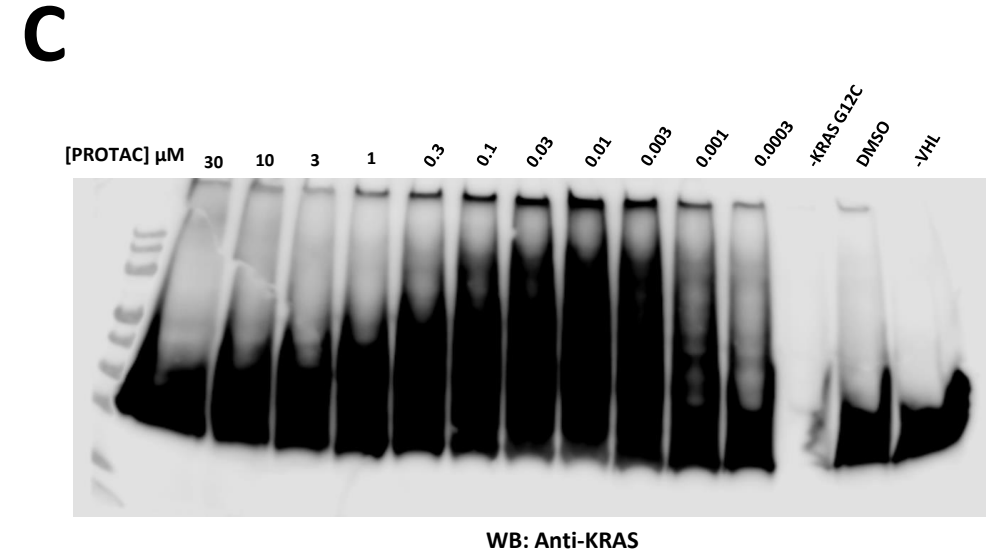
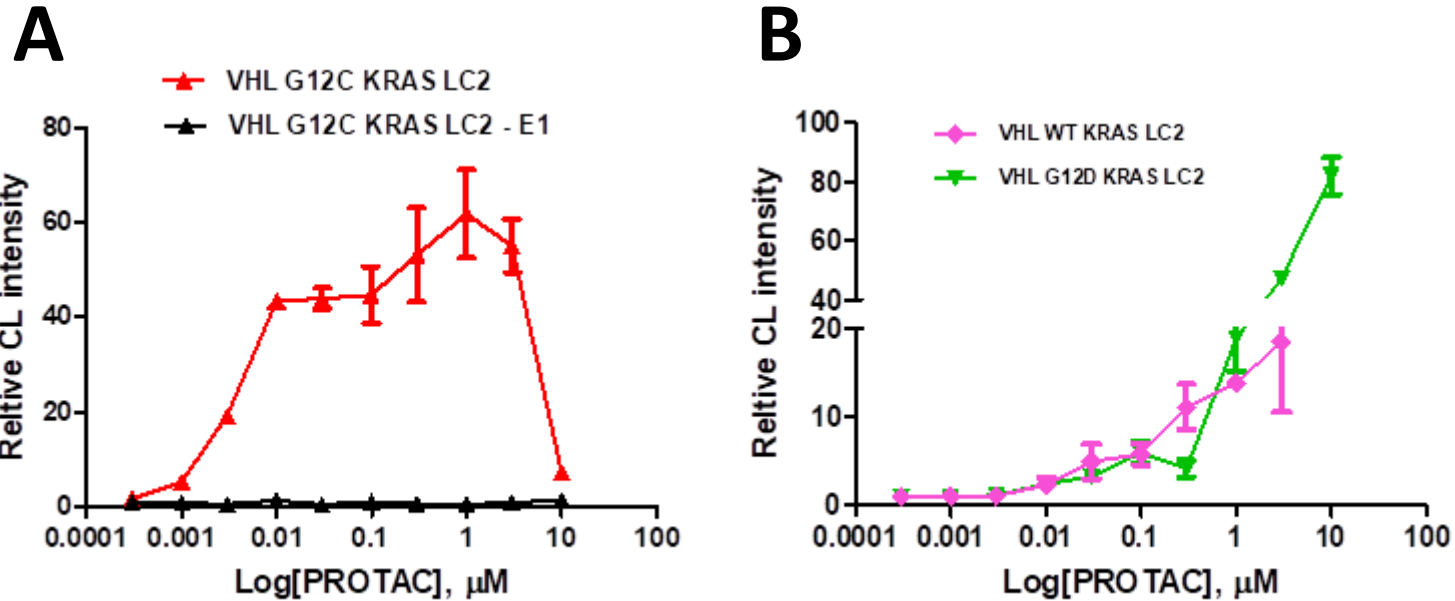


SIM1 - Trivalent

| 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

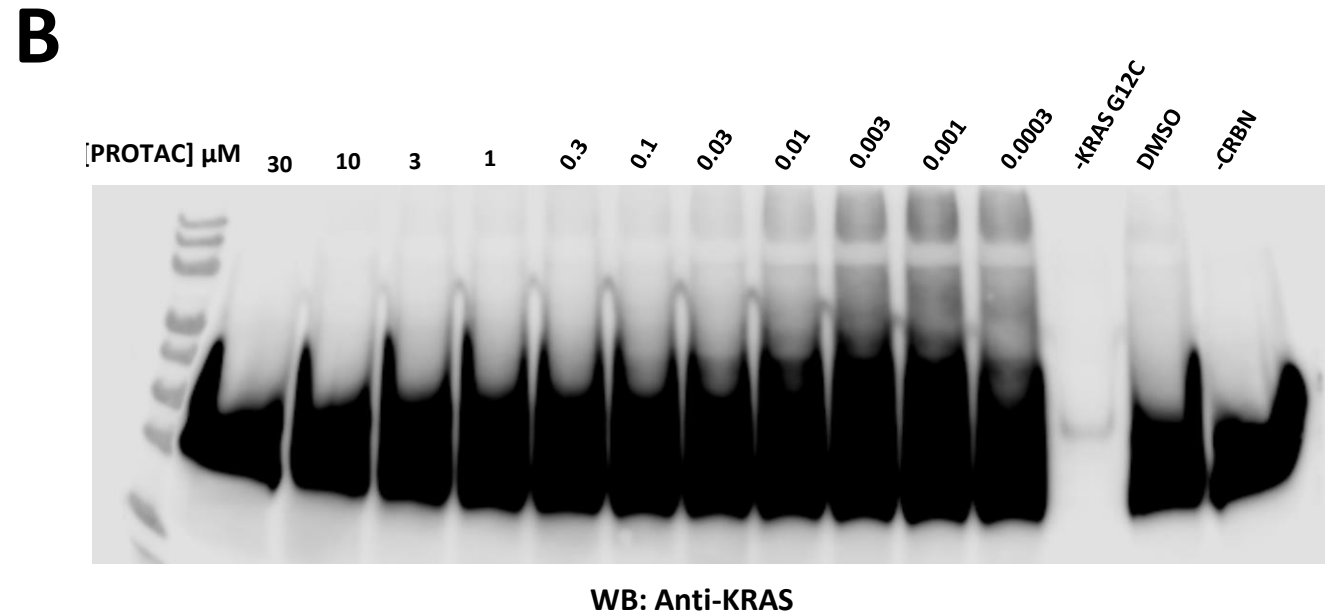
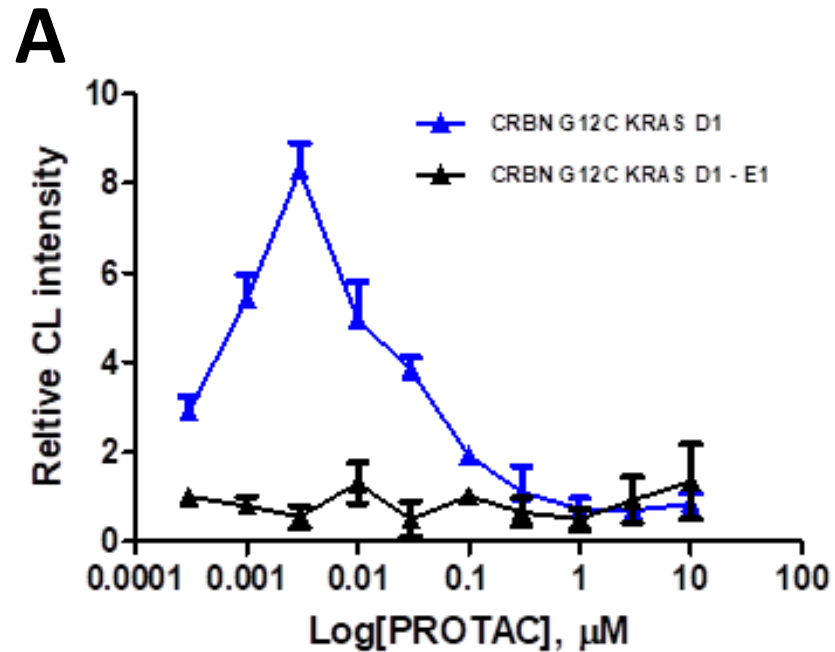
VHL PROTAC mediated in vitro ubiquitination



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.

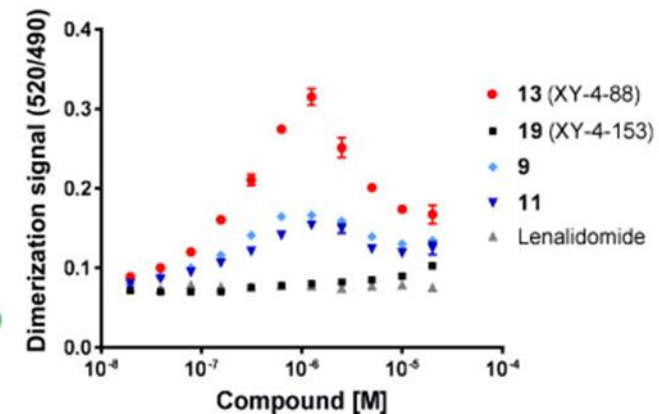
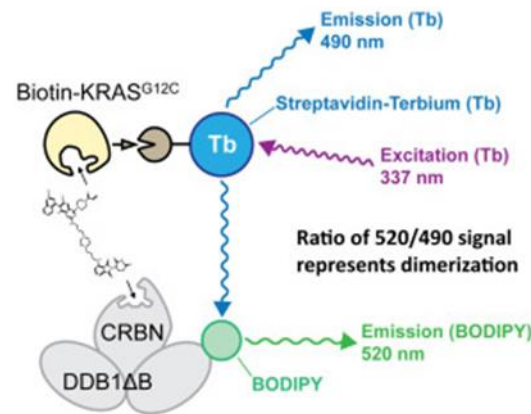
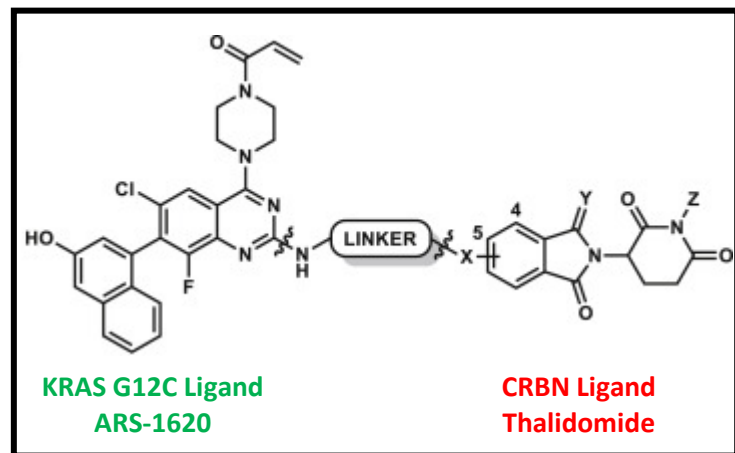
K-RAS PROTACs

CRBN PROTAC mediated in vitro ubiquitination

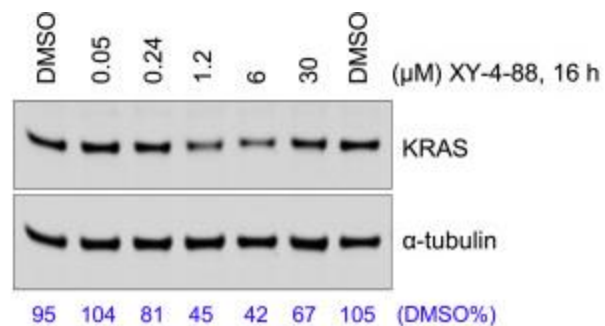


In vitro ubiquitination assay with KRAS G12C CRBN based degraders: ((A) CRBN-based PROTAC degrader 1 (compound 518) in a dose response study to monitor PROTAC mediated ubiquitination of KRAS G12C. CL intensities plotted in response to $\frac{1}{2}$ log dose response demonstrates PROTAC mediated ubiquitination.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

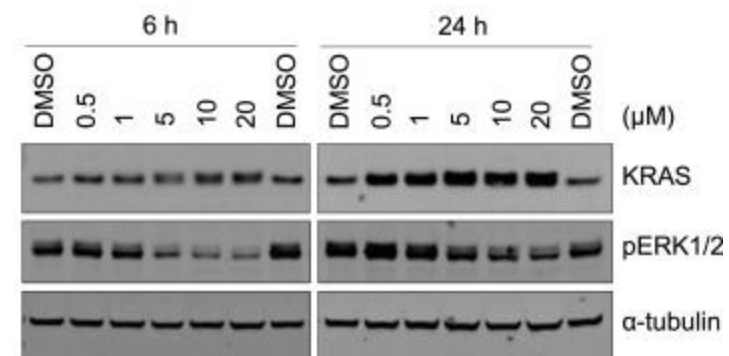
CRBN KRAS Non-degradative PROTACs



TR-FRET- based dimerization assay



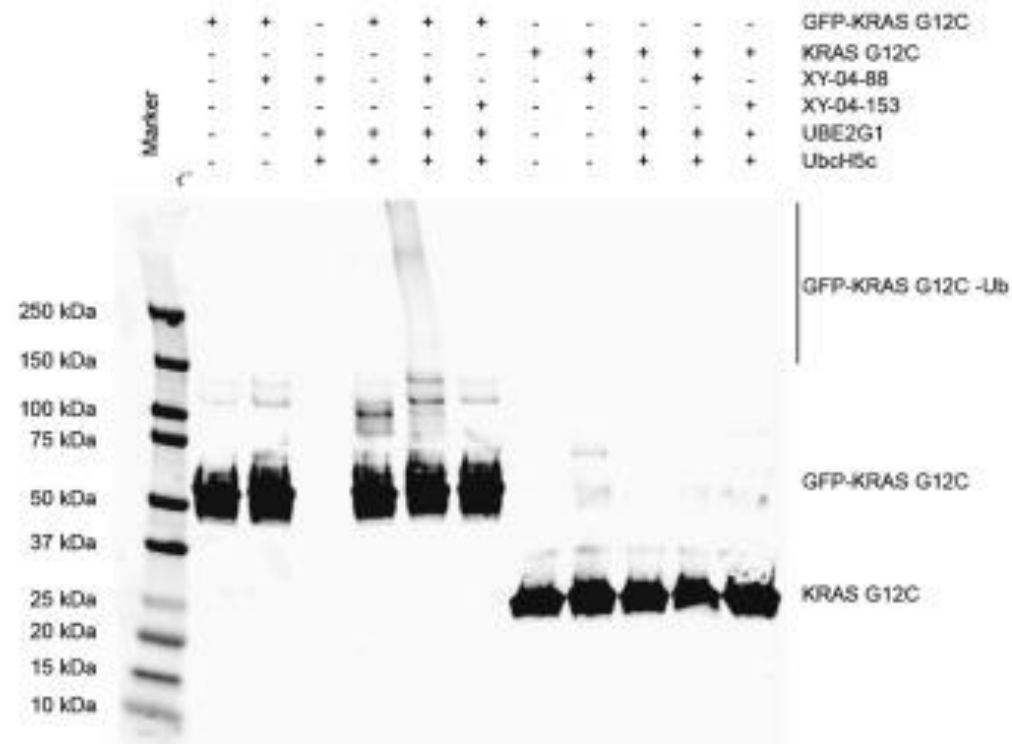
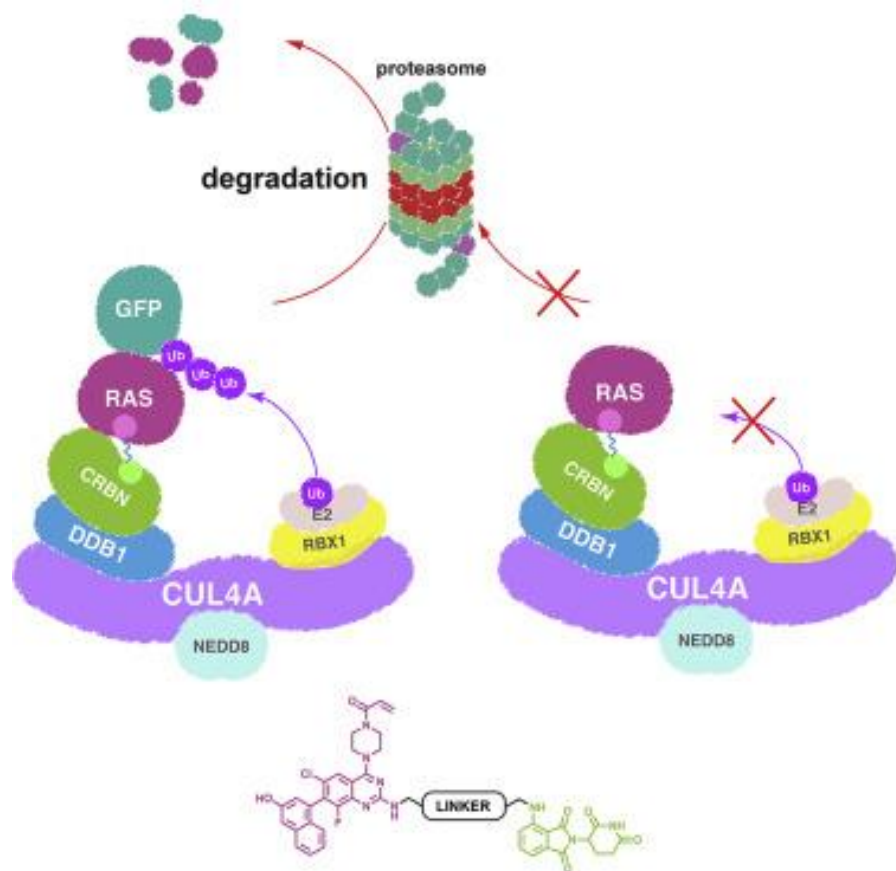
GFP-KRAS G12C Degradation



Endogenous KRAS G12C Degradation

Zeng, M., Xiong, Y., Safaei, N., Nowak, R. P., Donovan, K. A., Yuan, C. J., ... & Gray, N. S. (2020). Exploring targeted degradation strategy for oncogenic KRASG12C. *Cell chemical biology*, 27(1), 19-31.

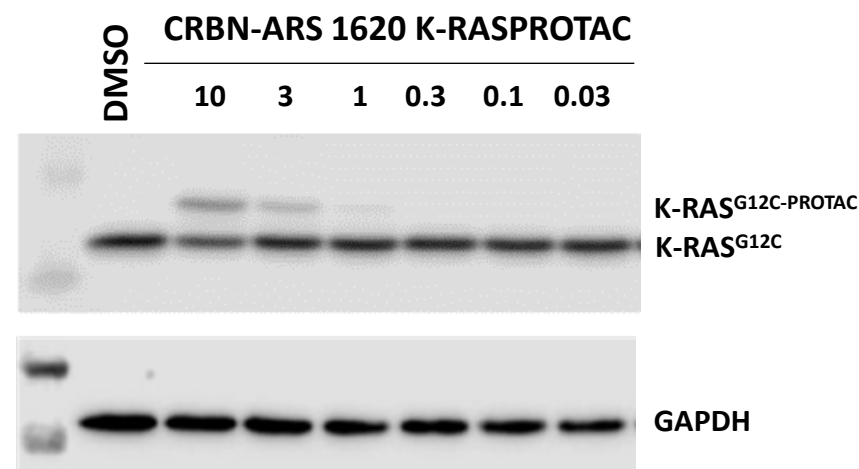
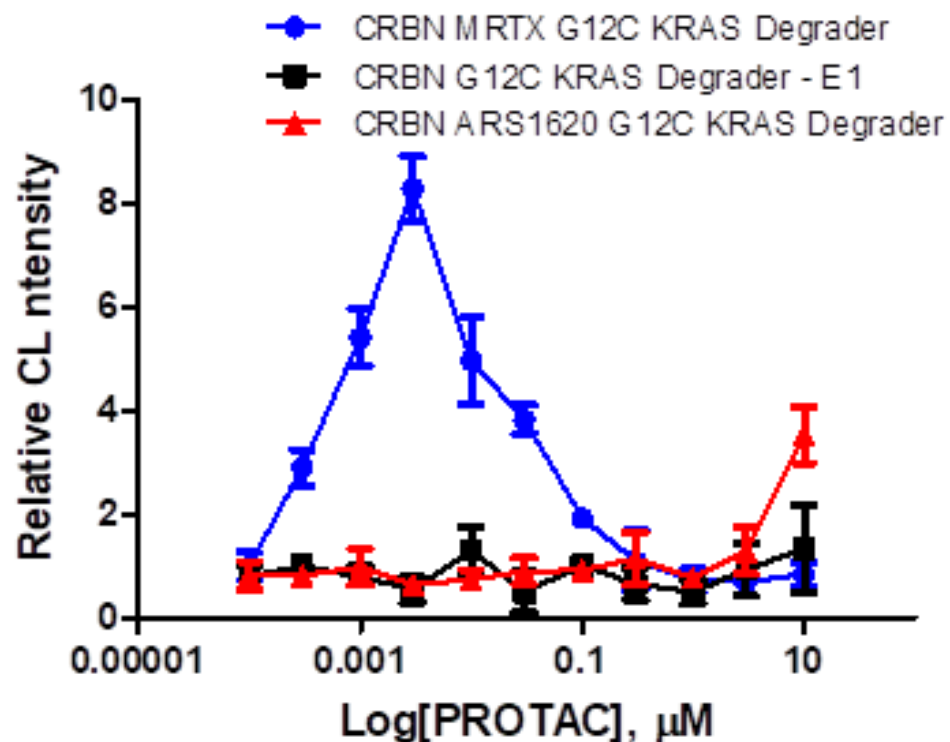
CRBN KRAS Non-degradative PROTACs



In vitro Ubiquitination Assay

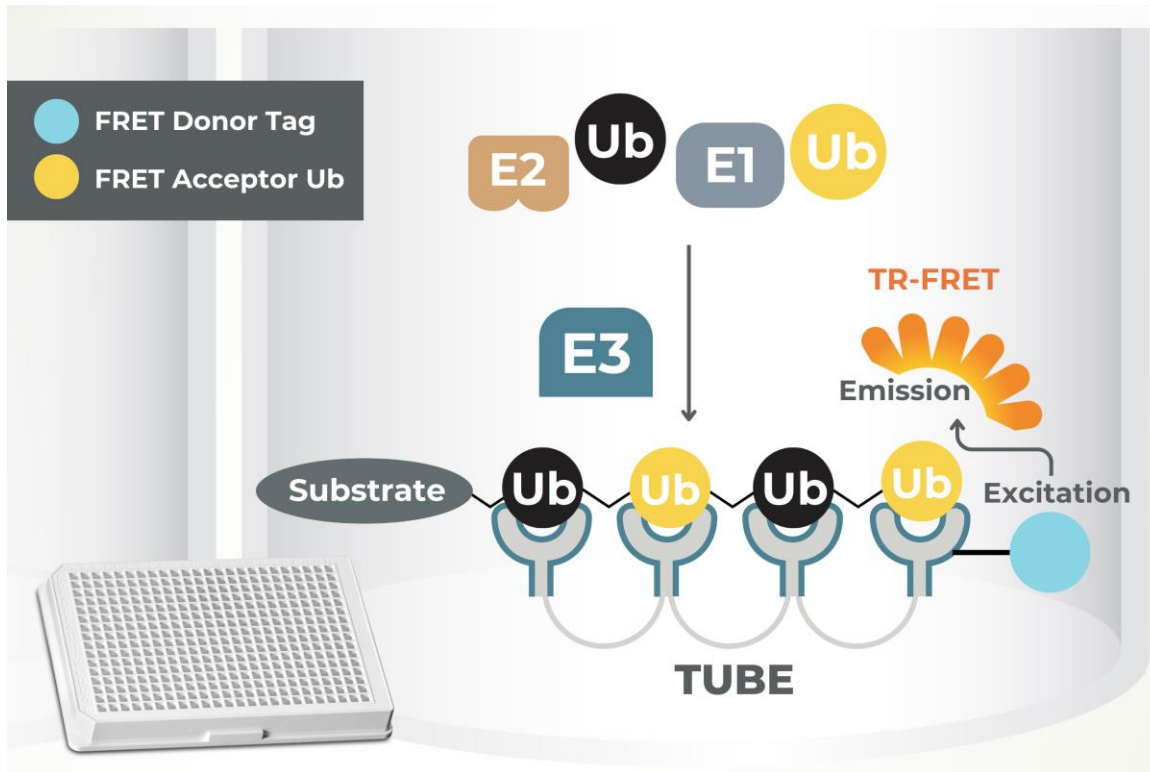
Zeng, M., Xiong, Y., Safaei, N., Nowak, R. P., Donovan, K. A., Yuan, C. J., ... & Gray, N. S. (2020). Exploring targeted degradation strategy for oncogenic KRASG12C. *Cell chemical biology*, 27(1), 19-31.

CRBN KRAS Non-degradative PROTACs



(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.

Novel E3 Ligase for KRAS

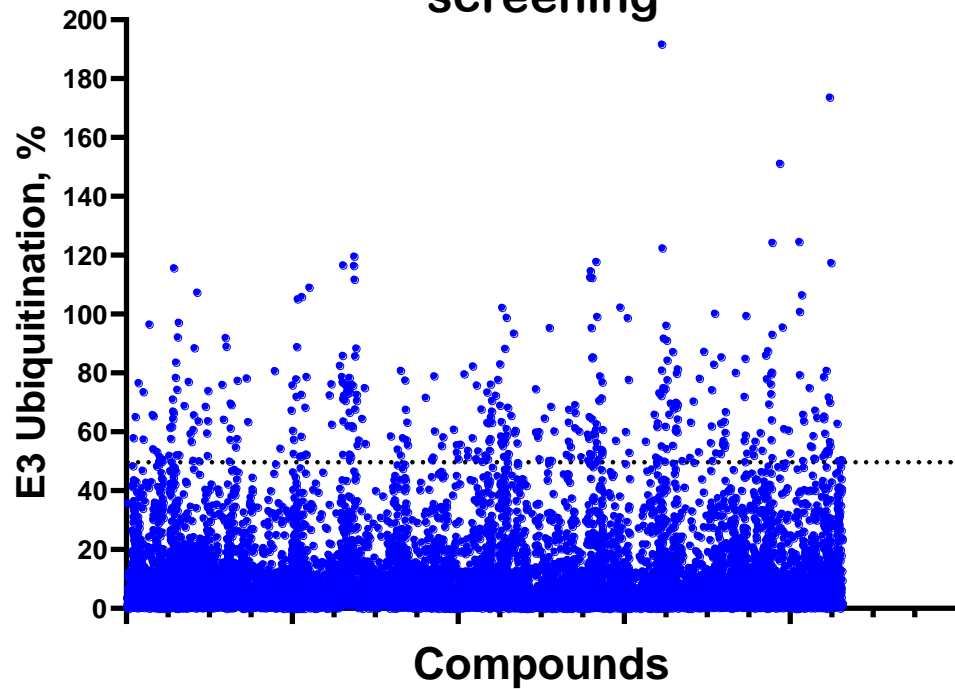


- ✓ High throughput screening for E3 ligase activators
- ✓ Homogenous assay for library screening
- ✓ Identification of novel E3 ligands
- ✓ SPR / TSA based confirmation and PROTACability

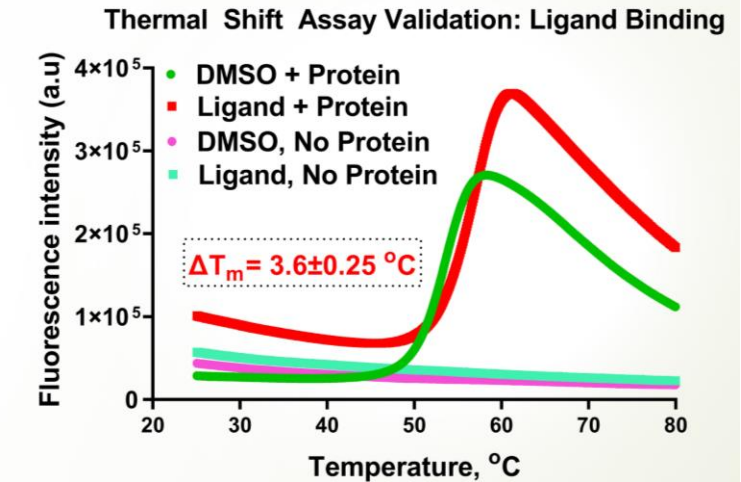
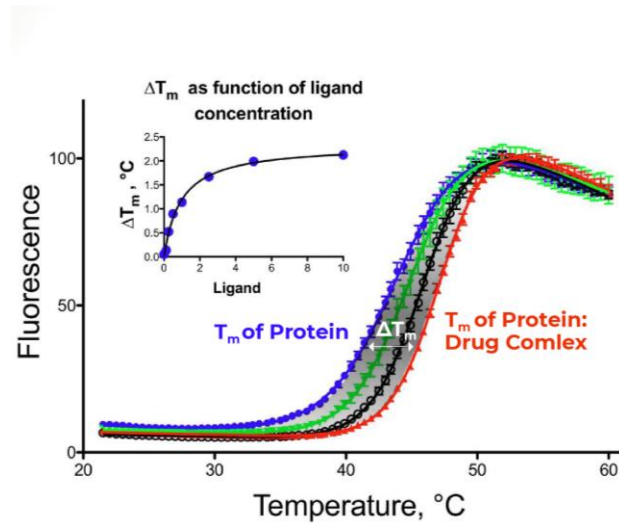
Discovery of Novel E3 Ligase for KRAS

Superior Properties as compared to VHL and CBRN

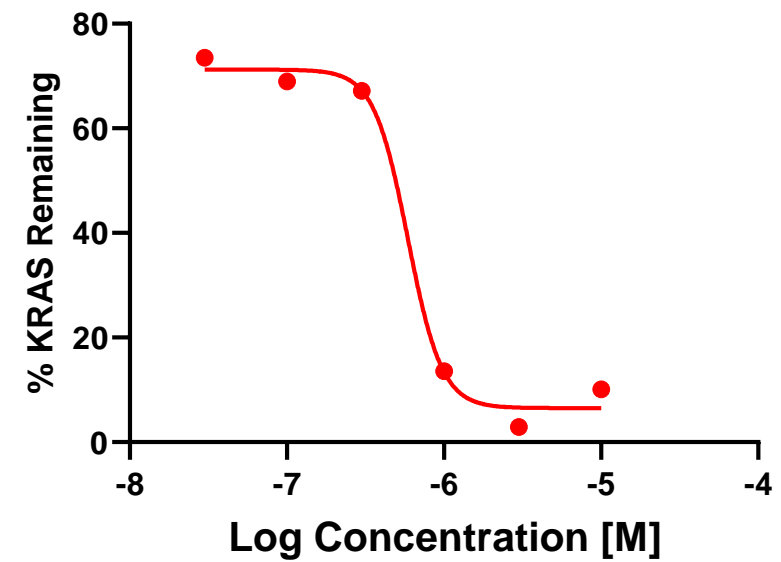
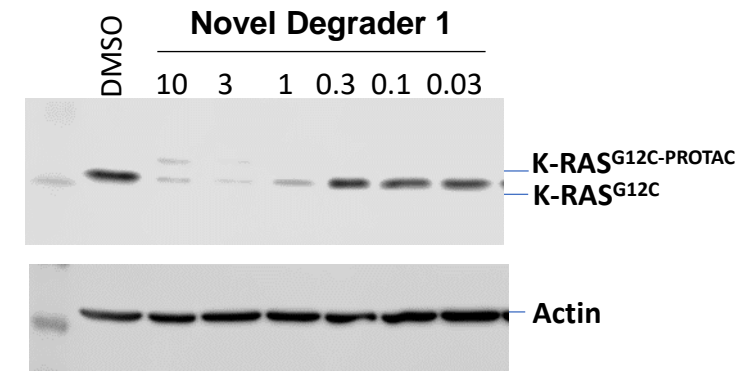
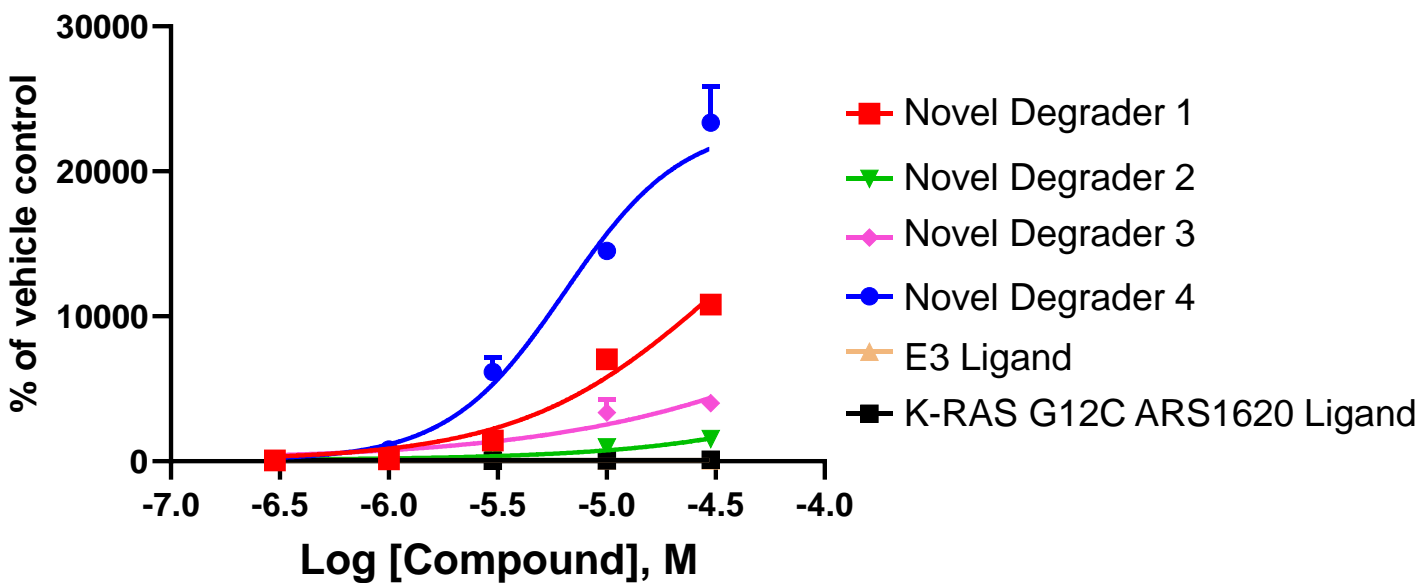
50K compound library screening



Thermal Shift Assay Validation

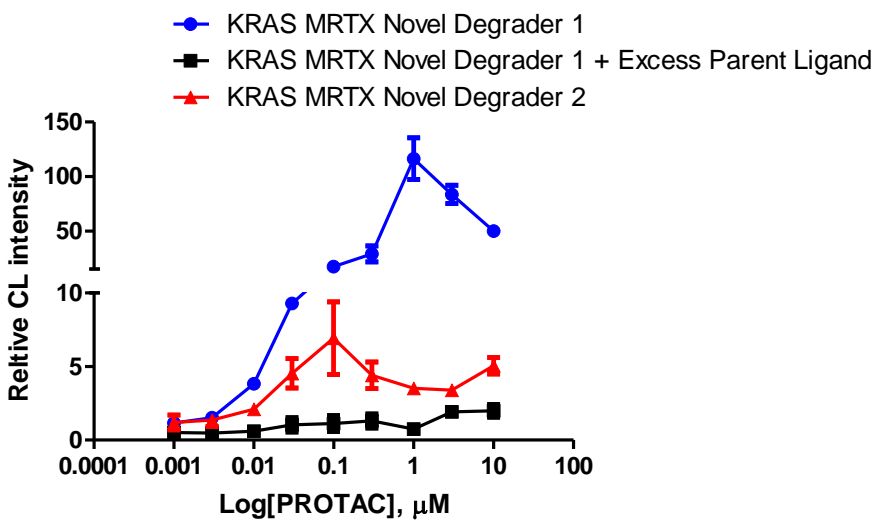


Novel E3 Ligand with ARS-1620 for KRAS Degradation

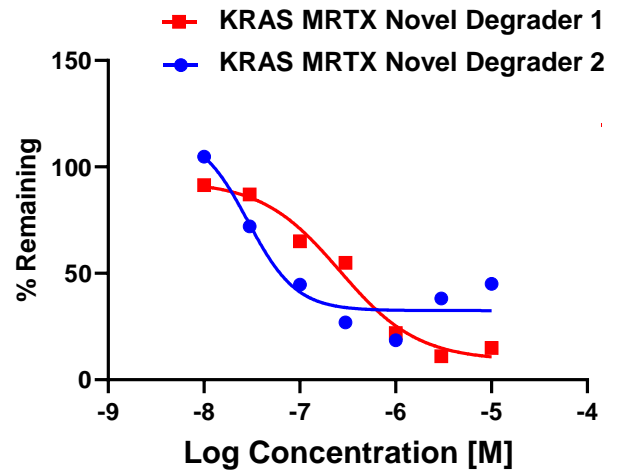
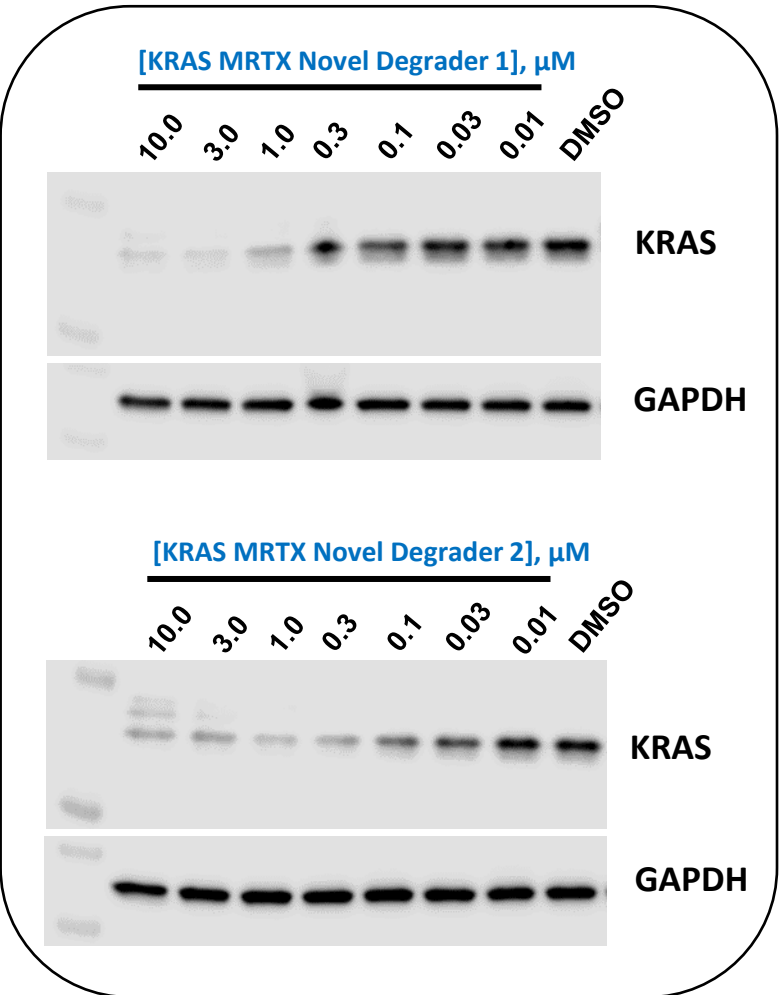


(A) *In vitro* ubiquitination assay with ARS 1620 KRAS G12C Novel E3 based degraders : dose response study to monitor PROTAC mediated ubiquitination of KRAS G12C. CL intensities plotted in response to 1/2 log dose response demonstrates PROTAC mediated ubiquitination. (B) Cell based assay to monitor degradation of KRAS G12C in H358 cells - PROTAC mediated degradation in a dose response study. (C) Representation of D_{max} as dose dependent degradation.

Novel E3 Ligand with MRTX-849 for KRAS Degradation

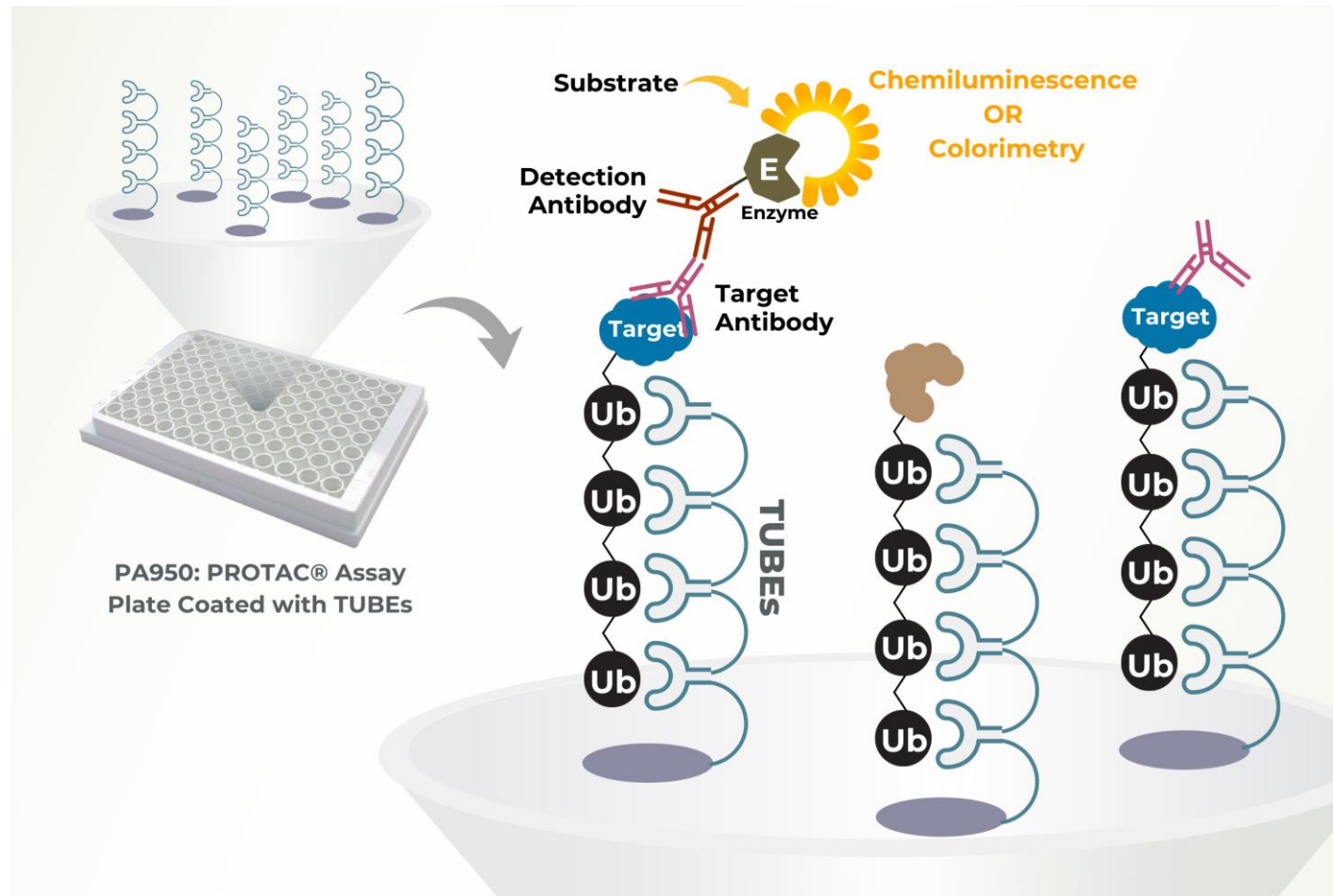


In vitro Ubiquitination



Cellular Degradation

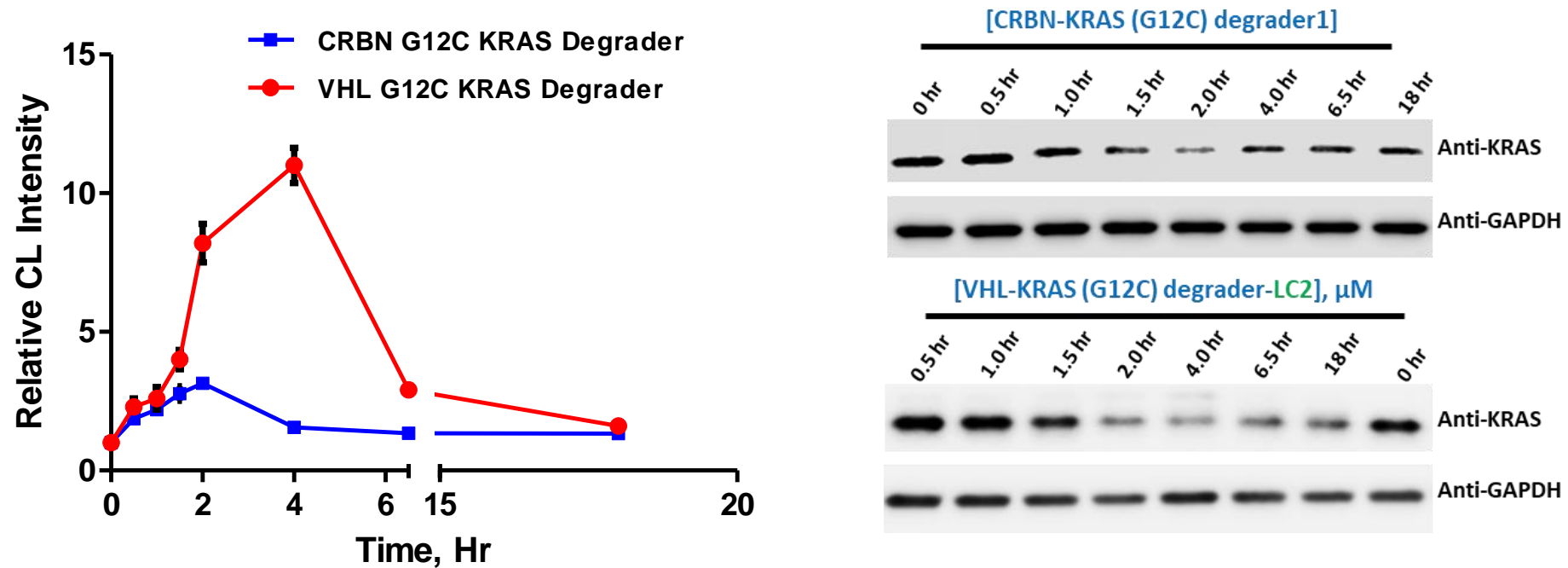
Validation – KRAS Cellular Ubiquitination and Degradation



CRBN & VHL KRAS Degraders

Monitor PROTAC mediated Cellular Ubiquitylation and Degradation

Time Course Study - HTS

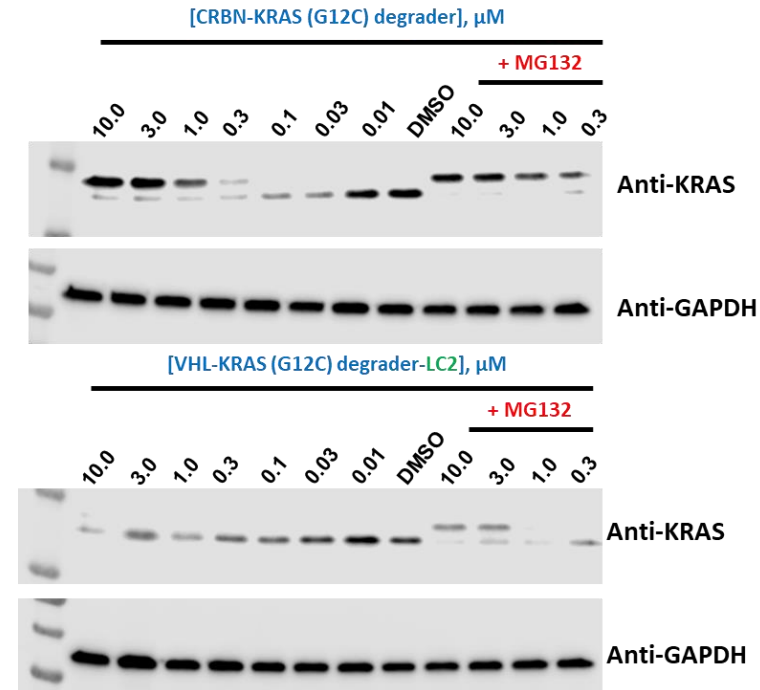
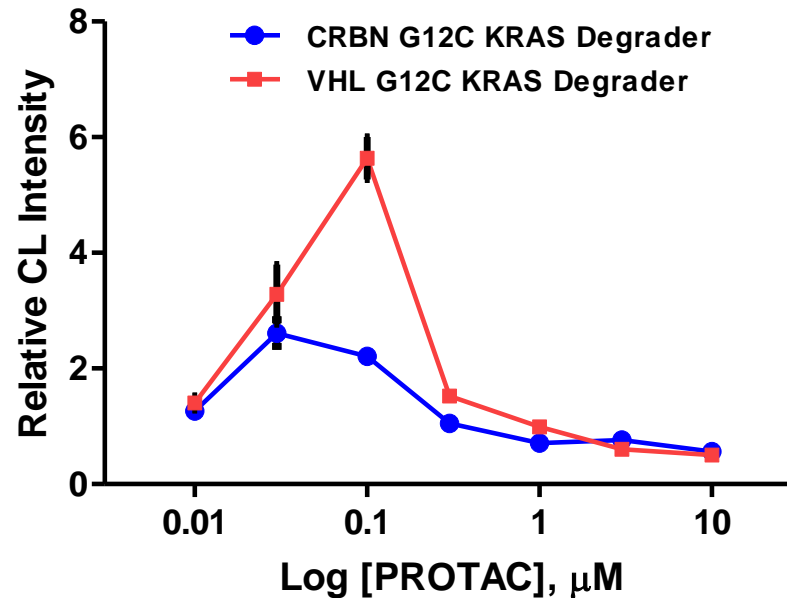


Monitoring Ubiquitination Kinetics – changes in PROTAC mediated ubiquitination profiles of endogenous KRAS and subsequent degradation in H358 cells. VHL and CRBN PROTACs designed with covalent ligands to engage KRAS G12C and successfully ubiquitinate and degrade within 2-4hrs of treatment.

CRBN & VHL KRAS Degraders

Monitor PROTAC mediated Cellular Ubiquitylation and Degradation

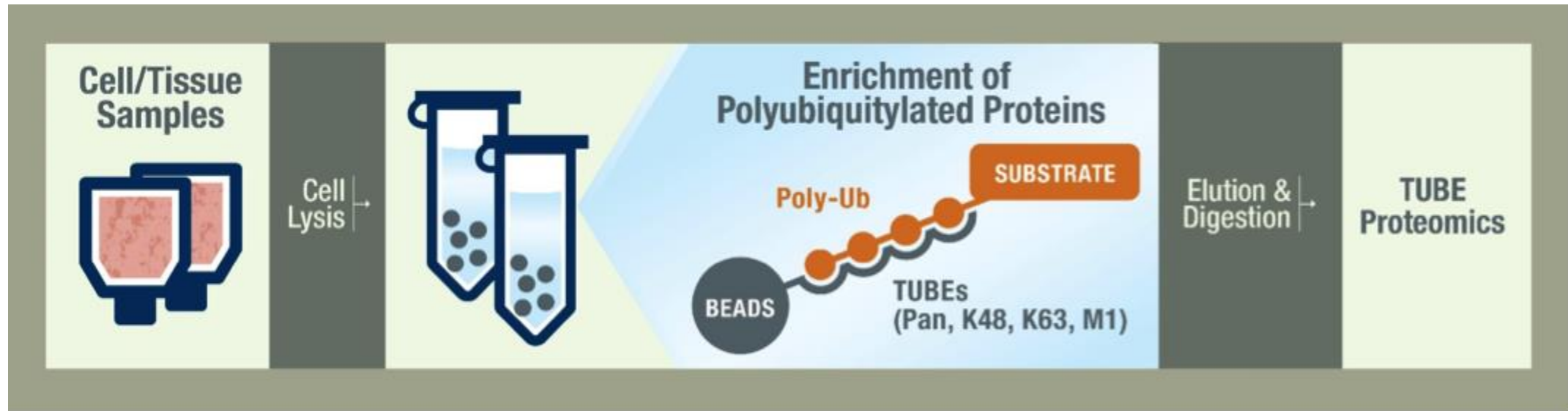
Dose Response Study - HTS



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 Mass spectrometry

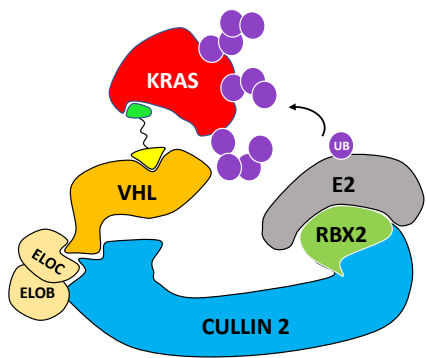
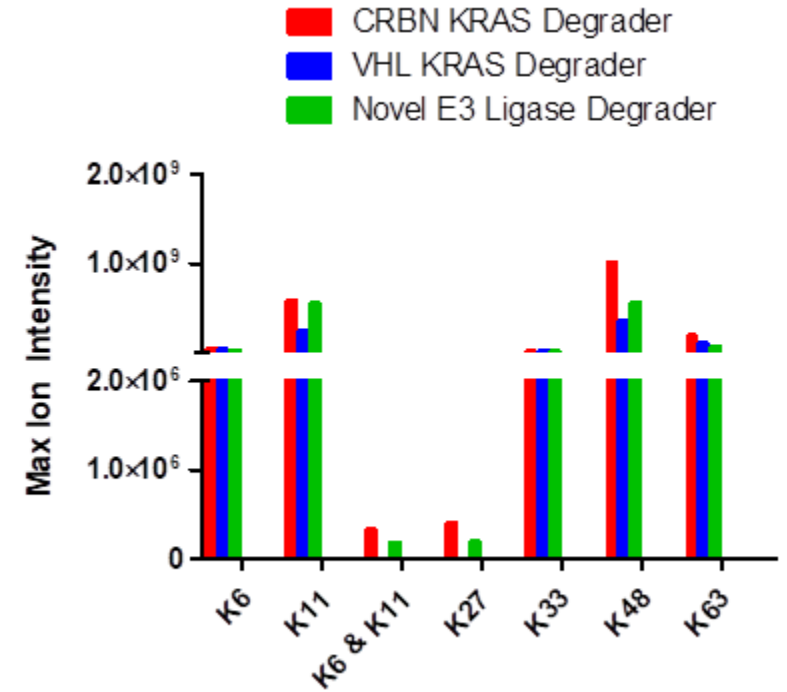
To Validate PROTAC Mediated Ubiquitination and Degradation



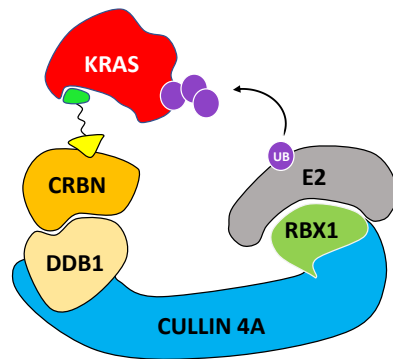
Mass Spectrometry Studies with KRAS Degraders

Comparative E3 Ligase Study – In vitro Ubiquitylation

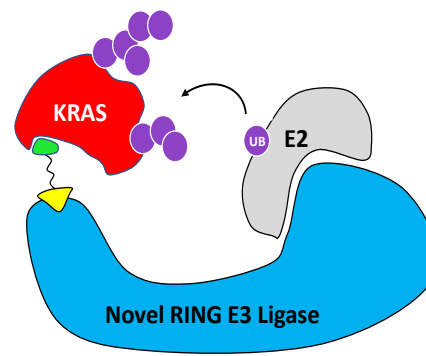
| K-RAS Degradator / Ubiquitinated Peptide | K86 | K103 | K127 | K146 | K183 |
|--|--------------|------|------|------|------|
| VHL based Degradator | High | High | High | High | High |
| CRBN based Degradator | High | Low | High | Low | Low |
| Novel E3 based Degradator | Confidential | | | | |



VHL E3 Ligase

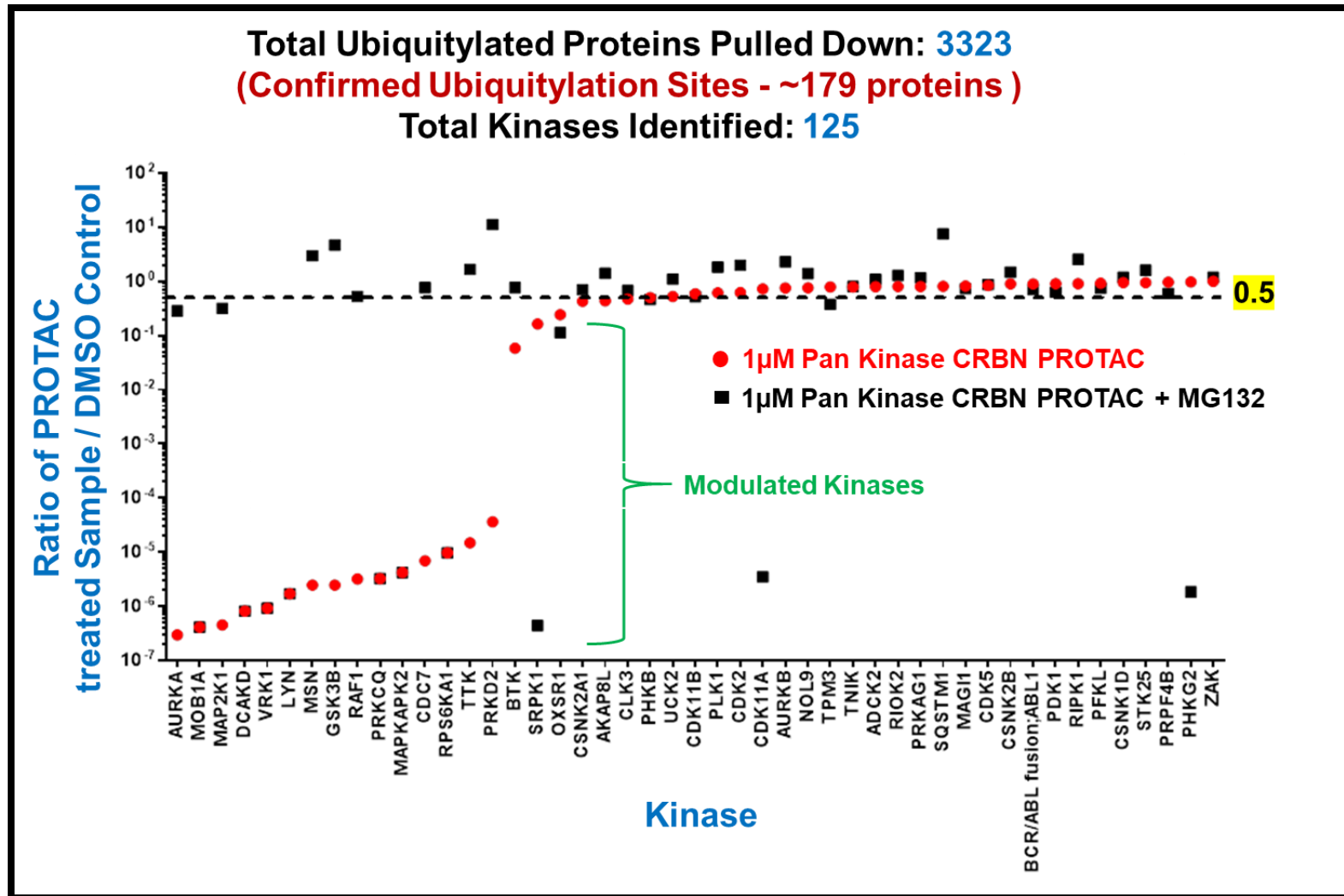


Cereblon E3 Ligase

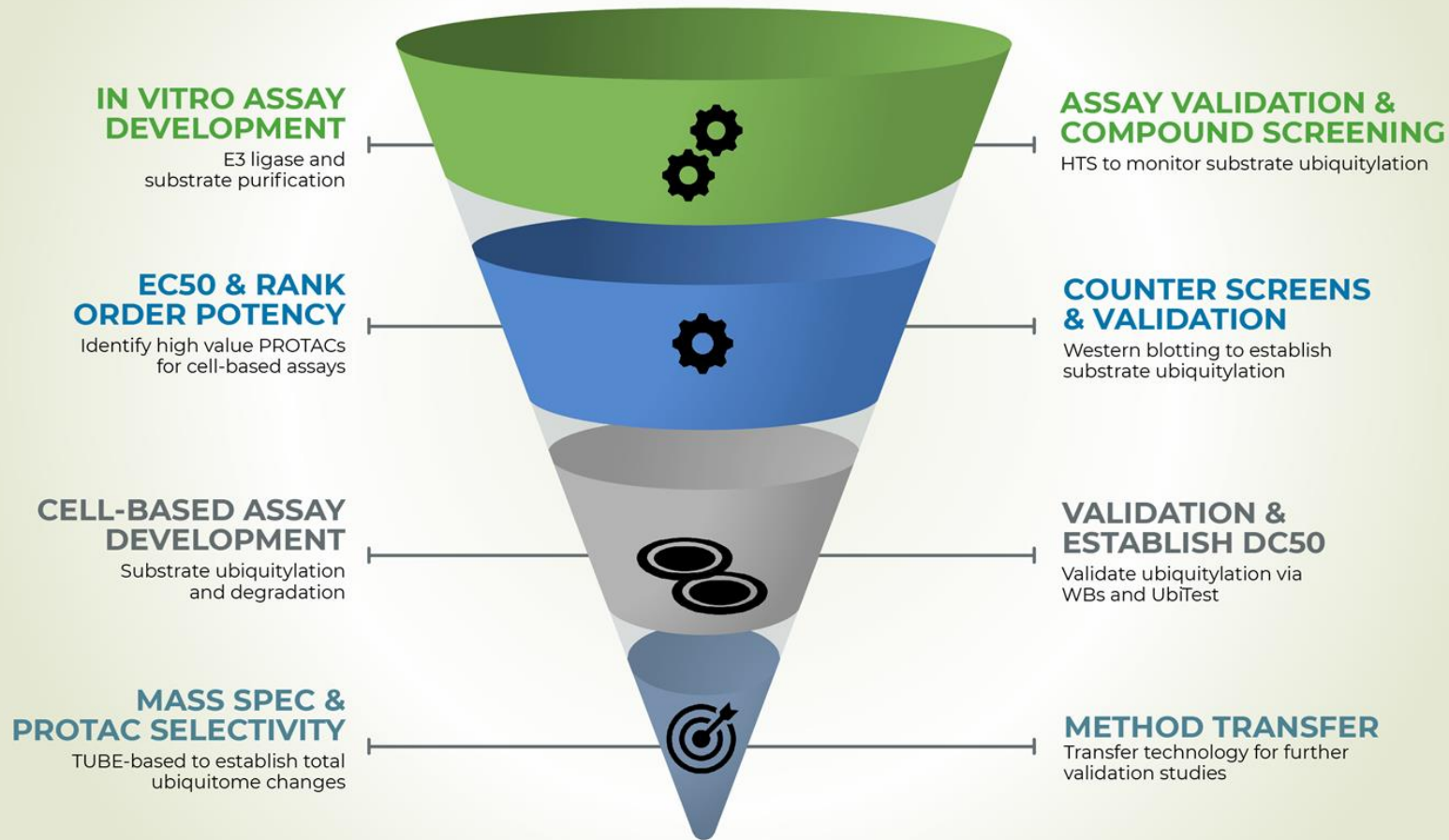


Novel E3 Ligase

Mass Spectrometry Studies with Pan Kinase Degradator



Pathway to PROTAC Drug Discovery



✓ **TUBEs based PROTAC® Assays provides a link between ubiquitination and degradation**

✓ **Robust SAR with true function of ubiquitination for development of potent degraders**

LifeSensors Leading the Way to UPS Drug Discovery

www.lifesensors.com

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