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# E3*scan*™ Ligand Binding Assay Platform and SPRINTer™ Cell Lines for Targeted Protein Degradation and PROTAC® Discovery

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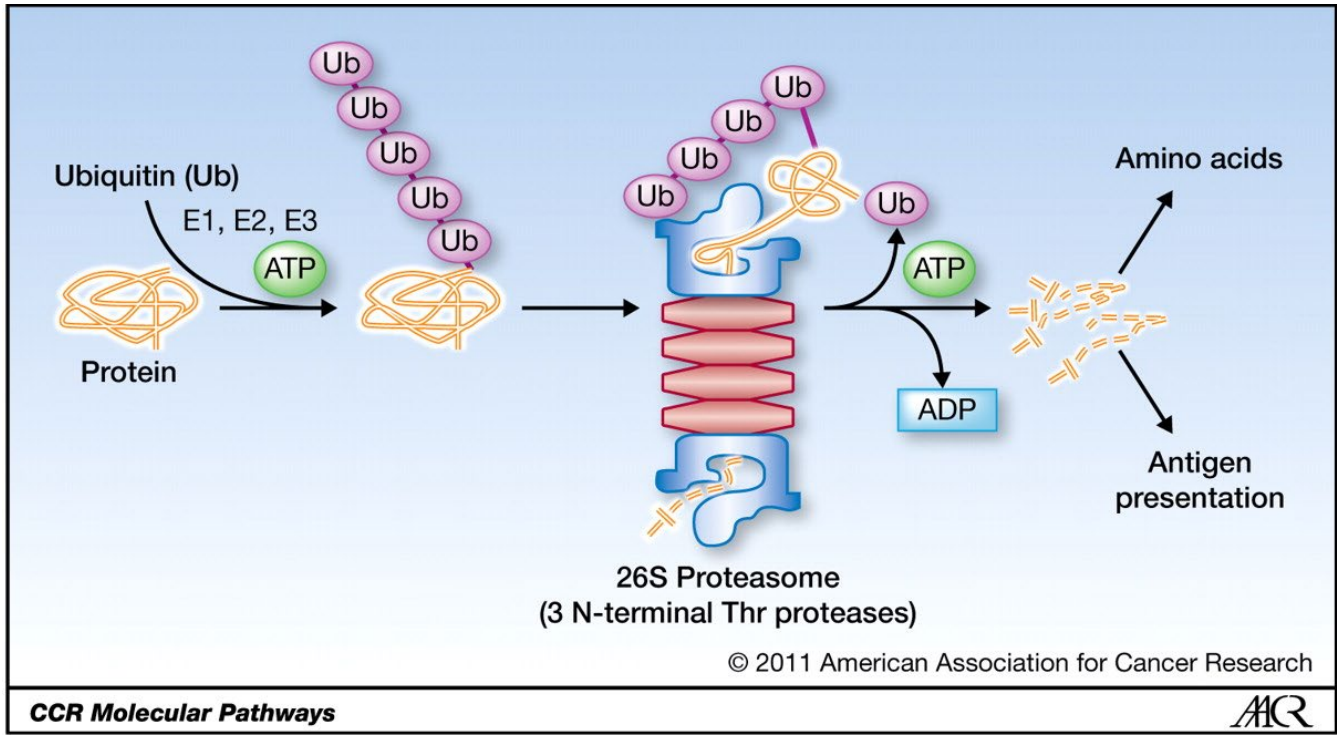
# Agenda

- Eurofins Discovery Targeted Protein Degradation Solutions Overview
- E3*scan*™ Technology and Assay Validation
    - CRBN with DDB1, VHL with Elongin B/C and other examples
- SPRINTer™ Biosensor Cell Lines For Screening Applications
- SPRINTer Assay Characterization
- Summary

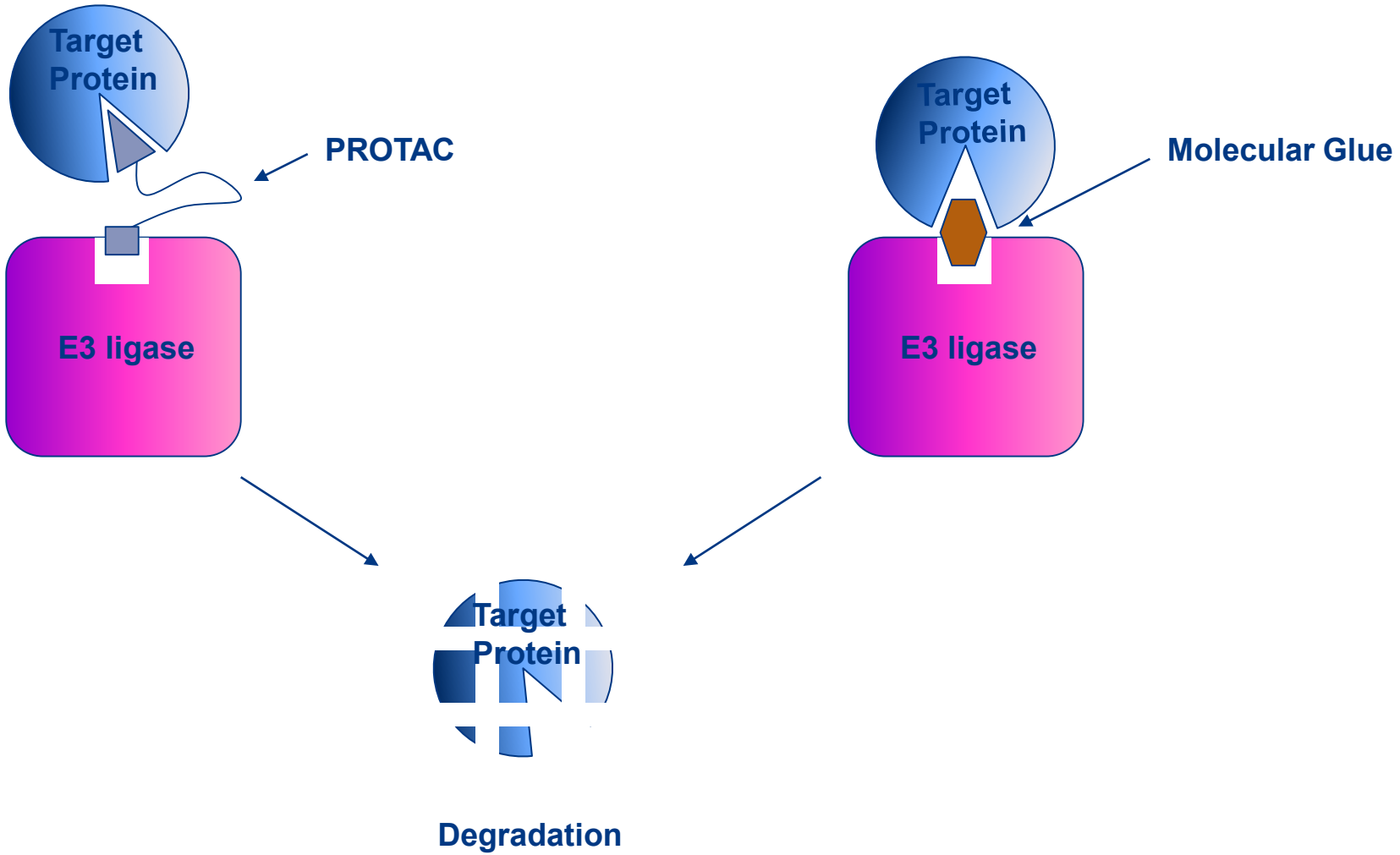
# Targeted Protein Degradation

The ubiquitin-proteasome system controls protein levels by tagging proteins with poly-ubiquitin (on specific lysine residues), which results in their degradation by the proteasome



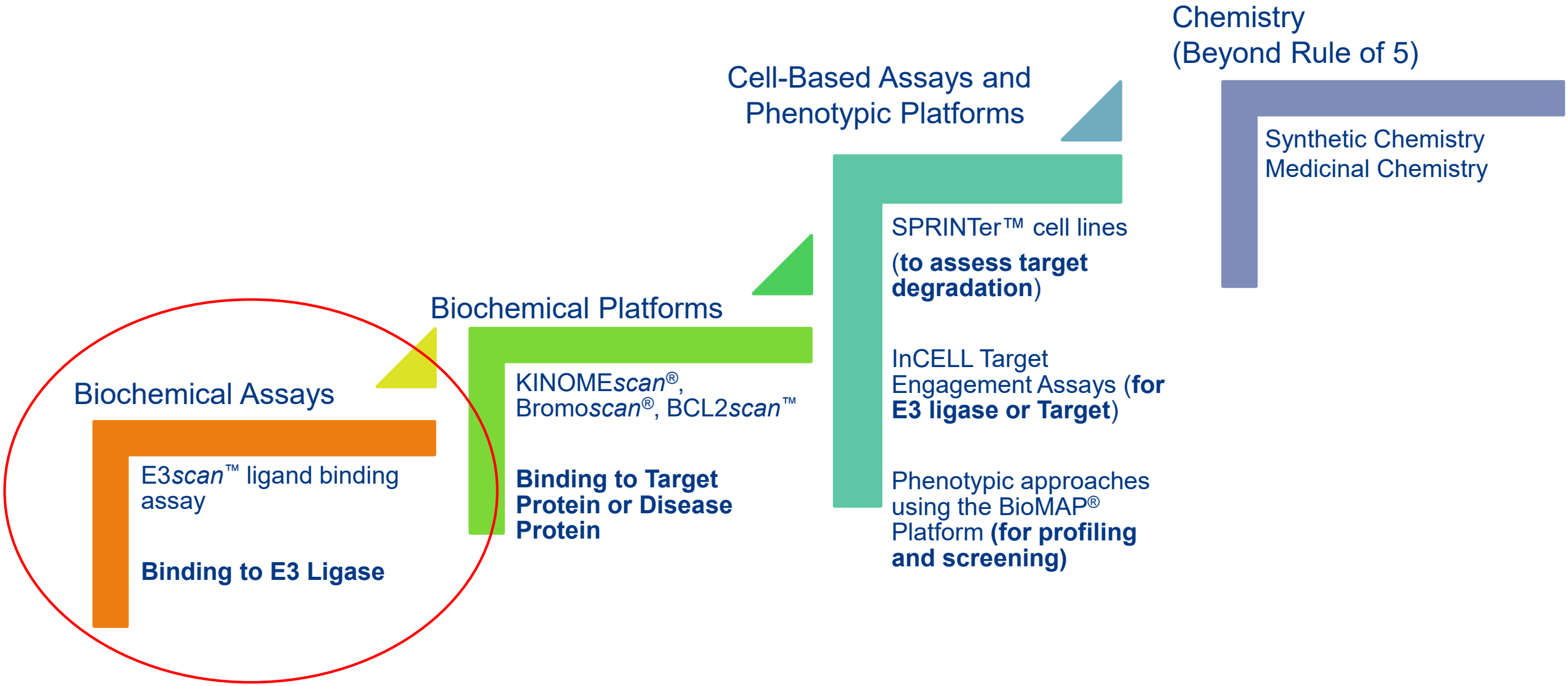

**Goal:** To manipulate the endogenous ubiquitin–proteasome system to achieve targeted degradation of specific proteins within cells

# Approaches in Targeted Protein Degradation: PROTAC® - Proteolysis-Targeting Chimera, and Molecular Glue



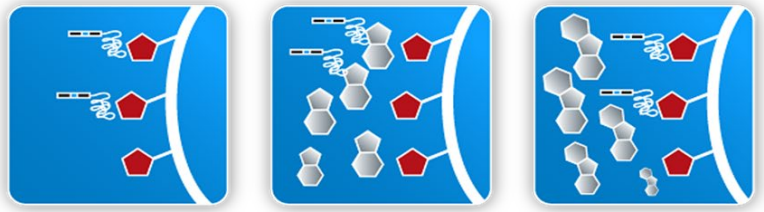

PROTAC is a trademark of Arvinas

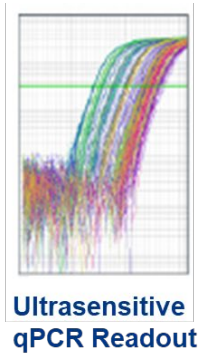
# Eurofins Discovery Protein Degradation Solutions



# Utilizing Novel Family-Wide Screening and Profiling Technology

*We can utilize our unique competition binding assays using DNA-tagged protein targets: qPCR readout. Protein purification not required*



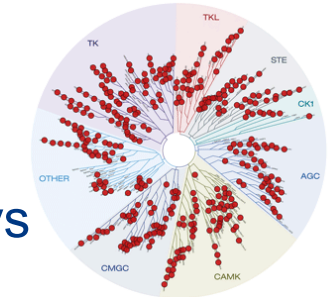




## E3*scan*™ - *NEW*

7 targets fully developed
9+ in development
500+ potential targets

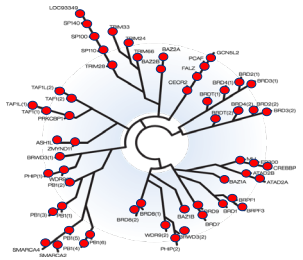
## KINOME*scan*®

489 assays




## BROMO*scan*®

40 assays



## BCL2*scan*™

All 5 anti-apoptotic BCL2 family proteins represented

# E3*scan*™ Assay Principle Using KINOME*scan*® Platform

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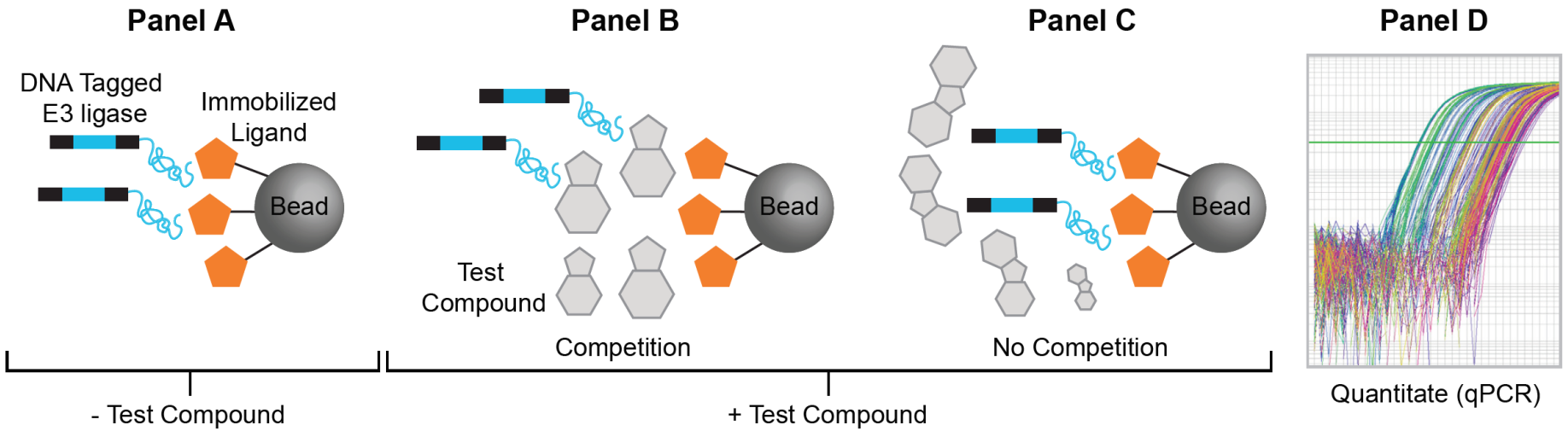
## Ligand binding site-directed competition binding assays

### Three key components

- E3 ligase tagged with DNA (low pM E3 ligase concentration in assay)
  - Expression in mammalian cells or by using proprietary T7 phage display system
- Known E3 ligase ligand (small molecule or peptide) immobilized on solid support
- Test compound or solvent control

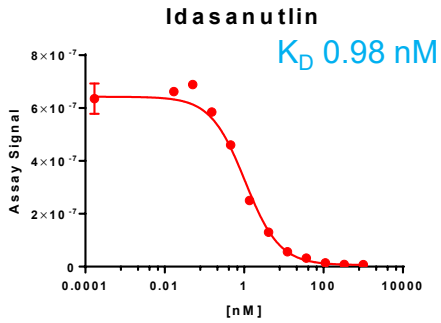
### Measure amount of E3 ligase captured by solid support in the presence or absence of a test compound (ultrasensitive qPCR readout)

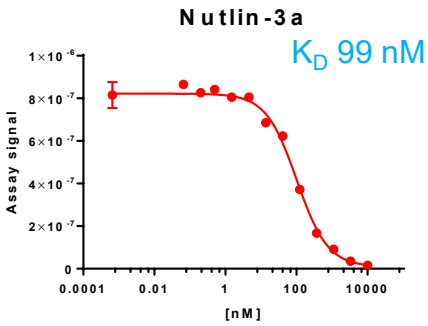


# Full Length MDM2 and MDMX E3*scan*™ Assay Validation Data

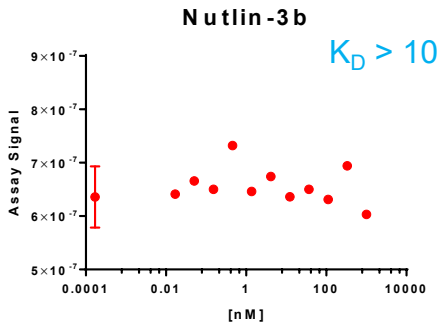
**MDM2**

MDM2 Positive controls:



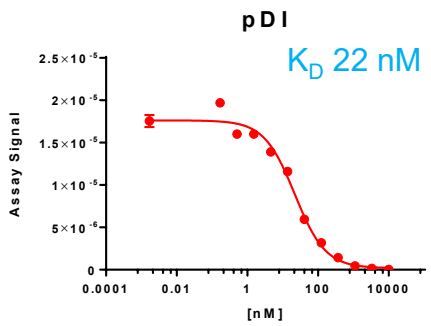




MDM2 Negative control:

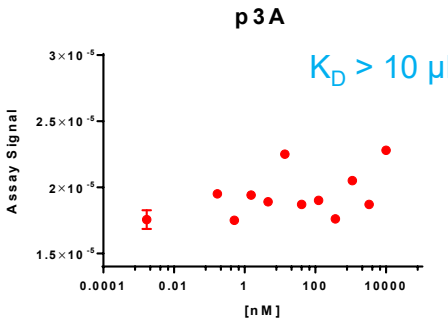



**MDMX**

MDMX Positive controls




MDMX Negative control:




## MDM2 and MDMX assay validation

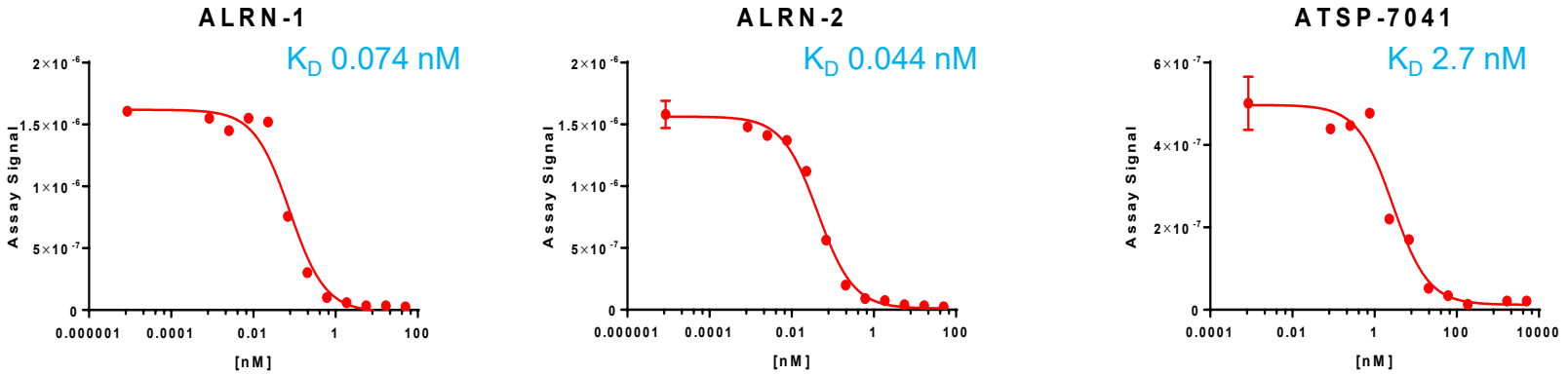
- Robust assay (assay window >50)
- Accurate data for control compound

| Compound Name | $K_D$ (nM) | Literature value |
|---|---|---|
| Idasanutlin | 0.98 | 6 nM by HTRF[1] |
| Nutlin-3a | 99 | 90 nM by SPR[2] |
| Nutlin-3b | >10,000 | 13.6 µM by SPR[2] |
| pDI | 17 | 100 nM by ELISA[3] |
| p3A | >10,000 | Non detected by ELISA[3] |

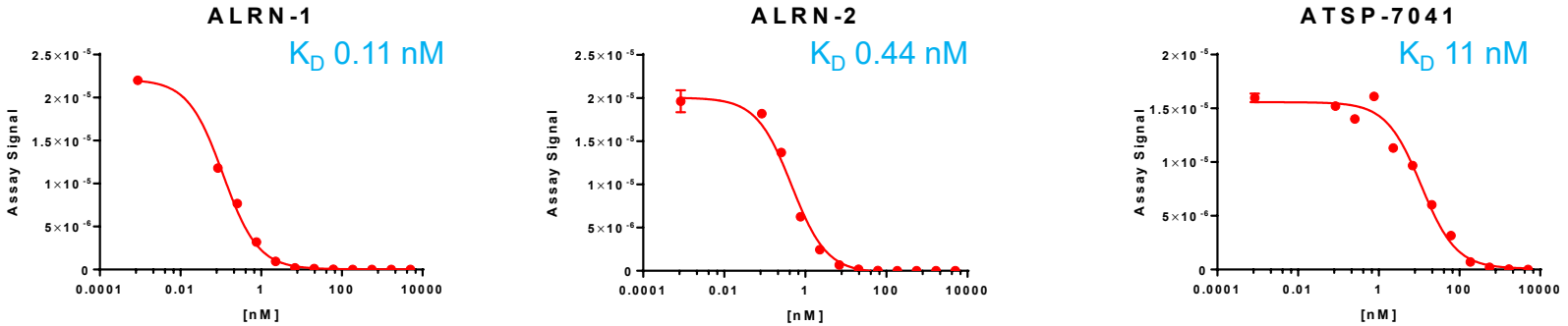

1. Ding et al, 2013
2. Vassilev et al, 2004
3. Hu et al, 2007

# MDM2 vs. MDMX E3*scan*™ Assay Validation Data - Aileron High-Affinity Stapled Peptides

## MDM2:




## MDMX:

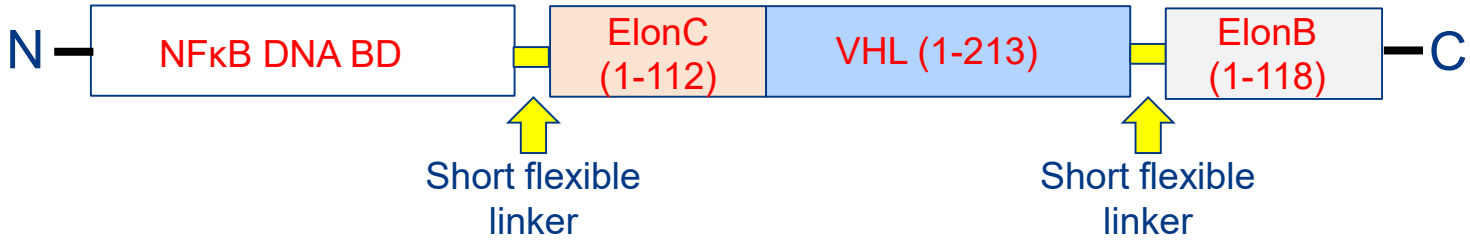



$K_D$ measurements for the interactions of Aileron's stapled peptides with MDM2 and MDMX.

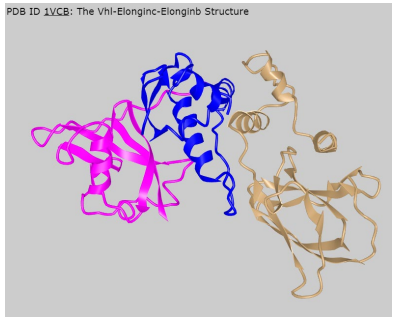
- The measured $K_D$s for ATSP-7041 stapled peptide are comparable to literature values measured using Biacore, 0.91 and 2.31 nM, respectively (Chang YS, et al. *PNAS*, 2013).

# VHL (elongin BC)
# E3*scan*™ Assay Validation Data

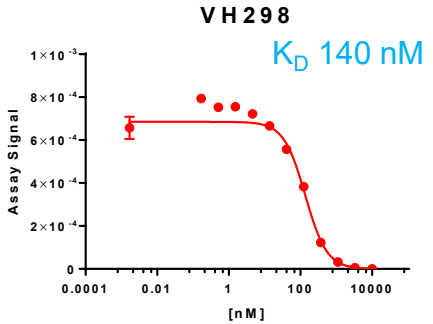
VHL fused to ElonC (NP_005639.1) and ElonB (NP_009039.1) – all full-length



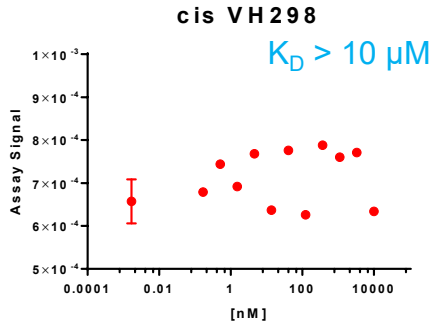

Inspired by PDB 1VCB




Positive control:




Negative control:

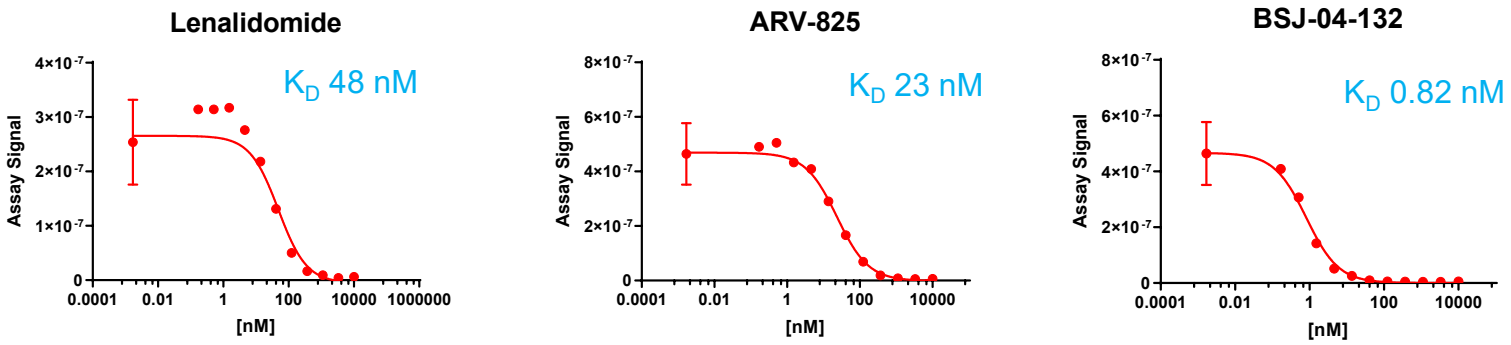



| E3 ligase | Compound | AVG $K_D$ (nM) | AVG Assay Window |
|---|---|---|---|
| VHL (elongin BC) | VH298 | 130 | 840 |
| VHL (elongin BC) | Cis VH298 | >10,000 | 0.9 |

## VHL (elongin BC) assay validation

- Robust assay (assay window >800)
- Accurate data for VH298, compare to 80-90 nM by FP and ITC (Frost J, et al. *Nat Commun*, 2016) and negative control Cis VH298 (no binding)
- Additional validation from compounds that cannot be disclosed

# Full Length CRBN E3*scan*™ Assay Validation Data




## CRBN assay validation

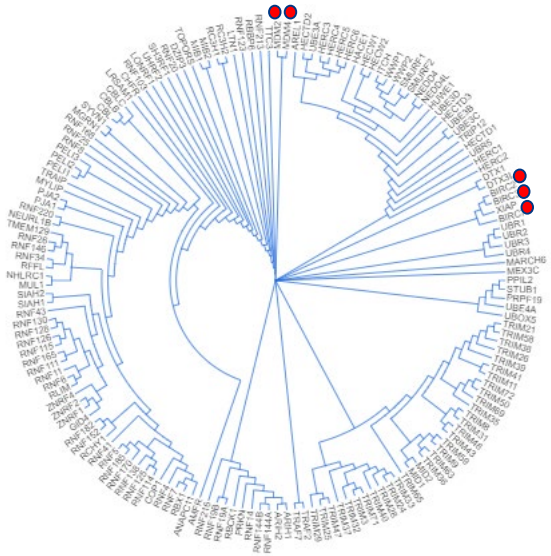
- Robust assay (assay windows >30)
- Accurate rank order $K_D$s for three control inhibitor compounds: Pomalidomide, Lenalidomide, Thalidomide (literature values obtained by FP are 157, 178, 250 nM respectively, Fischer ES, et al. Nature, 2014)
- Co-expression with DDB1 does not impact inhibitors $K_D$s and assay signals
- No binding detected for matching negative control PROTACs

| Compound Name | CRBN AVG $K_D$ (nM) | CRBN + DDB1 AVG $K_D$ (nM) | Compound Name | CRBN AVG $K_D$ (nM) | CRBN + DDB1 AVG $K_D$ (nM) |
|---|---|---|---|---|---|
| ARV-825 | 37 | 22 | Lenalidomide | 24 | 31 |
| BSJ-03-123 | 4.1 | 4.0 | Pomalidomide | 20 | 22 |
| BSJ-03-204 | 1.4 | 1.4 | Thalidomide | 48 | 44 |
| BSJ-04-132 | 1.3 | 0.77 | THAL SNS 032 | 18 | 13 |
| dBET1 | 3.8 | 4.8 | TL 12-186 | 10 | 5.7 |
| dBRD9 | 3.6 | 4.0 | TL 13-112 | 18 | 11 |
| dTAG-13 | 113 | 93 | ZXH 3-26 | 2.9 | 1.6 |

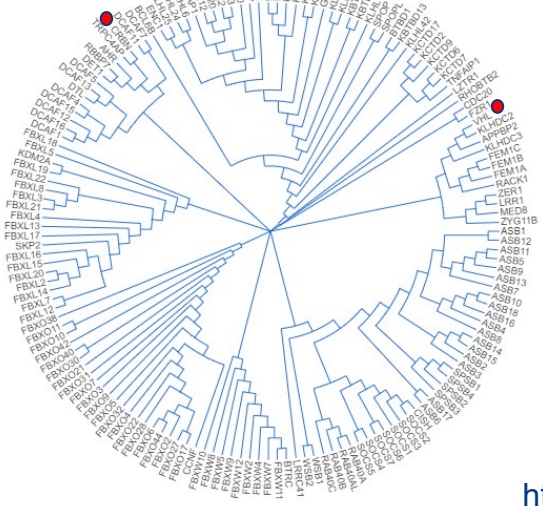
# E3*scan*™ Assay Development Summary

- We have developed and validated E3*scan* assays against MDM2, MDMX, VHL, CRBN, cIAP1, cIAP2, and XIAP
- All assays are robust and high throughput and give high quality $K_D$ curves
- Correct potency and rank order for the control inhibitors tested
- Assays do not approach the tight binding limit – even for pM compounds and stapled peptides
- More E3 targets in development, not limited by available small molecule ligand

**Simple E3 ligases tree**

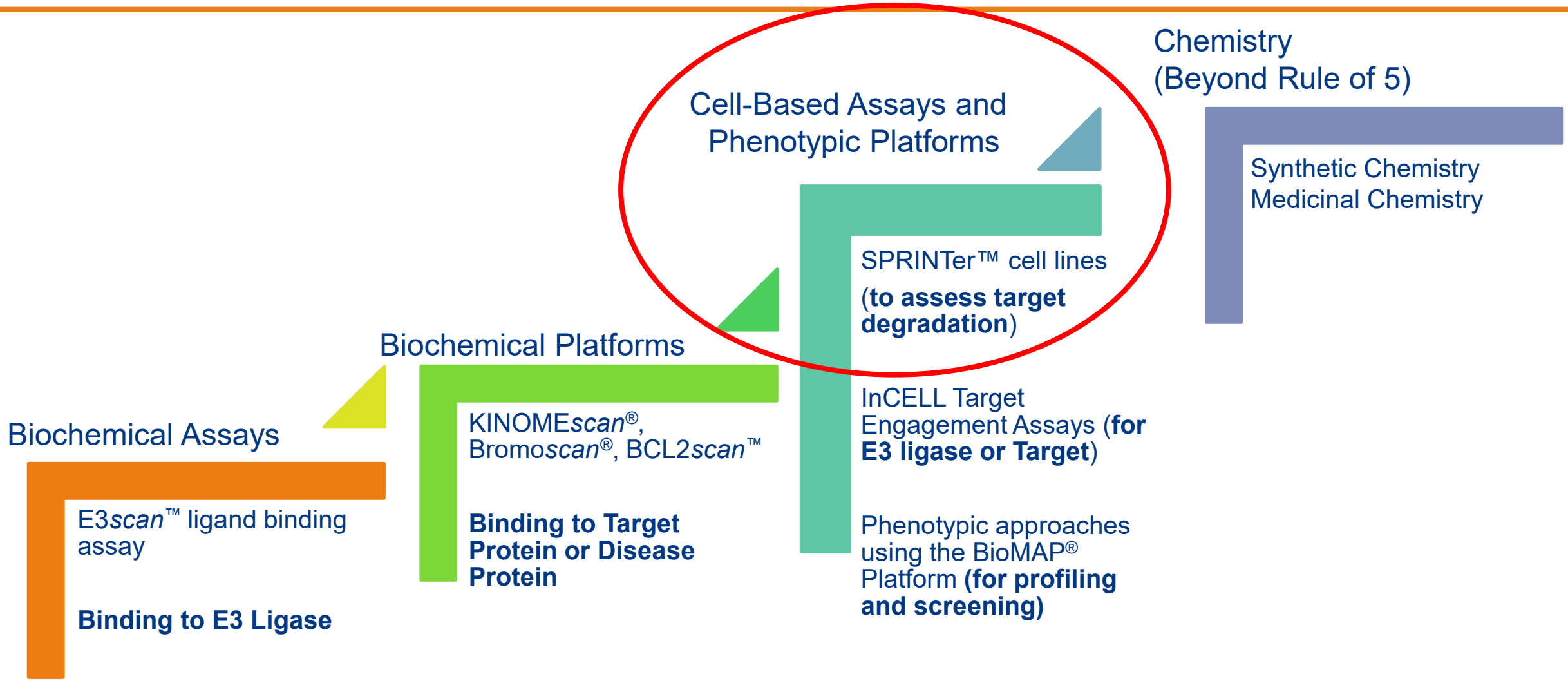


**Complex E3 ligases tree**

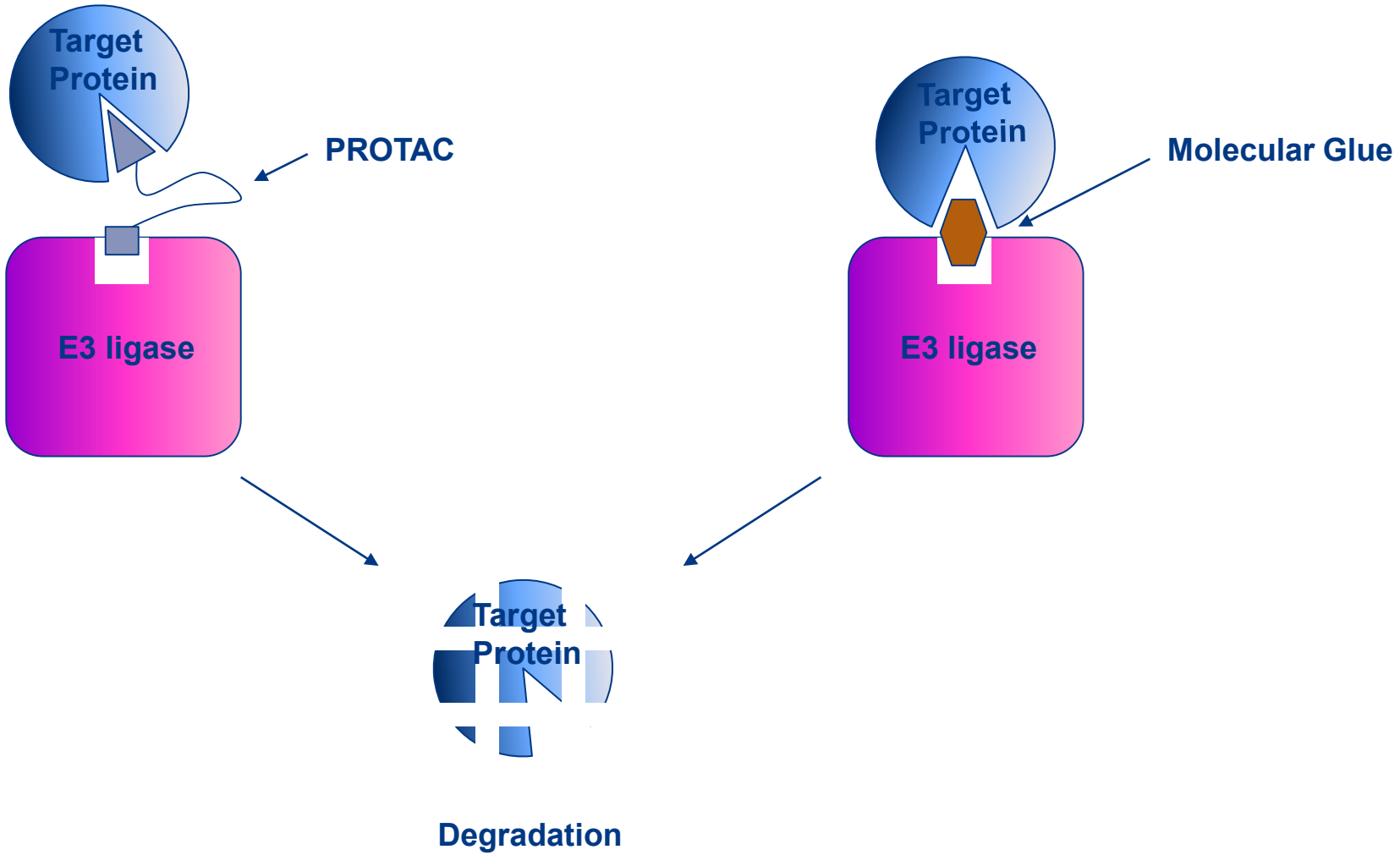


https://ubihub.thesgc.org/static/UbiHub.html

# Eurofins Discovery Protein Degradation Solutions



# Approaches in Targeted Protein Degradation: PROTAC® - Proteolysis-Targeting Chimera, and Molecular Glue

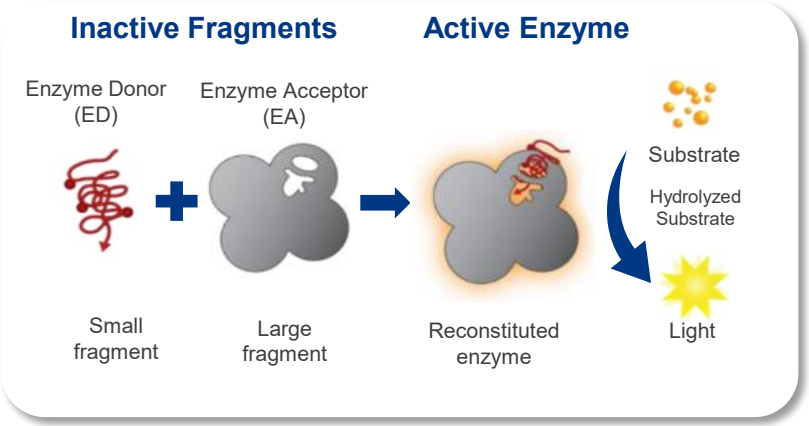



PROTAC is a trademark of Arvinas

# SPRINTer™ Cell-Based Assay Requirements

## Evaluating On-Target Activity of Targeted Degrader Molecules

- Compatible with disease cell models expressing physiologically relevant levels of the target protein and relevant E3 ligase(s)
  - Over-expression systems incompatible with certain degrader molecules, such as PROTACs
- A sensitive and scalable method for detection of protein turnover
  - Commonly utilized techniques include Westerns (low-throughput), ELISAs, or proliferation assays
- Robust, reproducible, and easy to implement

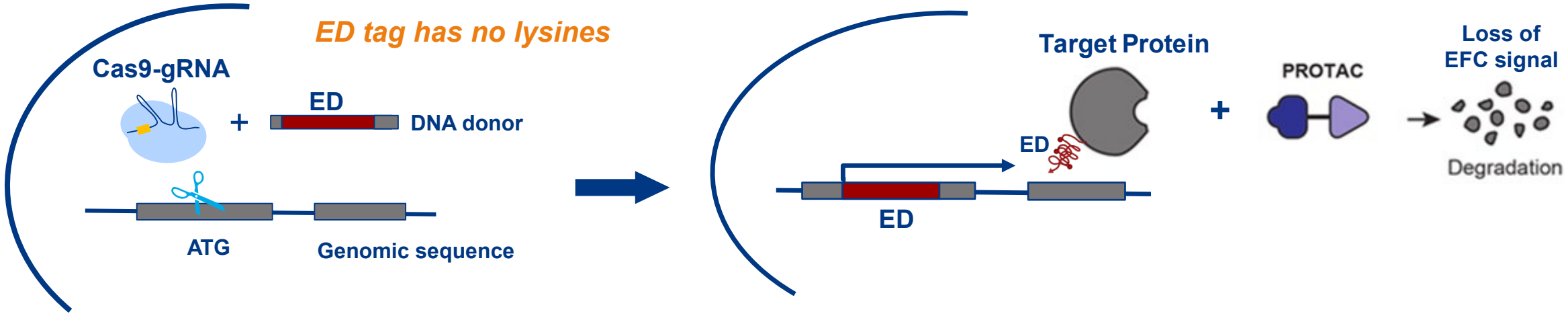



**Solution:**

**Enzyme Fragment Complementation (EFC)**

*Combines fM reporter sensitivity with ease of implementation (simple, homogeneous assay format)*

# Assay Concept: Employ CRISPR / Cas9 to Introduce ED Tag into Endogenous Target




## SPRINTer™ Assay Design: A Simple, Rapid and Homogeneous Assay Workflow

Seed stable biosensor cells in 96- or 384-well plate

Add degrader molecule(s)

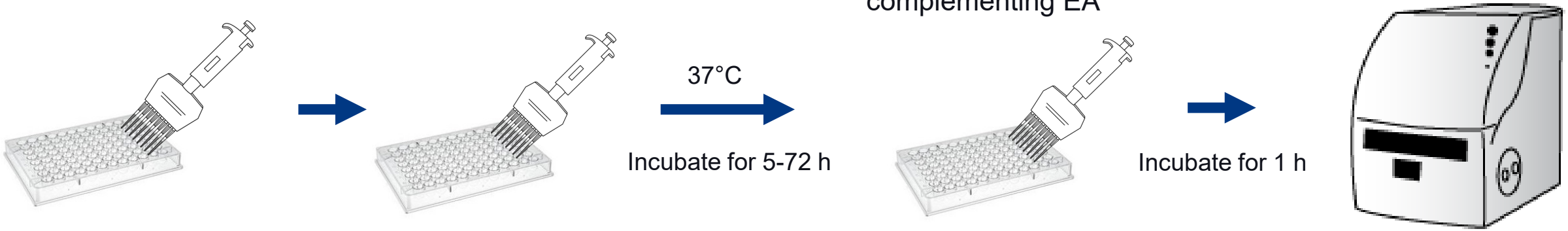
37°C

Incubate for 5-72 h

Add cell lysis reagent and detection reagent containing complementing EA

Incubate for 1 h

Read signal on plate reader



# SPRINTer™ Assays Proof-of-Concept

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## Building Biosensors for BRD4-Responsive Cancer Models

- Treatment with BET inhibitors (BETi) leads to decreases in c-Myc expression
- Several BETi (JQ1, I-BET-151, OTX015) with preclinical and clinical data supporting therapeutic use
- PROTACs targeting degradation of BRD4 also lead to decrease in c-Myc expression
- BETi and PROTACs to BRD4 are commercially available

### Cell Models

| Cell lines | Origins | BRD4 | c-Myc |
|---|---|---|---|
| K-562 | Chronic myeloid leukemia (CML) cell line | ++ | +++ |
| HCT116 | Colorectal carcinoma cell line | +++ | ++++ |

### Tool Molecules

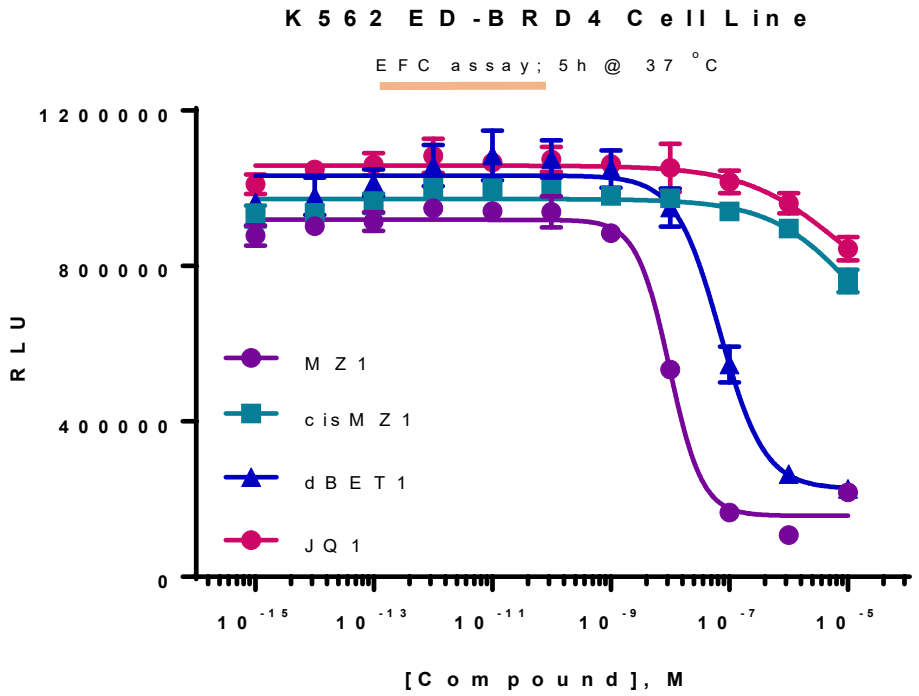
| Tool Molecules | BRD4 Ligand | E3 Ligase Ligand |
|---|---|---|
| MZ1* | JQ1 | VHL-1 (VHL) |
| dBET1* | JQ1 | Thalidomide (cereblon) |
| cisMZ1* | JQ1 | -- |
| JQ1 | JQ1 | N/A |

*PROTAC molecules

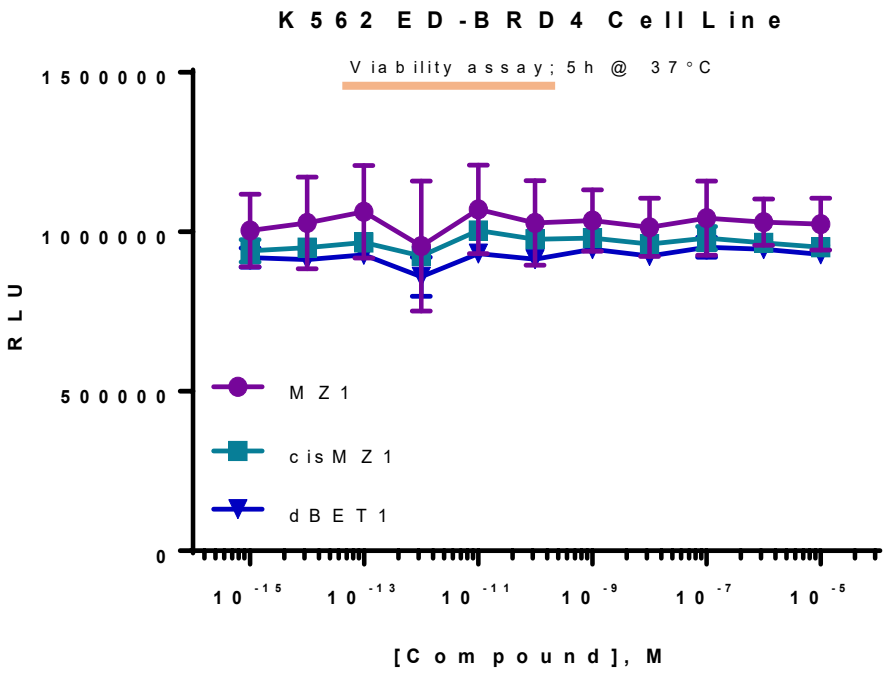


BET = Bromodomain & Extra-Terminal Motif; BRD4 = Bromodomain-containing protein 4

## Rapid Degradation of ED-BRD4 in K562 Cells with BRD4 Targeting Agents



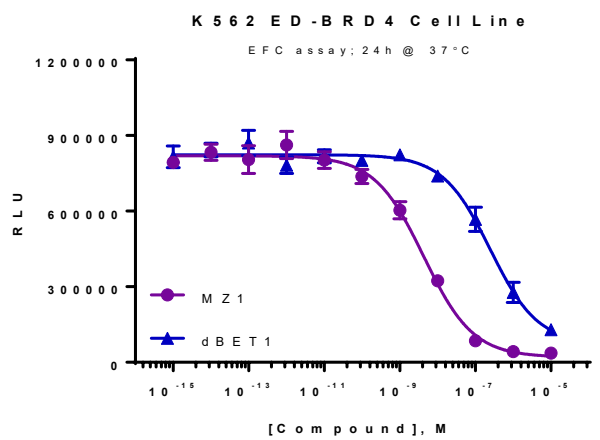

**EFC Assay** (functional)

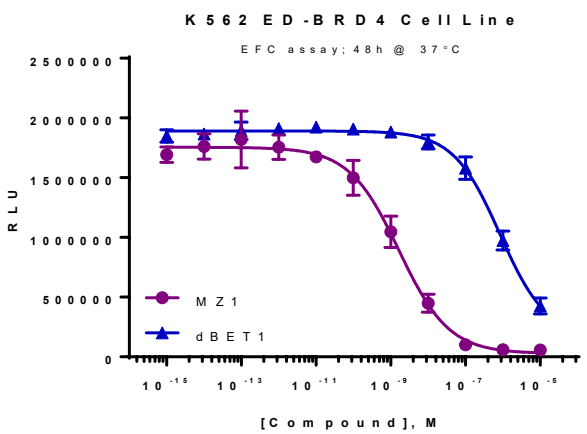


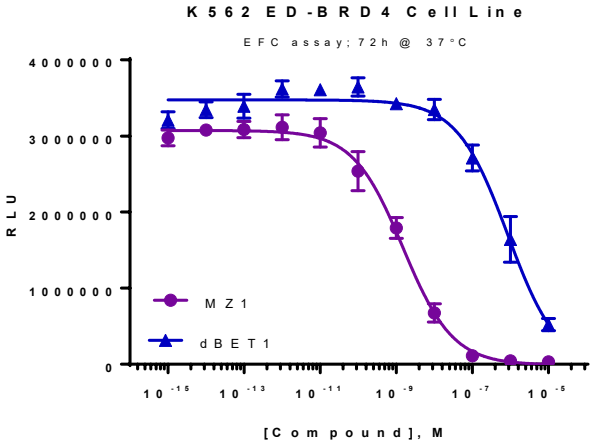

**Phenotypic Assay** (cell viability)

# Time Course for MZ1 and dBET1 Mediated Degradation of ED-BRD4 in K562 Cells and Cell Viability

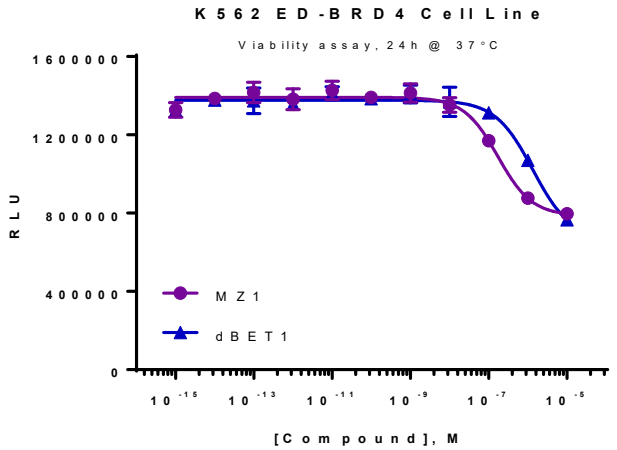
## EFC Assays (24, 48 & 72 hours)

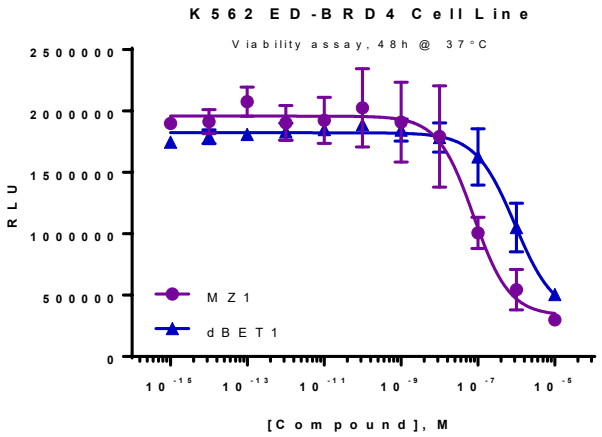


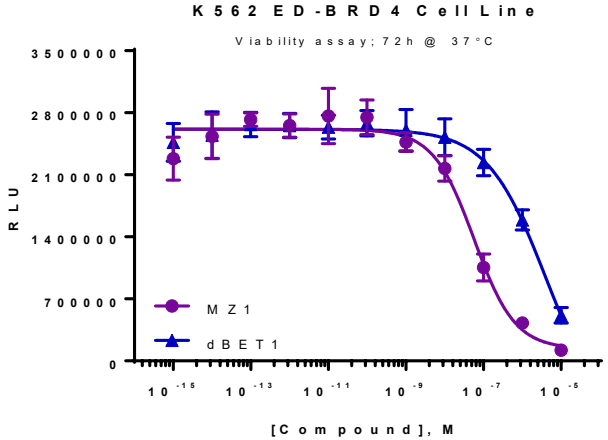




## Viability Assays (24, 48 & 72 hours)



# SPRINTer™ Assay Testing Results Summary

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## MZ1 Mediated Degradation of ED-BRD4 and Turnover of ED-c-Myc in K562 Cells

**EFC Assay** (functional)

| Biosensor | ED-BRD4 (K562) | | ED-c-Myc (K562) | | Fold Difference |
|---|---|---|---|---|---|
| | $EC_{50}$, nM | S/B | $EC_{50}$, nM | S/B | $EC_{50}$, nM |
| **5h** | 9.7 | 5.8 | 222 | 15 | 22.9 |
| **24h** | 4.3 | 18 | 51 | 14.3 | 11.9 |
| **48h** | 1.6 | 28 | 27 | 32 | 16.9 |
| **72h** | 1.5 | 66 | 13 | 14 | 8.7 |

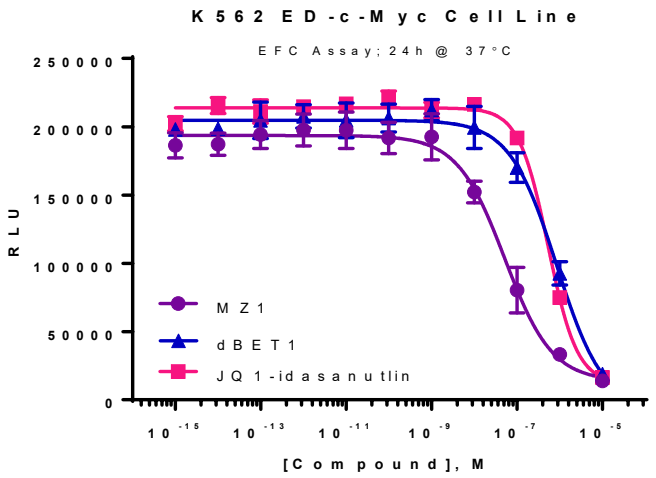
**Phenotypic Assay**
(cell viability)

| ED-BRD4 (K562) | |
|---|---|
| $EC_{50}$, nM | S/B |
| -- | -- |
| 172 | 1.6 |
| 77 | 6.3 |
| 57 | 19 |

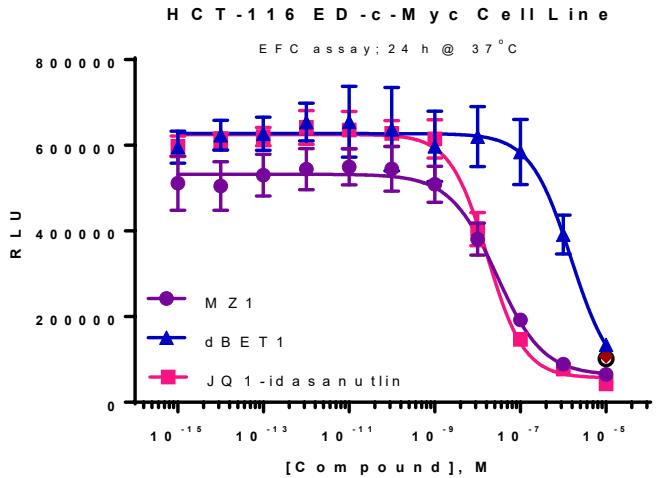
**EFC-based biosensor cells are fast and sensitive**

# SPRINTer™ Assay Characterization

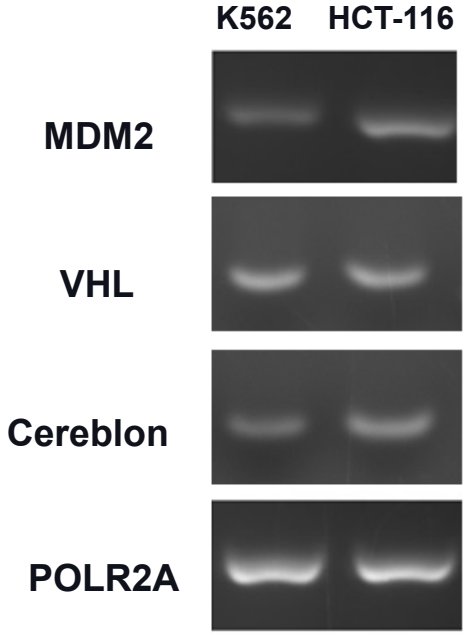
## Differential PROTAC Efficacies Among Cell Models Correlates with Relative E3 Ligase Expression




**MZ1 > dBET1 > JQ1-idasanutlin**




**MZ1 > JQ1-idasanutlin > dBET1**




| PROTAC | BRD4 ligand | E3 ligase ligand |
|---|---|---|
| JQ1-idasanutlin | JQ1 | idasanutlin (MDM2) |
| MZ1 | JQ1 | VHL-1 (VHL) |
| dBET1 | JQ1 | Thalidomide (cereblon) |

**Relevant cell model makes a difference**

# Conclusions

Cellular EFC Biosensor Cell Lines to Quantify Protein Turnover

- We have generated an **EFC-based assay platform** to quantify changes in **endogenous** protein levels in disease relevant cell models
- As a proof-of-concept, we have applied this assay platform for detection of drug-induced changes in endogenous **BRD4** levels and its downstream target, **c-Myc** in blood cancer (K-562) and colon cancer (HCT-116) cell models
- The **high sensitivity** of our assay platform allows the detection of target protein turnover induced by targeted degrader molecules, such as PROTACs, with more rapid kinetics than phenotypic endpoint assays (e.g. cell proliferation)

These SPRINTer™ biosensor cell lines provide a **screening platform** to identify new molecular entities that modulate oncogenic protein levels for therapeutic development

| SPRINTer Protein Turnover Biosenor Cell Lines |
|---|
| K562 BRD4 Cell Line |
| K562 c-Myc Cell Line |
| HCT-116 BRD4 Cell Line |
| HCT-116 c-Myc Cell Line |

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# Thank You

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