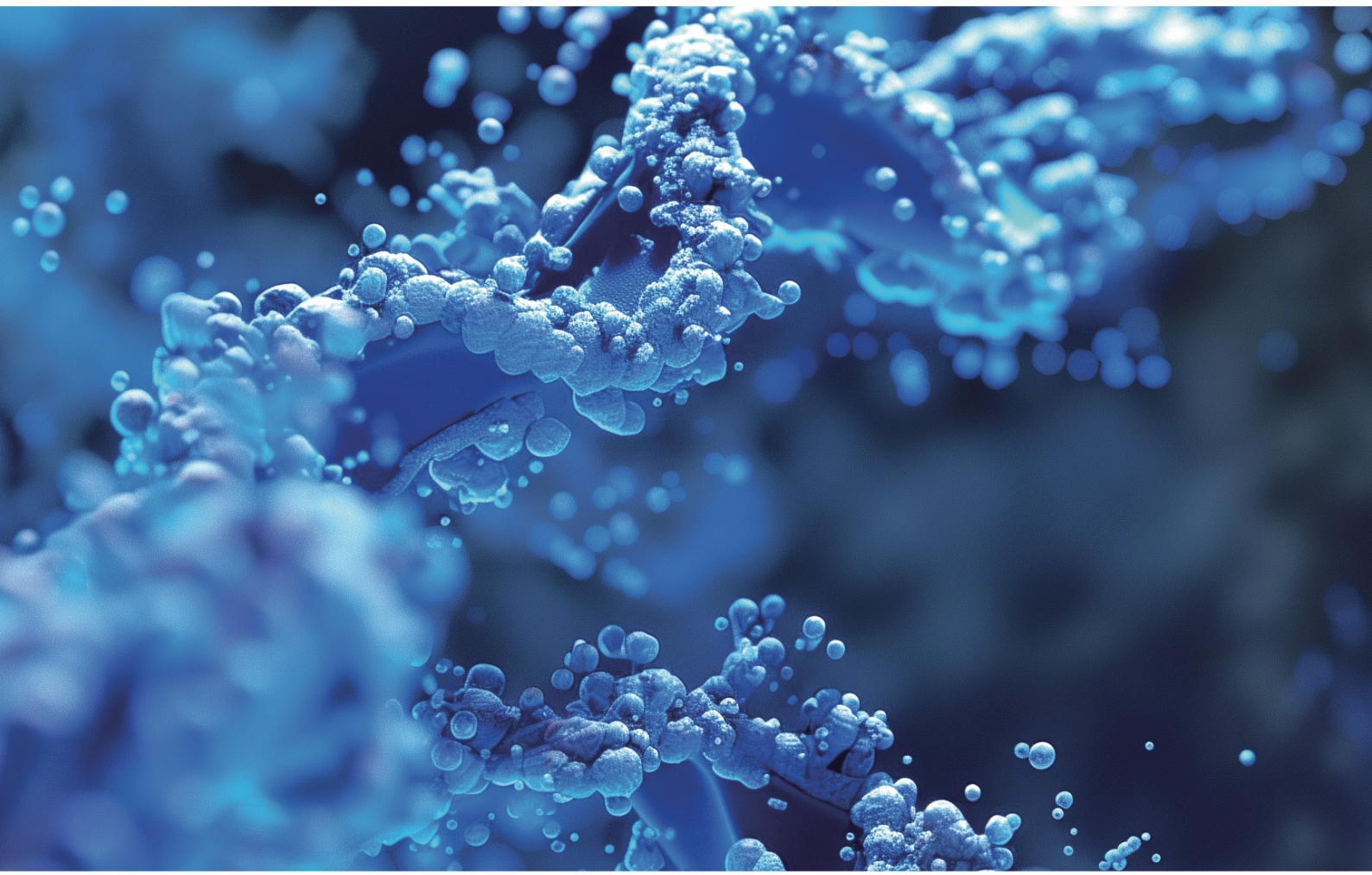
# Targeted Protein Degradation

## Drug Discovery Solutions



Innovative CRO+Explorer

ICE Bioscience

# Powering the Future of Degrader Therapeutics

Targeted Protein Degradation (TPD) is an innovative strategy that removes disease-related proteins directly, overcoming limitations of traditional inhibitors that merely block protein function. By harnessing the cell's own degradation machinery, TPD offers new therapeutic solutions for previously "undruggable" targets.

At ICE Bioscience, we specialize in supporting the development of these leading TPD modalities:

- PROTACs
- Molecular Glues
- DACs (Degrader-Antibody Conjugates)

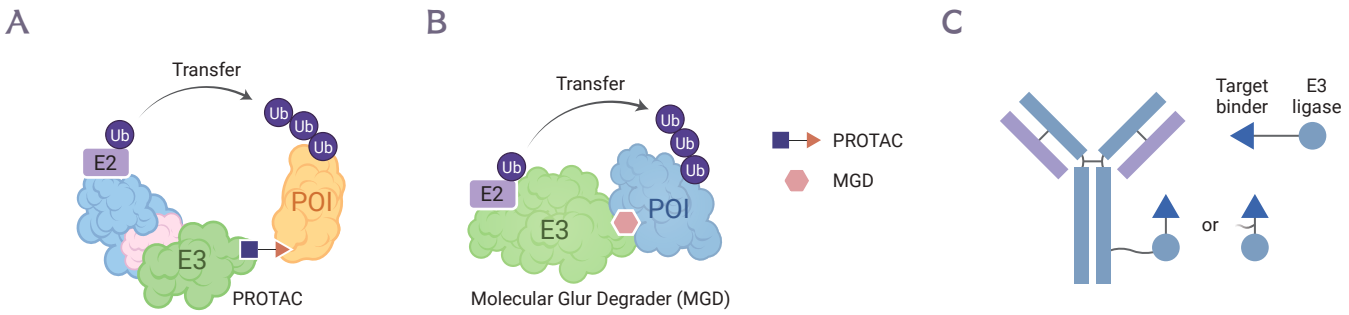
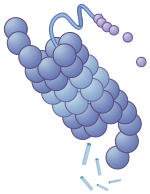



Figure 1. (A) A bifunctional molecule that links a target protein to an E3 ligase, inducing ubiquitination and degradation. (B) A small molecule that enhances the interaction between an E3 ligase and a target protein, promoting degradation. (C) DACs are made of an antibody for precise targeting, chemically linked to a degrader that induces protein degradation in specific cells.

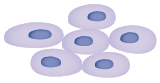
# Why Partner with Us?

- Seamless workflow: From in-house protein expression to complex assays, ensuring seamless integration.



- Comprehensive degradation analysis: High-throughput HiBiT assays and validation with Jess, ICW, and other classic methods.

- Mass spectrometry-based proteomics for TurboID labeling and protein degradation profiling.



- Diverse phenotypic screening tools for functional evaluation.

## IMPACT IN NUMBERS

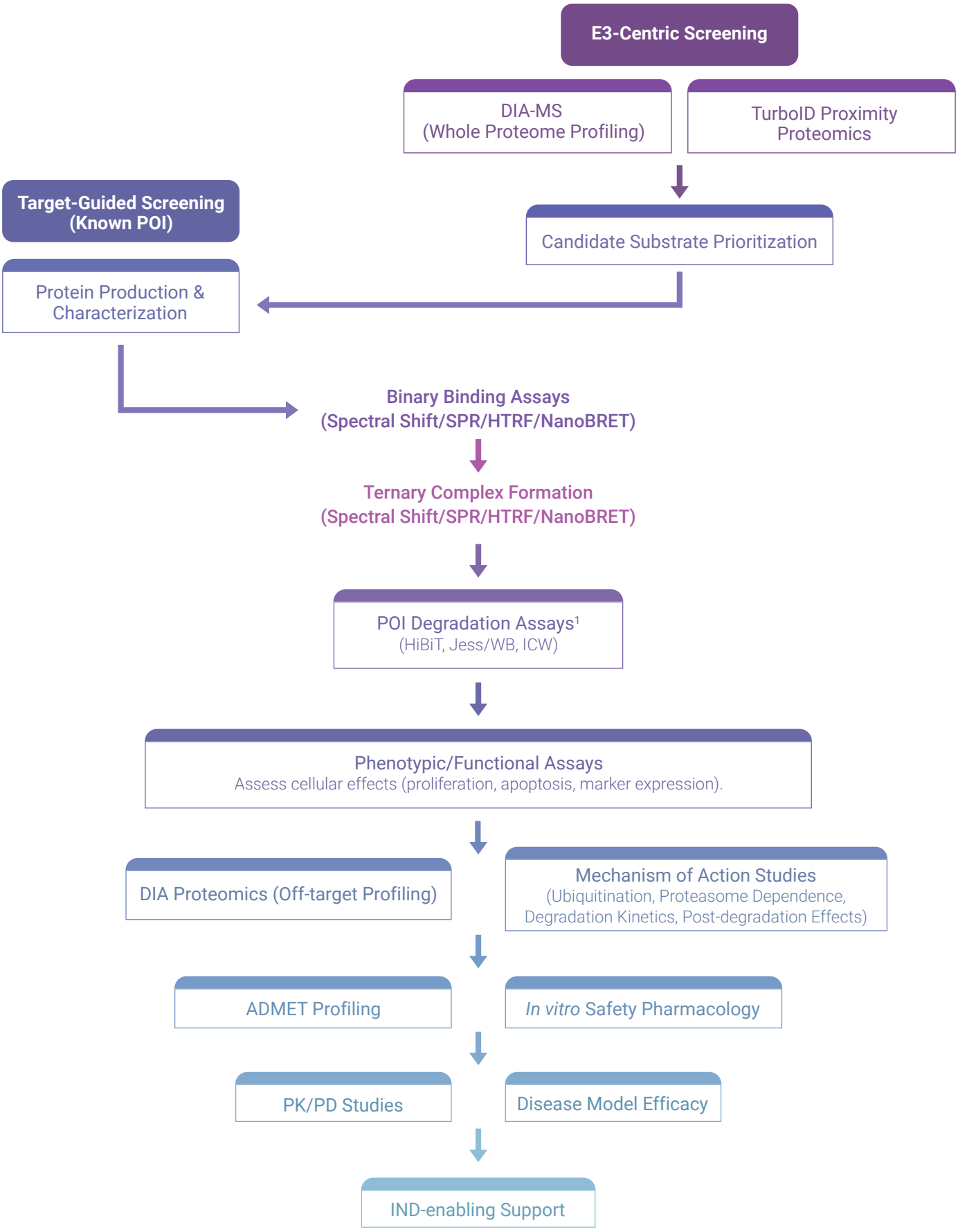
400+ Employees

15 Years of Excellence

1000+ Global Partnerships

10000+ Studies Annually

# Integrated TPD Screening Workflow

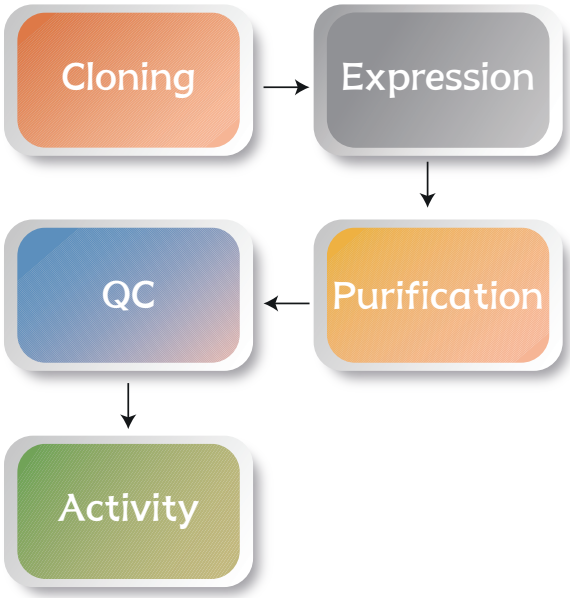



*[1] In some cases, a degradation-first screening strategy may be applied to quickly assess target degradation before detailed binding and complex formation studies.*

# Your TPD Starts Here: 700+ Ready-to-Use Proteins

- 700+ purified proteins, including 350+ kinases
- Specific activity and lot-to-lot consistency
- Covering Top TPD targets

√ KRAS and mutants
√ VAV1
√ NEK7
√ CDK2
√ BTK and mutants
√ c-Myc
√ EGFR and mutants
√ IRAK4
√ HPK1
√ BRD4
√ STAT6
√ GSPT1
√ IKZF1
√ PLK3
…



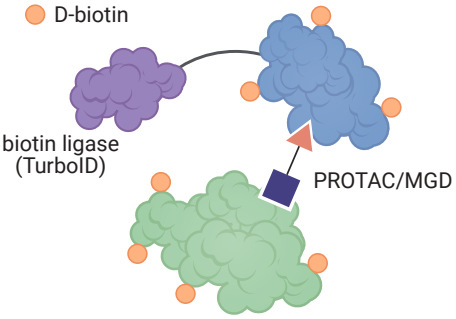

# Target Validation

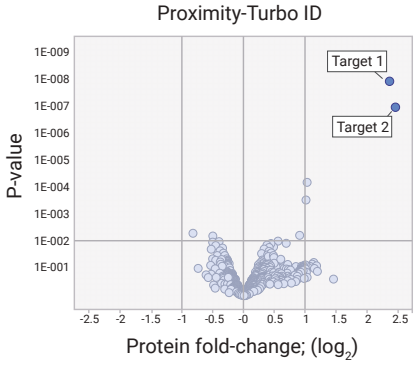
Target validation is essential in TPD drug discovery to assess whether a protein is biologically relevant and susceptible to degradation. We provide comprehensive validation services to confirm target feasibility, degradability, and therapeutic potential, supporting efficient TPD research.

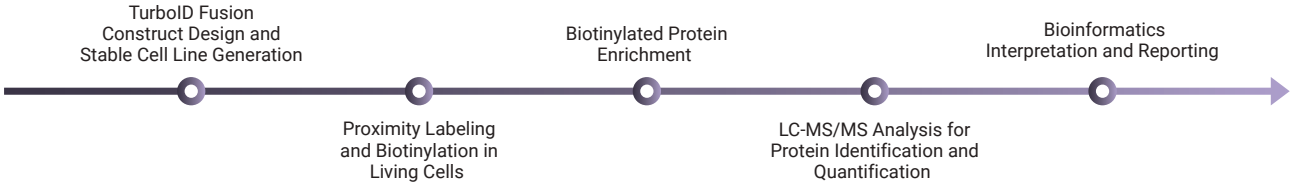
| Assay Category | Assay Type | Description and Purpose |
|---|---|---|
| Genetic Modulation | - CRISPR/Cas9 Knockout (KO) | Uses CRISPR or RNAi to reduce or eliminate target protein expression, confirming its essentiality for cellular functions or disease pathways. |
| | - RNA Interference (RNAi) | |
| Expression and Localization | - qPCR | Evaluates the mRNA and protein levels of the target and determines its subcellular or tissue localization, validating its relevance in specific biological contexts. |
| | - Western Blotting | |
| | - Immunohistochemistry (IHC) | |
| Protein Production | - Recombinant Protein Expression and Purification | Supplies high-quality proteins for use in binding assays, complex formation studies, or functional and mechanistic research. |
| Protein Stability and Turnover | - Protein t1/2 Assay | Measures the natural degradation rate (half-life) or ligand-induced stability changes of the target protein to assess its suitability for therapeutic degradation strategies. |
| | - Thermal Shift Assay (TSA)/CETSA | |
| Functional Dependency | - Cell Viability Assays | Evaluates how modulating the target protein affects survival, proliferation, or signaling pathway activity, establishing its role in cellular and disease phenotypes. |
| | - Reporter Gene Assays | |

# TurboID Proximity Proteomics

TurboID proximity labeling combined with quantitative proteomics provides a powerful platform for mapping protein interaction networks and uncovering the mechanisms of TPD. By biotinylating proteins in close proximity to a bait protein in living cells, TurboID captures dynamic protein interactions under physiological conditions, allowing downstream mass spectrometry to identify and quantify associated partners.



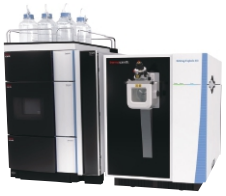




# DIA Proteomics for Substrate Discovery

Our DIA (Data-Independent Acquisition)-based proteomics service combines automated, high-throughput sample prep with the ultra-fast, high-resolution Orbitrap Exploris™ 480 or Astral MS platform, enabling early identification of degrader substrates. From large-scale screening to precise degradation profiling, we deliver deep, reliable proteome insights—fast.

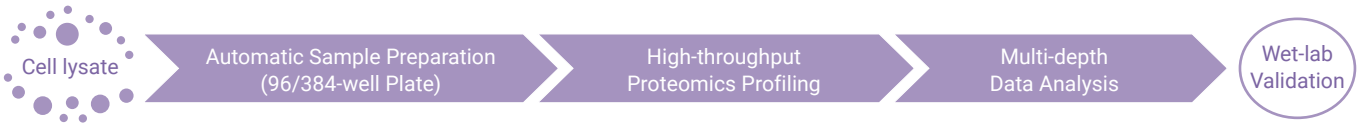
***Key Advantages***

- *High Sensitivity – Capable of single-cell level proteomics*
- *High Throughput – Process up to 60–180 samples per day (SPD)*
- *High Resolution – Up to 80,000 resolving power for precise quantitation*
- *Deep Proteome Coverage – Quantifies over 5,000 proteins per run*

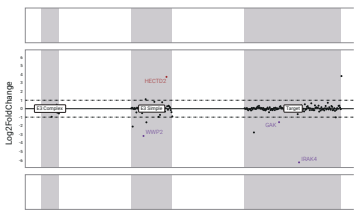


Orbitrap Exploris™ 480

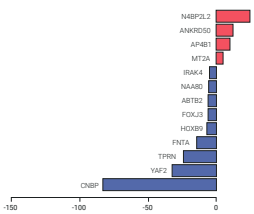
Orbitrap Astral




Multi-lane Volcano



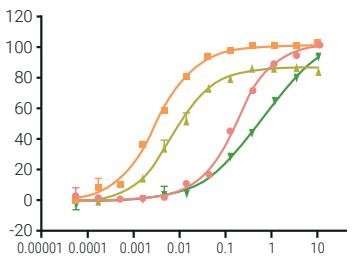
Hitsocre



| Gene | Ratio T1/C | Ratio T2/C | Rank |
|---|---|---|---|
| A | XXX | XXX | I |
| B | XXX | XXX | II |
| C | XXX | XXX | III |

Multi-depth Data Analysis

Multi-Complex Validation




Wet-lab Validation

# Binary and Ternary Complex Formation Assays

Complex formation assays enable comprehensive characterization of binary interactions between proteins and ligands, as well as ternary complex formation with E3 ligases.

- **Biochemical Assays:** Using techniques such as TR-FRET, we evaluate binary interactions between protein-of-interest (POI) and ligands or binders.

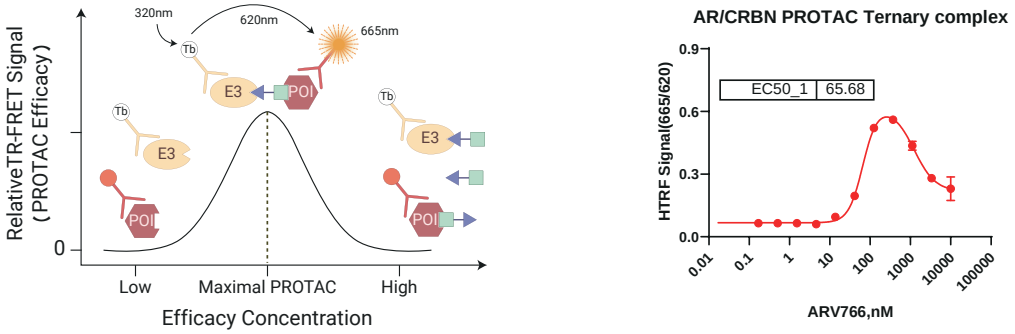



Figure 2. Complexes are formed among target–PROTAC-E3 ligase, with Tb as the donor fluorophore and AF488 as the acceptor fluorophore. A typical bell-shaped dose–response curve is shown.

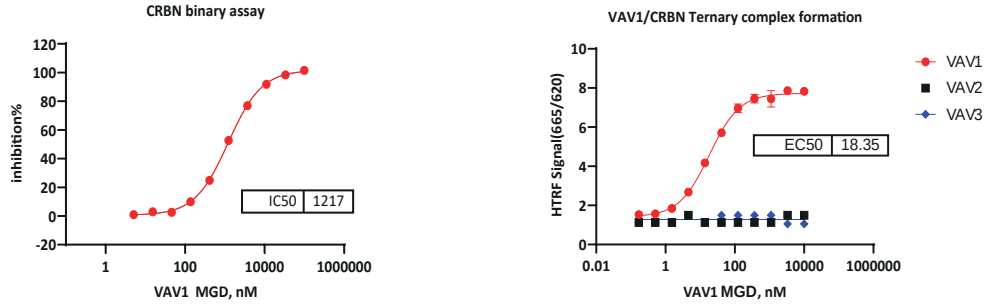



Figure 3. TR-FRET dose-response curves for binary and ternary complex formation in a molecular glue degrader (MGD) system.

- **Biophysical Assays:** Advanced tools like Spectral Shift (SpS), Temperature-Related Intensity Change (TRIC) and SPR, allow us to characterize the kinetic and thermodynamic properties of binding events, offering mechanistic insights into complex formation.

NanoTemper Dianthus

***Features***

- *Single-plate assay for simultaneous binary and ternary affinity measurement.*
- *Rapid, high-throughput binding detection.*
- *Solution-Based Analysis, eliminating immobilization issues.*

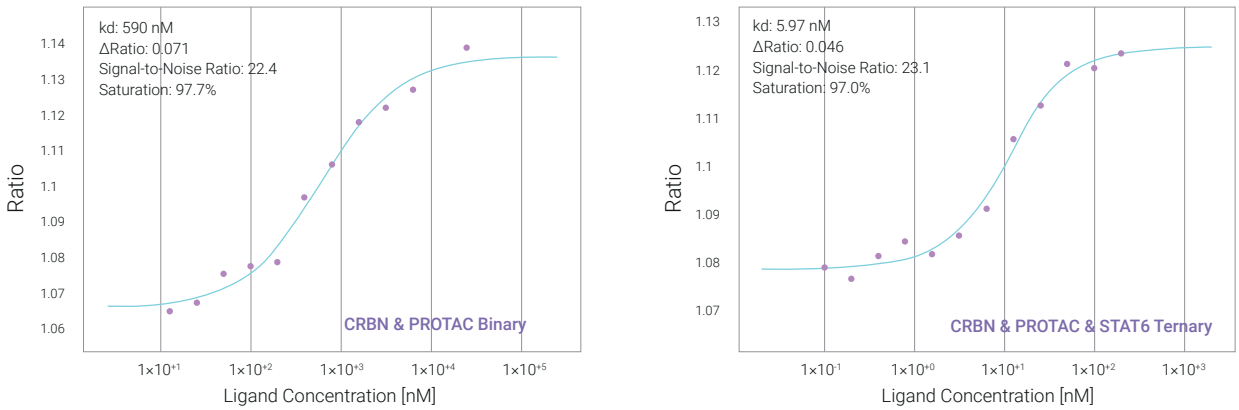



Figure 4. Spectral Shift assays demonstrate the binding affinity of a PROTAC to CRBN (binary complex, left) and the formation of a ternary complex with STAT6 (right).

- **Cell-based Assays:** Cell-based NanoBRET and NanoBiT assays enable the validation of binary and ternary interactions in their native context.

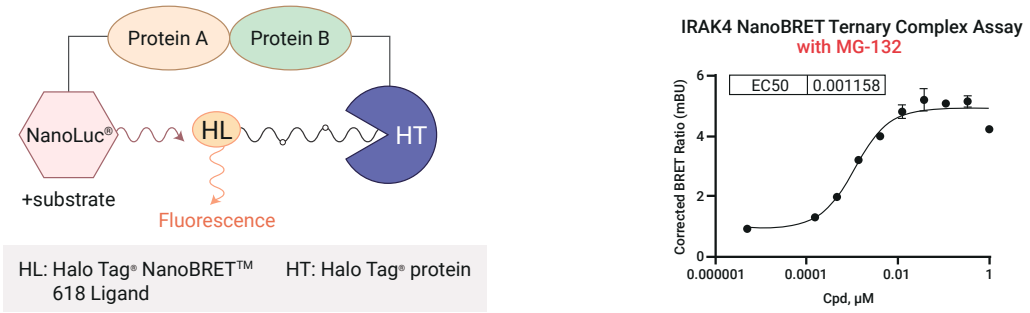



Figure 5. NanoBRET measures ternary complex formation by detecting energy transfer between luminescent and fluorescent tags on proteins, indicating their proximity. This assay shows the concentration-dependent formation of the IRAK4 ternary complex in the presence of MG-132.

# Protein Degradation Assays

## HiBiT-Based *In Vitro* Lytic Assays

HiBiT, an 11-amino acid tag from NanoLuc® luciferase, enables real-time detection of PROTAC and Molecular Glue -induced protein degradation. Upon target degradation, HiBiT signal decreases as it no longer binds LgBiT to form an active luciferase. Integrated via CRISPR/Cas9 or transient expression, HiBiT provides a quantitative, high-throughput solution for evaluating degrader efficacy in live cells.

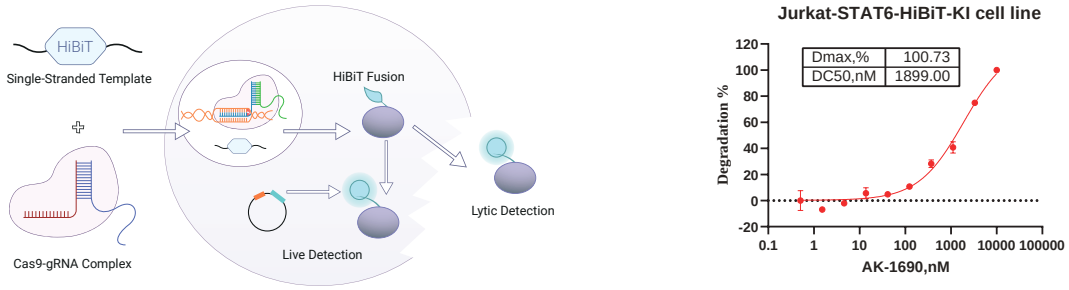
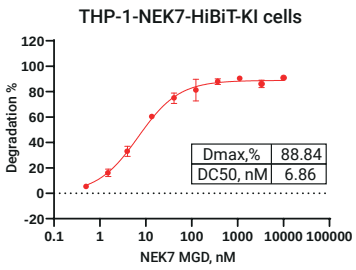



Figure 6. HiBiT-based endogenous tagging and luminescent detection of target proteins.

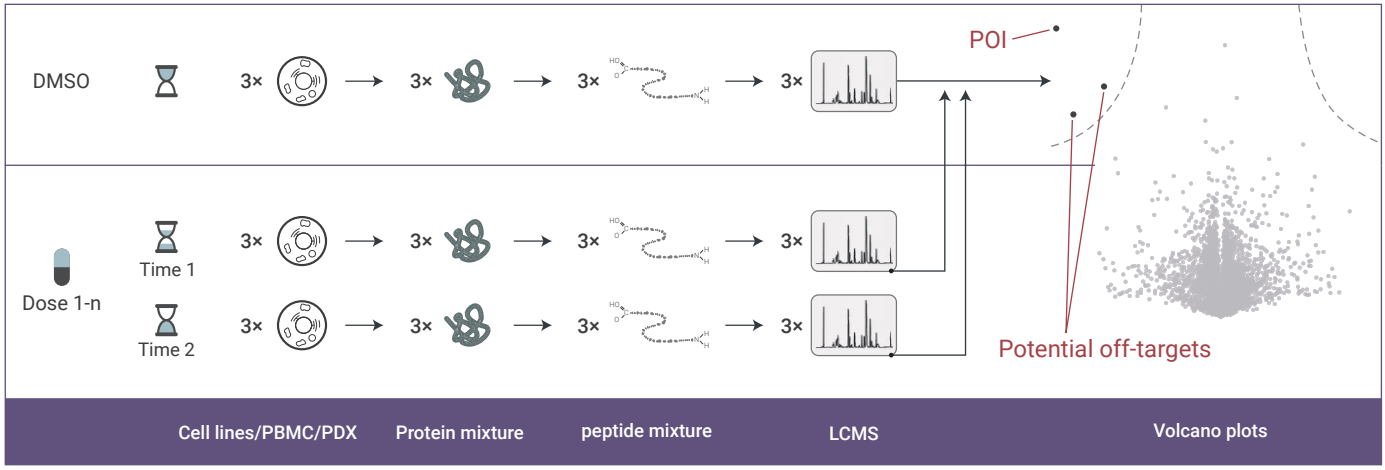
***Features***

- *High-Throughput & Fast*
- *Covering Top TPD targets*
- *Custom HiBiT Cell Line Development*




## DIA Proteomics for Off-target Profiling

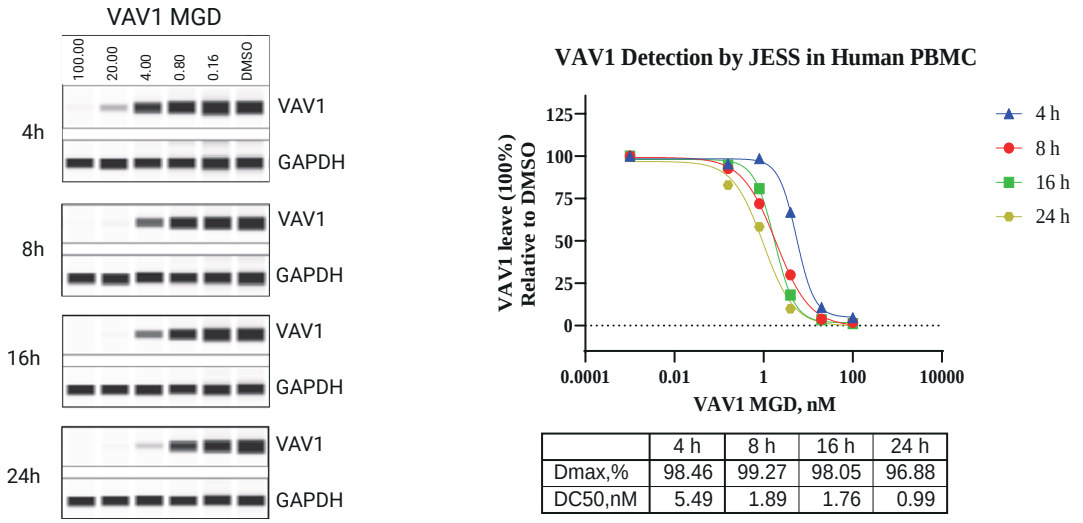
Our DIA-based proteomics enables quantitative and unbiased off-target analysis for molecular glue degraders and PROTACs, facilitating early identification of unintended substrate interactions.



## Classic Protein Degradation Assays

Protein degradation assays deliver quantitative, reproducible data to confirm target degradation, evaluate dose/time responses, and detect off-target effects. By integrating high-sensitivity, high-throughput technologies, our assays accelerate target validation, degrader optimization, and drug development.

| Category | Assays |
|---|---|
| Traditional Protein Detection | WB, Jess (Simple Western), ICW |
| Homogeneous HTS | HTRF, AlphaLISA |
| Multiplex & High-Sensitivity | MSD, Flow Cytometry |
| Imaging-Based Assays | HCA (High Content Analysis) |




| | 4 h | 8 h | 16 h | 24 h |
|---|---|---|---|---|
| Dmax,% | 98.46 | 99.27 | 98.05 | 96.88 |
| DC50,nM | 5.49 | 1.89 | 1.76 | 0.99 |

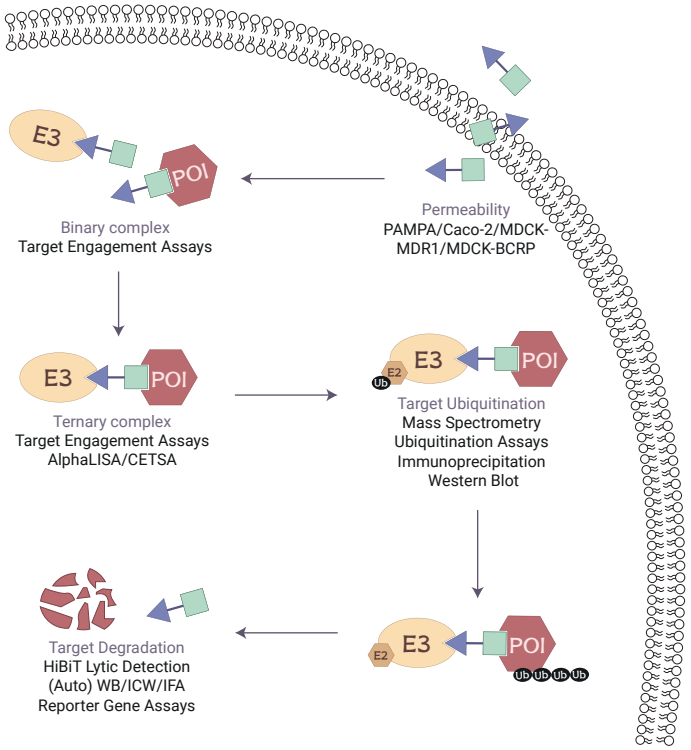
Figure 7: Time-dependent degradation of VAV1 in human PBMCs measured by Jess.

# Confirm the Mechanism Behind Your Degrader

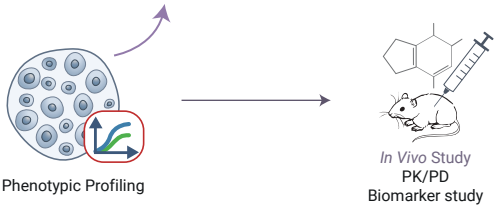
Understanding how your degrader works is critical for optimizing efficacy, selectivity, and therapeutic impact. Our comprehensive MOA services provide key insights into ubiquitination, degradation pathways, and functional consequences.

| Question | Our Approach |
|---|---|
| Is the POI ubiquitinated? | WB, PRM-MS, *in vitro* ubiquitination assay |
| Does degradation rely on the proteasome? | Proteasome Inhibitor Assays (MG-132), CHX Chase |
| What are the kinetics of degradation? | HiBiT Assay, MSD, ICW, Time-Course WB |
| What happens after POI degradation? | Phosphoproteomics, Pathway Assays, Phenotypic Analysis |

# From Target Engagement to Phenotypic Impact




| Phenotypic Assays | Examples and Techniques |
|---|---|
| Cell Viability and Proliferation | - CellTiter-Glo for cell panel screening |
| | - Ki-67 staining and live-cell imaging |
| Apoptosis and Cell Death | - Annexin V/PI staining, Caspase activity assays, and TUNEL staining for apoptosis. |
| | - LDH release assays for necrosis. |
| Cell Cycle Dynamics | - Flow cytometry with PI/DAPI. |
| | - BrdU/EdU incorporation assays for S-phase detection. |
| Immune and Inflammatory Response | - Cytokine secretion assays (e.g., ELISA, AlphaLISA). |
| Morphology and Differentiation | - High-content imaging (HCA) for cell/nuclear morphology. |
| | - Immunofluorescence for differentiation markers. |




# DMPK and *In Vivo* Pharmacology

Our DMPK service assays provide comprehensive support for PROTAC discovery, lead optimization, clinical candidate selection, and IND filing processes.

| | Lead Discovery and Optimization | Clinical Candidate Selection | Clinical Candidate Characterization and IND Filing |
|---|---|---|---|
| **Biotransformation** | ▪ Metabolic Soft Spot Analysis<br>▪ Reactive Metabolite Screening<br>▪ Special Metabolizing Enzyme Phenotyping | ▪ Metabolism in Hepatocytes Across Species<br>▪ ADME in BDC Rats<br>▪ Radiolabeled Tissue Distribution Study in Rats | ▪ Radiolabeled ADME Study in Animals<br>▪ Radiolabeled Tissue Destruction Study<br>▪ Metabolizing Enzyme Mapping |
| ***In Vitro* DDI/ADME** | ▪ Solubility and Permeability<br>▪ P-gp/BCRP Substrate Analysis<br>▪ Plasma Protein Binding<br>▪ Metabolic Stability | ▪ CYP Inhibition and Induction<br>▪ Transporter Inhibition | ▪ Transporter Substrate Analysis |
| **Bioanalysis/PK** | ▪ PK Screening in Animals<br>▪ PK/PD in Pharmacological Model | ▪ PK/TK in Animals<br>▪ Biomarker Exploration<br>▪ Tissue Distribution Studies of the Parent<br>▪ Excretion Studies of the Parent | ▪ Full PK Studies in Animals<br>▪ Tissue Distribution Studies of the Parent<br>▪ Excretion Studies of the Parent |

**Efficacy Studies**

- Cancer Models
- Neurodegenerative Disease Models
- Inflammatory and Autoimmune Disease Models
- Disease Model Development and Customization

***In Vivo* Mechanistic Studies**

- *In Vivo* Target Degradation
- Pathway Analysis
- Tissue Distribution Studies
- Biomarker Development

# Decoding the Mechanism and Impact of a VAV1 Molecular Glue

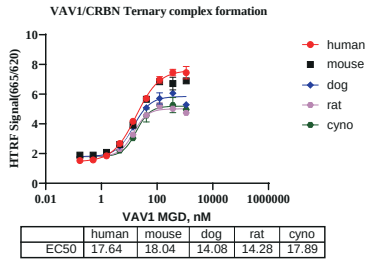
## Background

VAV1 is a key signaling protein in T cell activation and immune regulation, with aberrant expression linked to autoimmune diseases and cancer. Despite its biological relevance, VAV1 has long been considered undruggable. Recent advances in molecular glue degraders (MGDs) have enabled selective targeting of such proteins. Building on this concept, our study aims to evaluate a VAV1-directed MGD using a suite of *in vitro* and *in vivo* assays to characterize its degradation efficiency, mechanism of action, and functional impact.

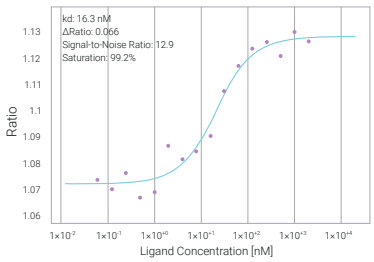
## Results

This case study uses a non-disclosed VAV1 degrader compound to showcase the capabilities of our assay platforms for molecular glue evaluation.

### 1. VAV1 Binary and Ternary Complex Formation




| | human | mouse | dog | rat | cyno |
|---|---|---|---|---|---|
| EC50 | 17.64 | 18.04 | 14.08 | 14.28 | 17.89 |

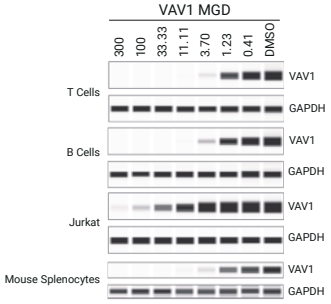


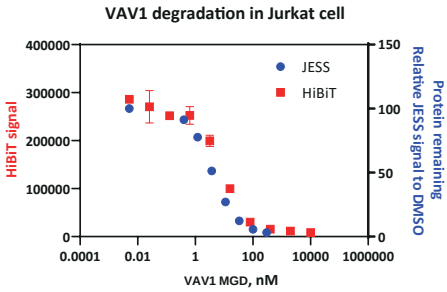

| Complex Type | Assay Format | Value |
|---|---|---|
| Binary Complex | HTRF ($IC_{50}$) | 1.2 μM |
| | Spectral Shift (Kd) | 511 nM |
| Ternary Complex | HTRF ($EC_{50}$) | 18 nM |
| | Spectral Shift (Kd) | 16.3 nM |
| | NanoBRET ($EC_{50}$) | 18 nM |

Figure 8: Evaluation of VAV1-targeting molecular glue complex formation. Left: Ternary complex formation across VAV1 proteins from different species, detected via HTRF. Middle: Spectral shift assay showing ternary binding with a determined Kd of 16.3 nM. Right: Summary of binary and ternary complex formation using recombinant human VAV1, assessed by HTRF, spectral shift, and NanoBRET PPI assays.

### 2. VAV1 Degradation Assays

### 2.1 *In vitro* HiBiT Assays and Simple Western Assays in Primary Immune Cells

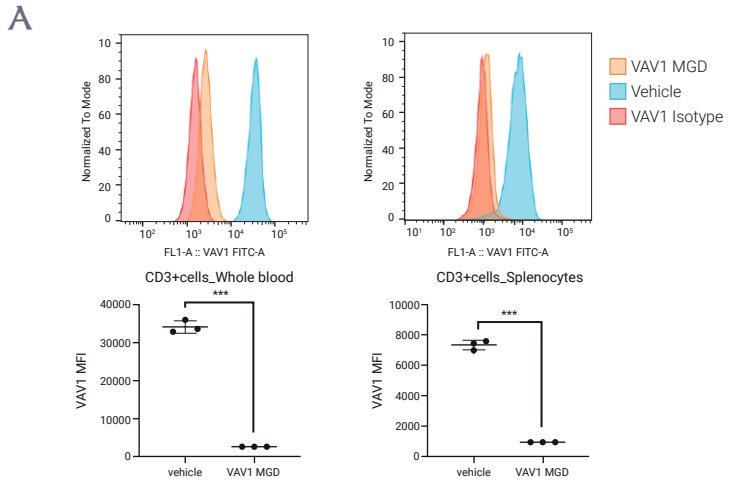



| Cell Type / Assay | $DC_{50}$ (nM) |
|---|---|
| Jurkat (HiBiT) | 10 |
| Jurkat (JESS) | 4 |
| Primary T cells (JESS) | 0.64 |
| Primary B cells (JESS) | 0.88 |
| PBMCs (JESS) | 0.99 |

Figure 9: Quantification of VAV1 degradation across cell types. Left: Representative protein detection by JESS showing dose-dependent VAV1 degradation across multiple cell types. Middle: Dose–response curves in Jurkat cells show strong agreement between HiBiT (red) and JESS (blue) platforms. Right: Summary of $DC_{50}$ values derived from HiBiT and JESS assays, indicating potent and consistent degradation across multiple immune cell types.

### 2.2 *In vivo* VAV1 Degradation Analysis via Flow Cytometry and Jess



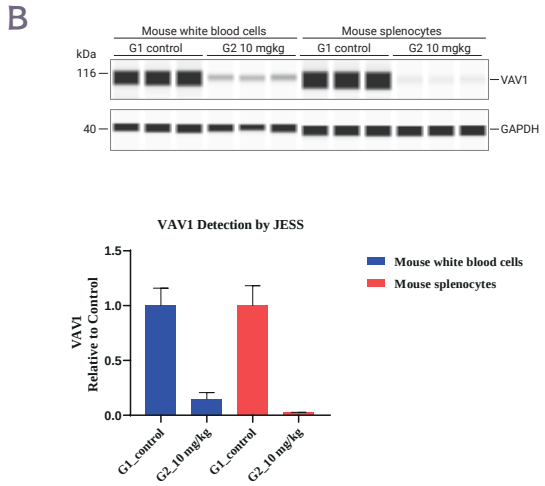




Figure 10: *In vivo* degradation of mouse VAV1 (A) Flow cytometry detection of VAV1 levels in CD3⁺ T cells from mouse whole blood and splenocytes 24 hours after oral dosing with VAV1 MGD. MFI reduction indicates effective *in vivo* degradation. (B) Jess-based quantification of VAV1 in mouse white blood cells and splenocytes confirms protein level reduction at 10 mg/kg dosing.

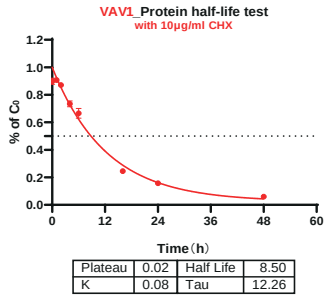
### 3. Functional Assays in Primary Immune Cells

To evaluate the immunomodulatory effects of the VAV1 degrader, we conducted functional assays in primary immune cells. These assays provide insights into the compound's impact on immune cell function and inflammatory signaling.

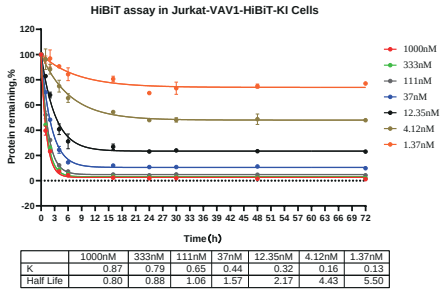
| Complex Type | Functional Assay | $IC_{50}$ (nM) |
|---|---|---|
| T cells | Proliferation (Flow cytometry) | 0.35 |
| | CD69 Detection (Flow cytometry) | 0.32 |
| | IL-2 Secretion | 0.35 |
| B cells | CD69 Detection (Flow cytometry) | 0.77 |
| | IL-6 Secretion | 0.75 |
| Th17 polarization | IL-17A Secretion (ELISA) | 1.95 |

### 4. MOA Studies

We provide rapid, multi-angle analysis of degradation mechanisms using CHX chase, time-course assays, and pathway validation to confirm proteasome dependency and degrader selectivity.

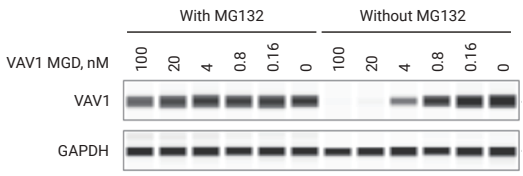



| | | | |
|---|---|---|---|
| Plateau | 0.02 | Half Life | 8.50 |
| K | 0.08 | Tau | 12.26 |




| | 1000nM | 333nM | 111nM | 37nM | 12.35nM | 4.12nM | 1.37nM |
|---|---|---|---|---|---|---|---|
| K | 0.87 | 0.79 | 0.65 | 0.44 | 0.32 | 0.16 | 0.13 |
| Half Life | 0.80 | 0.88 | 1.06 | 1.57 | 2.17 | 4.43 | 5.50 |

Figure 11: VAV1 undergoes dose- and time-dependent degradation. Left: CHX chase assay confirms active protein turnover. Right: Time- and dose-dependent degradation kinetics of VAV1 measured by HiBiT assay.



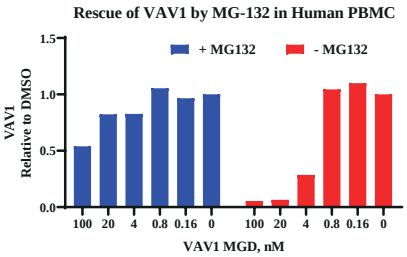




Figure 12: VAV1 degradation is mediated by the proteasome pathway. Treatment with the VAV1 molecular glue induces significant degradation of VAV1 protein, which is rescued by the proteasome inhibitor MG132. This indicates that VAV1 is degraded via a proteasome-dependent mechanism.

### 5. DIA-MS Off-Target Profiling

Unbiased, high-depth proteomic profiling to identify on- and off-target effects of degraders. Quantify thousands of proteins per sample across time points and doses to support selectivity assessment and mechanism validation.

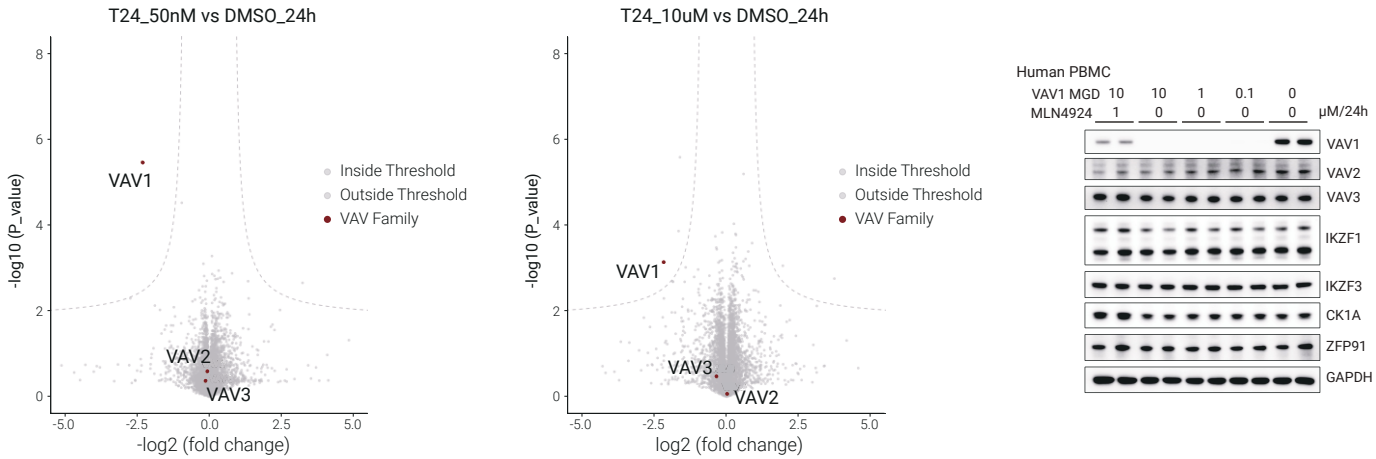



Figure 13. DIA-based proteomic profiling and Western blot confirm selective VAV1 degradation in human PBMCs. PBMCs were treated with VAV1 MGD (50 nM and 10 μM, 24 h). DIA profiling showed significant VAV1 degradation, with minor effects on VAV2 and VAV3. Western blot (right) validated these results.

ICE Bioscience was founded in 2010 as an Innovative CRO+ Explorer company. We specialize in early drug discovery services, spanning from target validation to the identification of pre-clinical candidates. We stand out for our collaborative spirit and expertise in boldly exploring new therapeutic target research. Our commitment to drug discovery services, delivered with enthusiasm and professionalism, empowers clients to overcome challenges, address scientific puzzles, and fulfill our promises to clients, communities, the environment, and global health.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