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Targeted Protein Degrader Discovery Using the E3*scan*™ Technology Platform and SPRINTer™ Cell Lines

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Discovery On Target September 28th, 2021

Agenda

- Targeted Protein Degradation (TPD) Introduction
- Overview of Eurofins Discovery TPD Portfolio
- E3*scan*™ Technology and Proof of Concept
- Other Biochemical Assays for Targeted Protein Degradation
- Eurofins Screening Capabilities
- SPRINTer™ Platform: A Cell-Based Functional Assay
- Q&A

TPD Represents A Compelling New Drug Discovery Strategy

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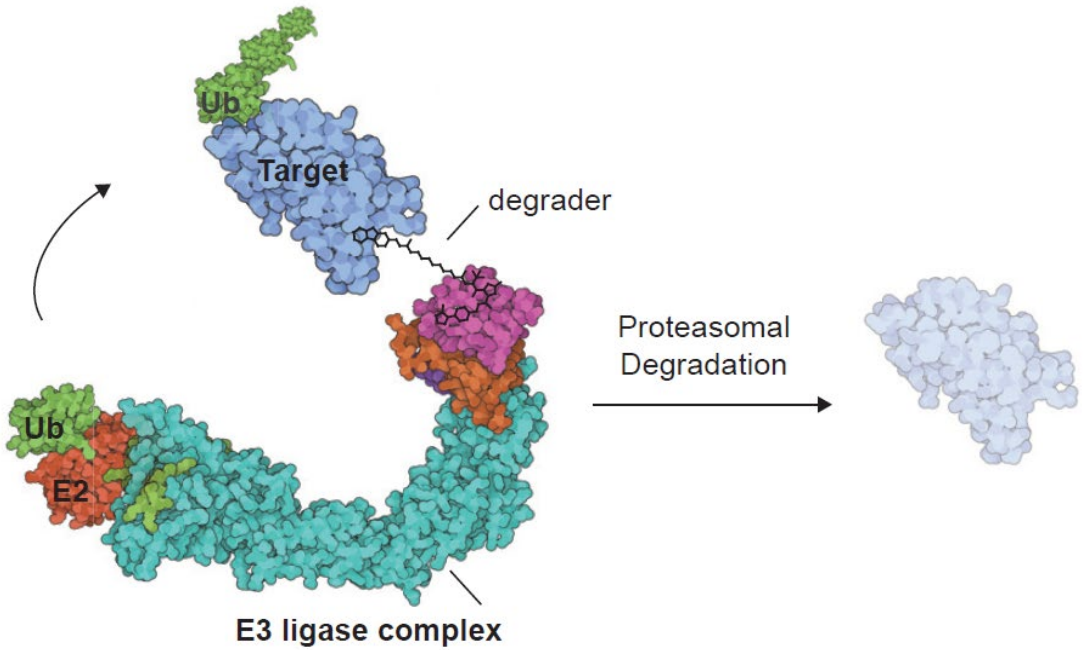
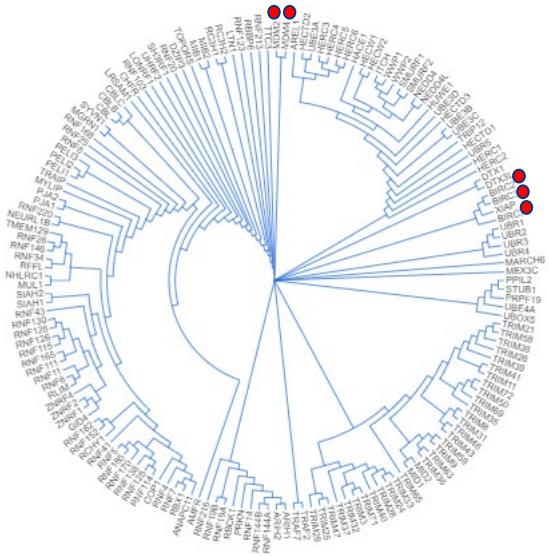
Goal: Expanding the TPD toolbox with more options for E3 ligase targets



Donovan et al., 2020, Cell

Simple E3 ligases tree



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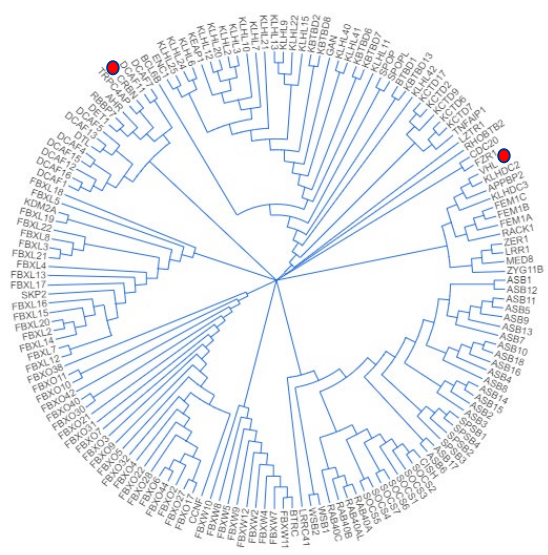
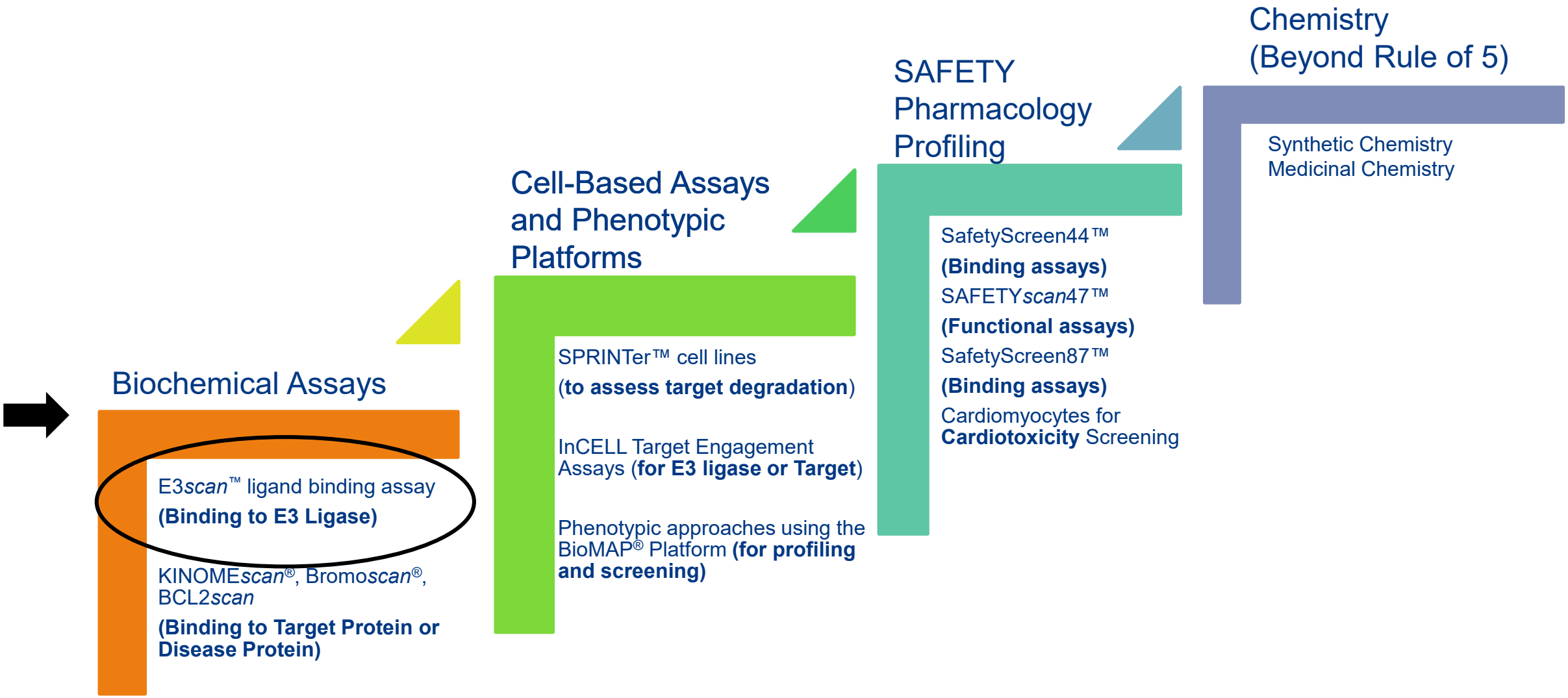


https://ubihub.thesgc.org/static/UbiHub.html

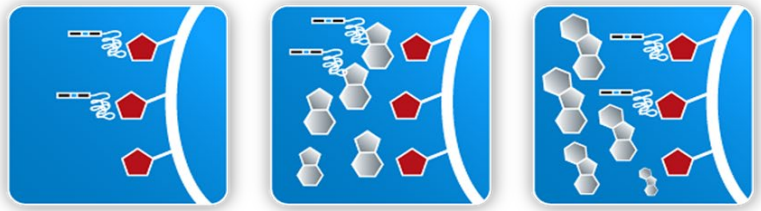
~1% of the entire 600 E3 ligase family has been explored in TPD

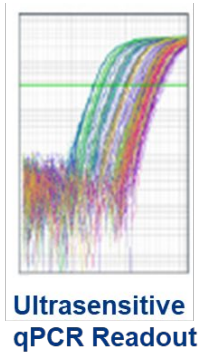
Eurofins Discovery TPD Portfolio: Assays and Analysis



Expanding the Novel E3 Family-Wide Screening and Profiling Technology

Unique competition binding assays with DNA-tagged protein targets and qPCR readouts eliminate the need for protein purification



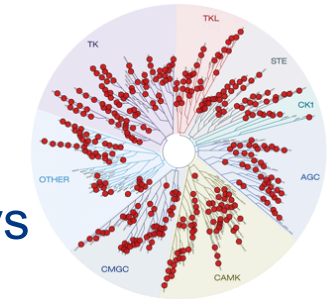


E3*scan*™ - *NEW*

12 family members are represented, and BIR domain selectivity available

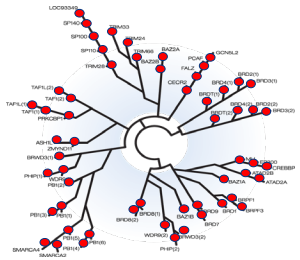
KINOME*scan*®

489 assays



BROMO*scan*®

40 assays



BCL2*scan*

All 5 anti-apoptotic BCL2 family proteins represented

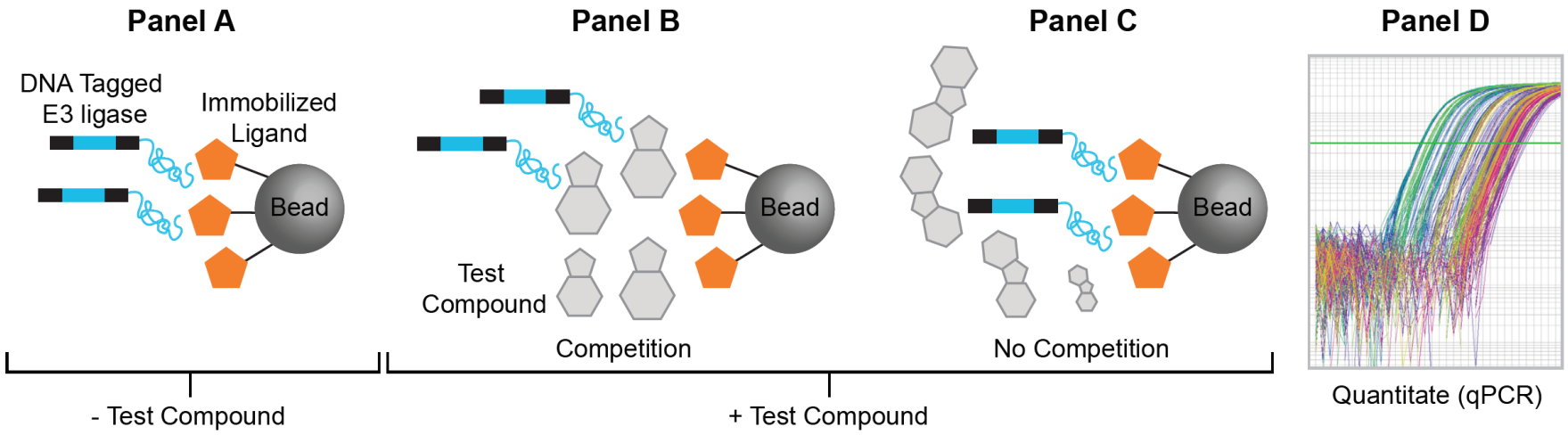
E3*scan*™ Assay Principle Using KINOME*scan*® Platform

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)



Advantages of E3*scan*™ Platform

- Broad sensitivity & dynamic range (pM to mM K_D values)
- All assays run in parallel on single platform
- The largest assay panel available on a single technology platform
- High throughput, run in 384-well format
- Rapid turnaround time of 5 days for weekly submissions
- 20 days turnaround time for library screens
- Custom assay development

E3*scan* Assays currently available

cIAP1 BIR2	CRBN	VHL-ELO_B/C
cIAP1 BIR3	CRBN-DDB1	XIAP BIR3
cIAP1 FL	ITCH WW1-2	XIAP FL
cIAP2 BIR2	MDM2	WWP1
cIAP2 BIR3	MDMX	WWP2
cIAP2 FL	NEDD4 WW2-3	SMURF2

Previously Launched E3*scan*™ Assays

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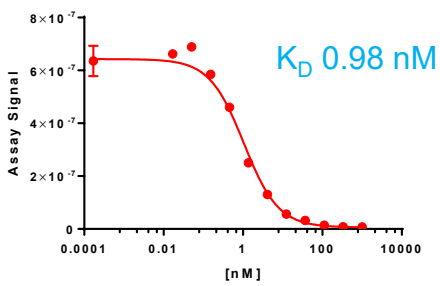
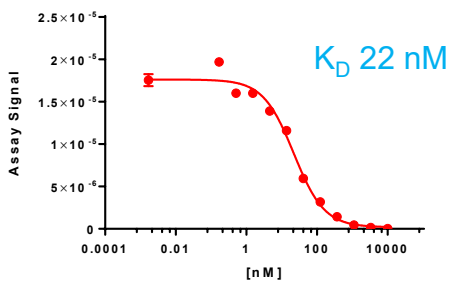
MDM2

Idasanutlin



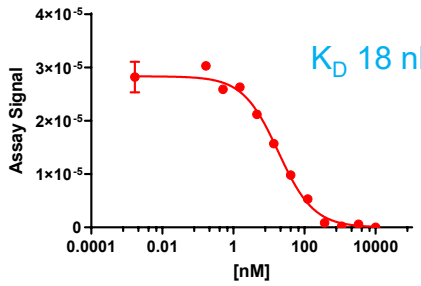
MDMX

pDI



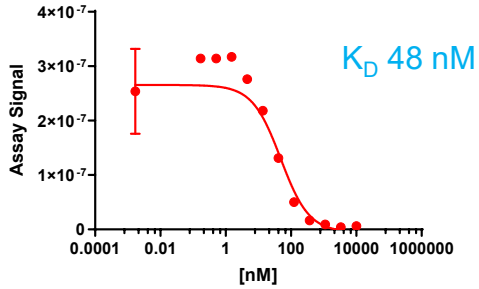
VHL-ELOB-ELOC

VH298



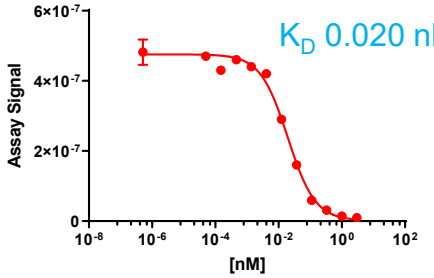
CRBN-DDB1

Lenalidomide



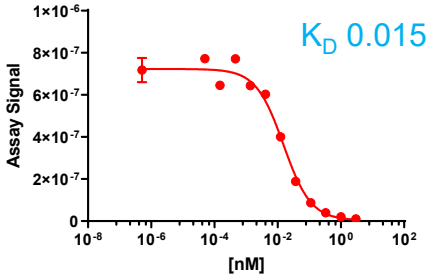
cIAP1

AZD5582



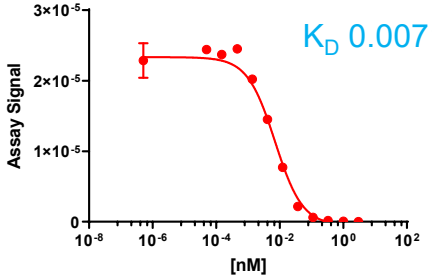
cIAP2

AZD5582



XIAP

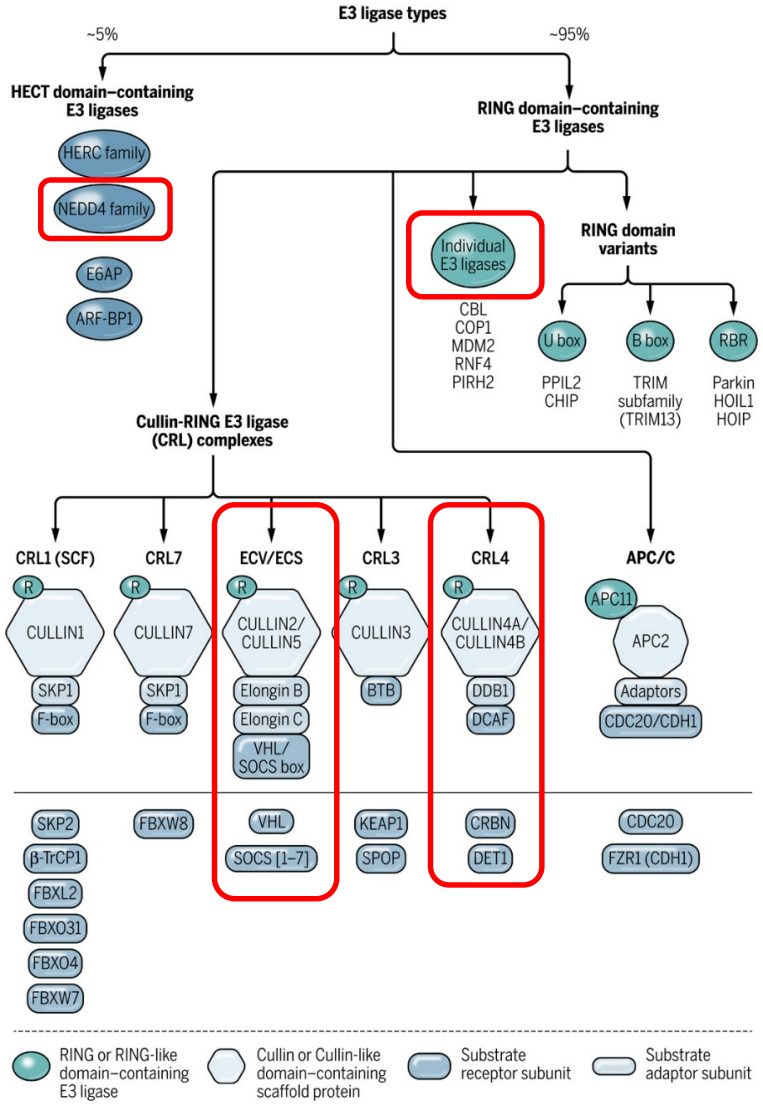
AZD5582



Individual BIR domain assays are now available, data presented in our poster

- We have previously launched E3*scan* assays for 7 commonly used E3 ligase targets in TPD
- 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

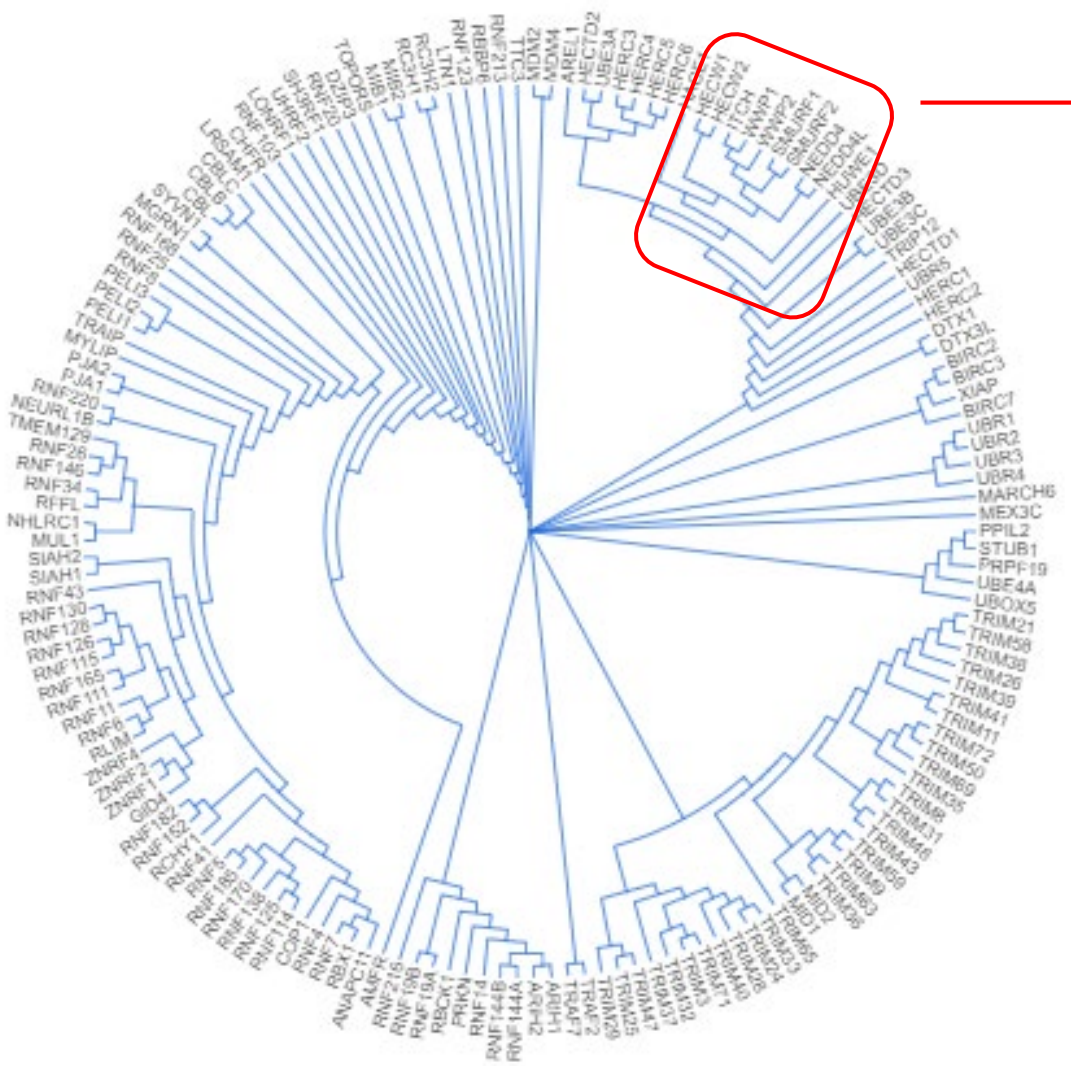
Classification of E3 Ligase Proteins and Complexes

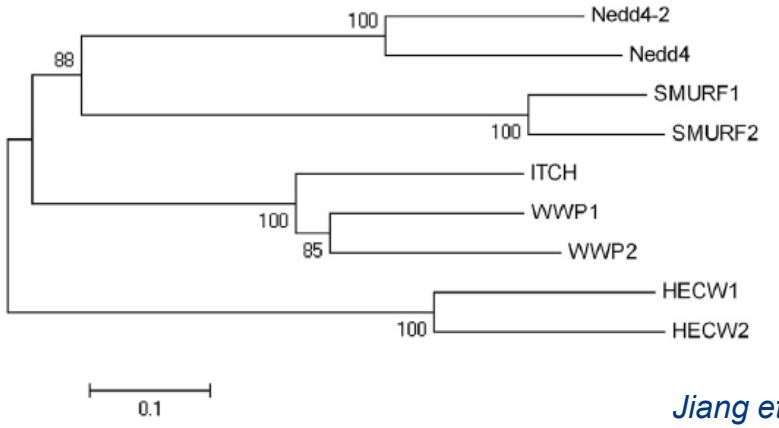


Mészáros et al. 2017, Science Signaling

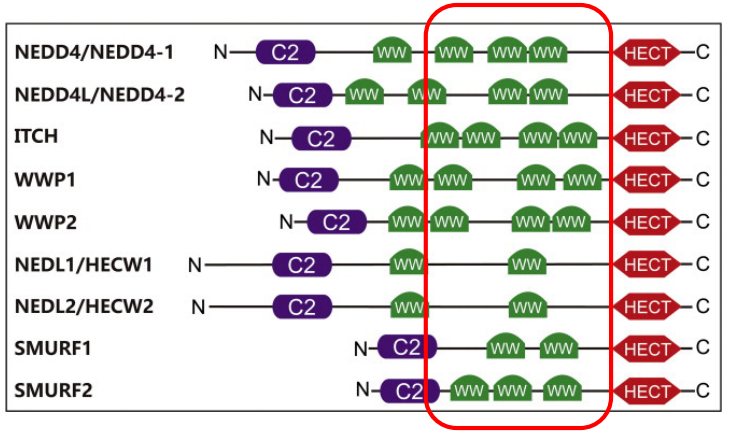
HECT-NEDD4 E3*scan*™ Assay Development and Validation

Simple E3 ligases tree





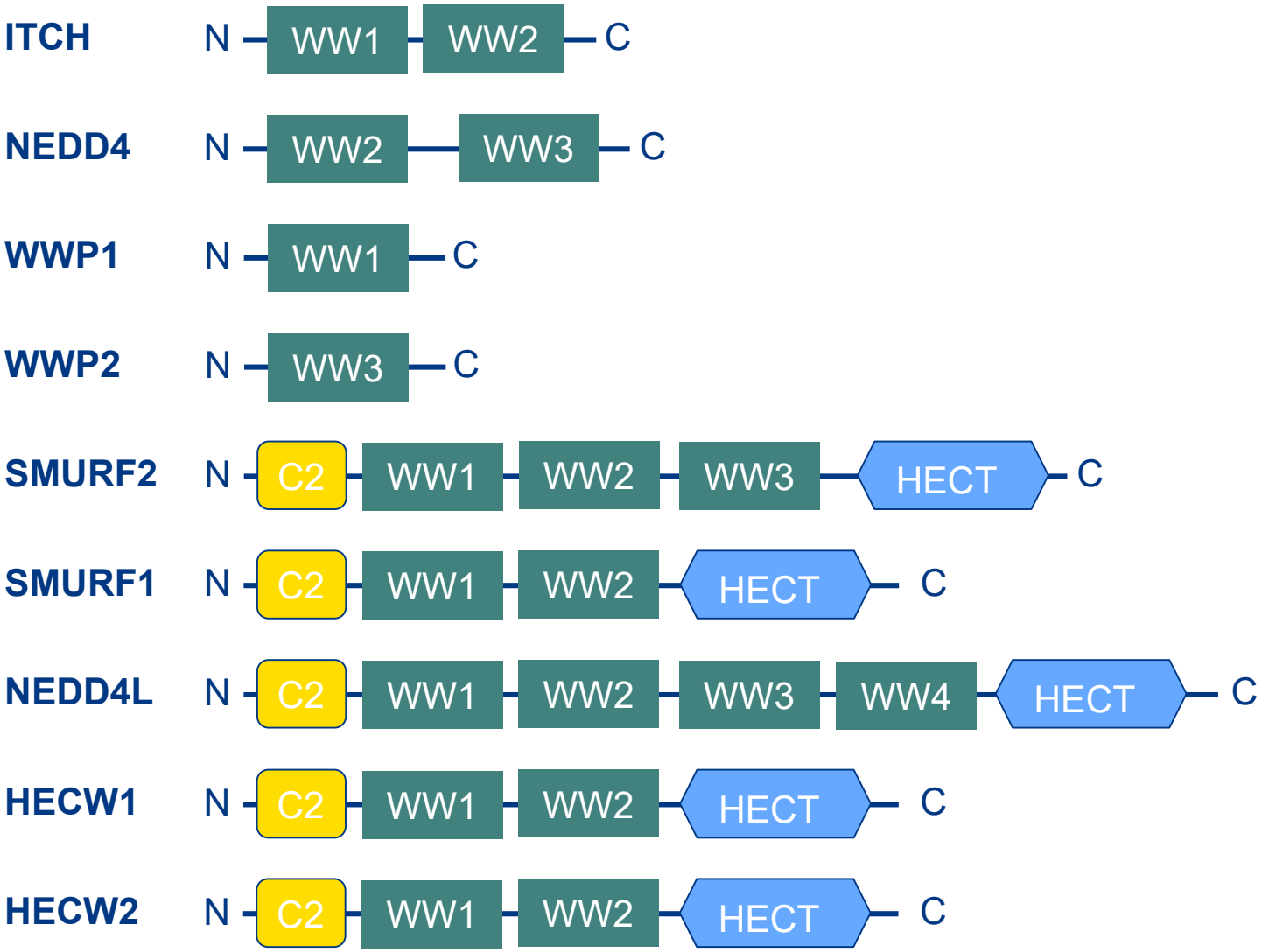
Jiang et al., 2015, FEBS Letters



Zou et al., 2015, BBA

PPxY

HECT-NEDD4 Sub-family Constructs used for E3*scan*™ Assay Development

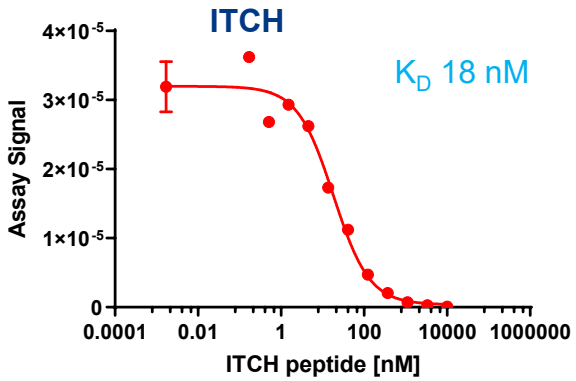


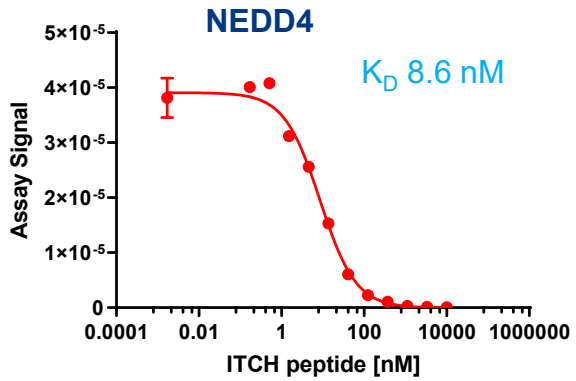
HECT NEDD4 Sub-family E3*scan*™ Assays: Summary Table

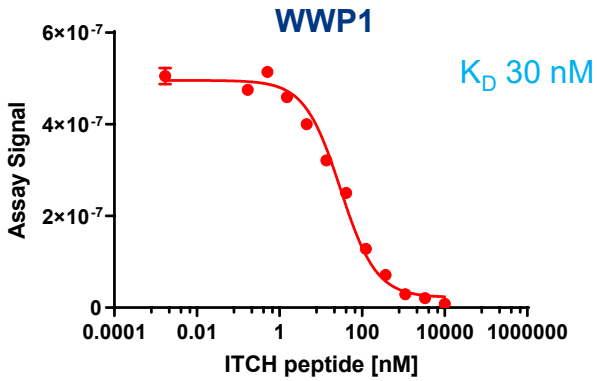
Peptide Name	Derivation	AVG K_D (nM)				
		NEDD4 E3*scan*	ITCH E3*scan*	WWP1 E3*scan*	WWP2 E3*scan*	SMURF2 E3*scan*
ITCH	TXNIP substrate of ITCH[1]	5.7	22	24	749	1492
NEDD4-2	ARRDC3 substrate of NEDD4[2]	108	47	54	1819	1597
WBP-1	YAP ligand[3]	>10,000	>10,000	1663	>10,000	>10,000
WBP-2A	YAP ligand[3]	625	>10,000	3264	>10,000	>10,000
Smad7	Smad7 adaptor of Smurf2[4]	1495	629	323	2233	258

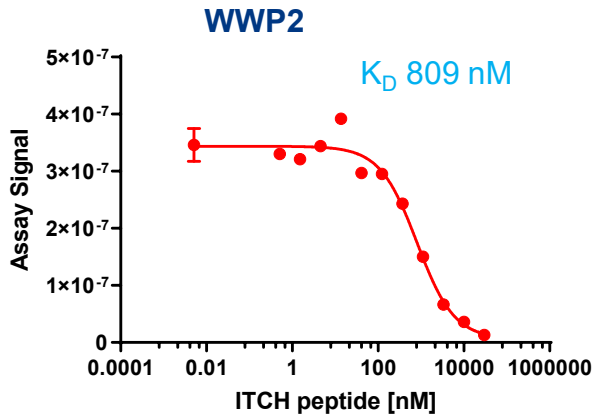
1. Liu et al., 2016, Biochem J
2. Qi et al., 2014, JBC
3. Pirozzi et al., 1997, JBC
4. Chong et al., 2006, JBC
5. Hu et al., 2004, Proteomics

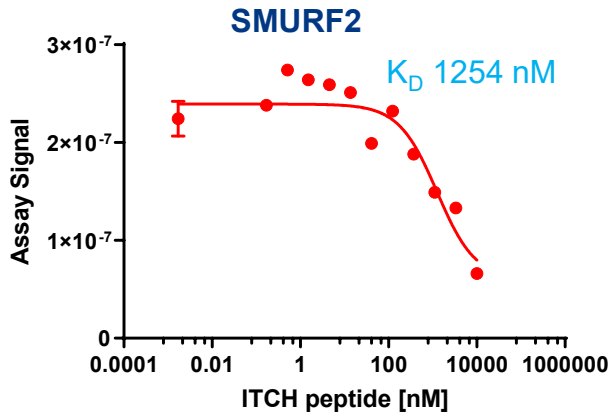
ITCH Peptide Across E3*scan*™ Assays











- ITCH peptide containing two PPxY motifs from a substrate of ITCH (TXNIP) was used as reference control peptide
- As expected, ITCH is binding potently to its substrate peptide
- NEDD4 and WWP1 also bind potently to the ITCH substrate peptide, but not WWP2 and SMURF2

HECT NEDD4 Sub-family E3*scan*™ Assays: Summary Table

Peptide Name	Derivation	AVG K_D (nM)				
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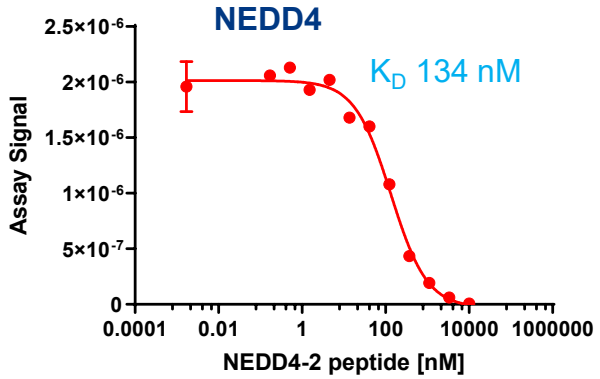
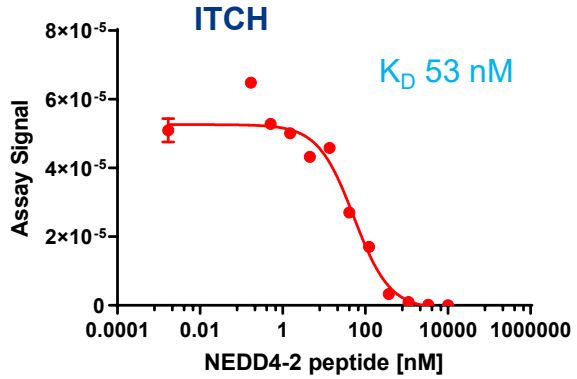
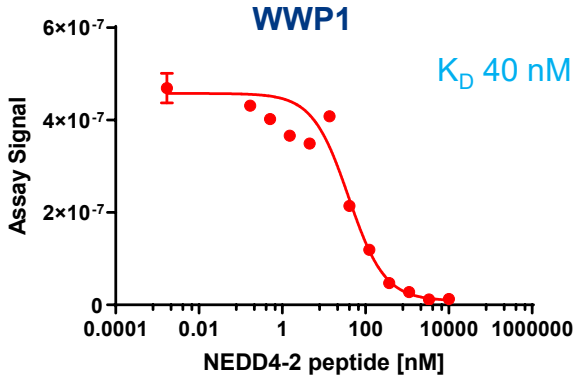
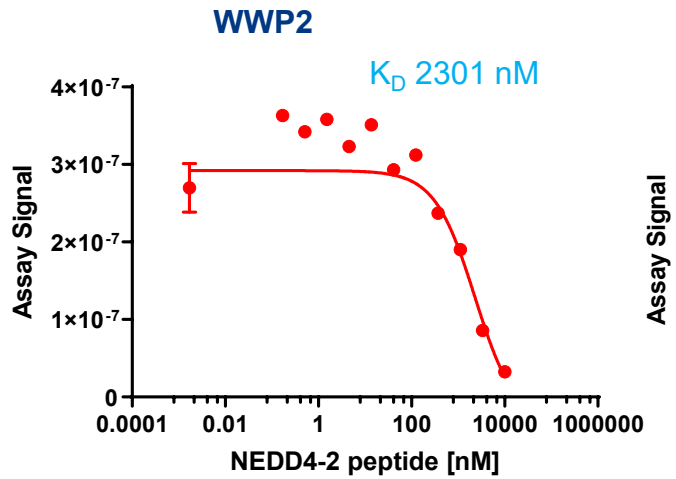
5. Hu et al., 2004, Proteomics

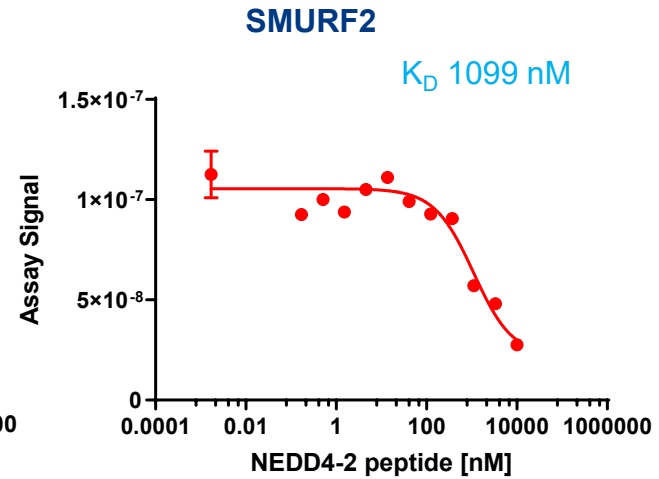
NEDD4-2 Peptide Across E3*scan*™ Assays











- NEDD4-2 peptide containing two PPxY motifs from a substrate of NEDD4 (ARRDC3) was used as reference control peptide
- As expected, NEDD4 is binding potently to its substrate peptide
- ITCH and WWP1 also bind potently to the NEDD4 substrate peptide, but not WWP2 and SMURF2

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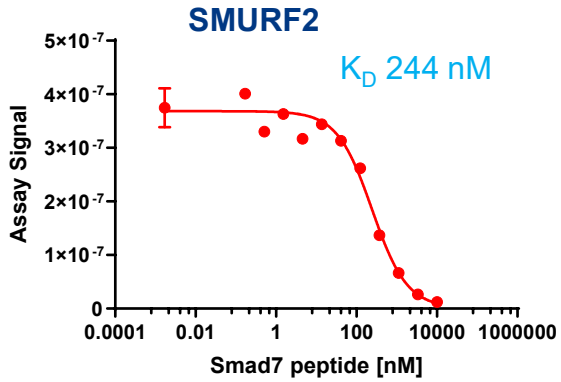
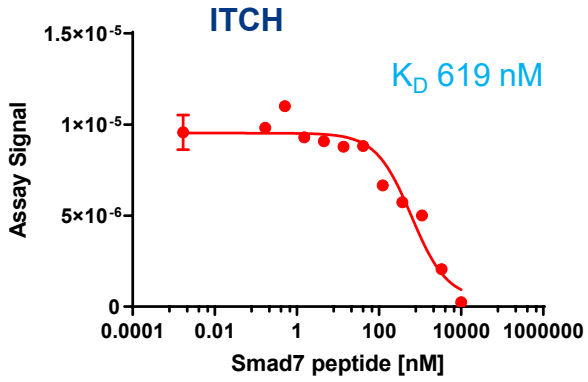
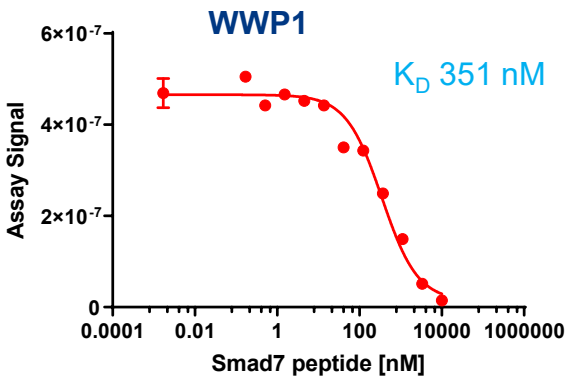
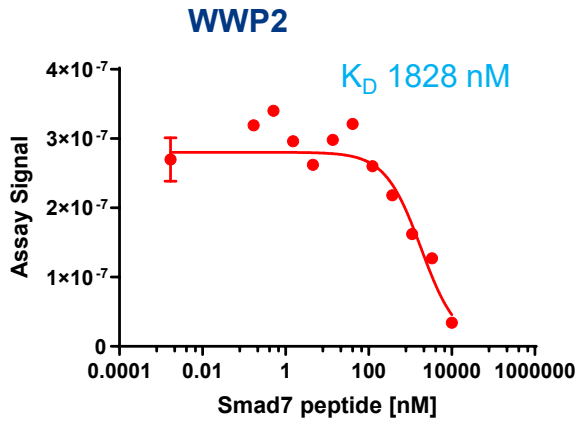
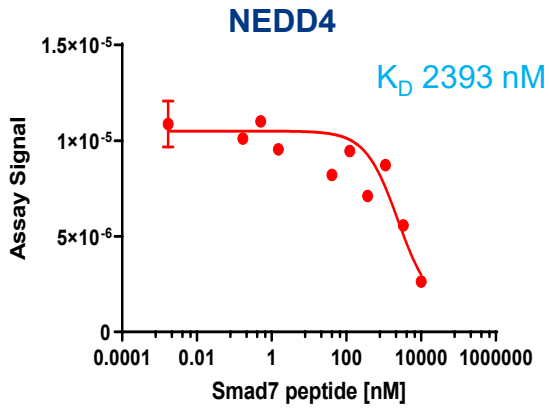
Smad7 Peptide Across E3*scan*™ Assays











- Smad7 peptide containing a PPxY motif from an adaptor protein of SMURF2 (Smad7) was used as reference control peptide
- As expected, SMURF2 is binding most potently to its adaptor protein peptide
- WWP1 and ITCH also show some binding to the SMURF2 adaptor protein peptide, but not WWP2 and NEDD4

HECT NEDD4 Sub-family E3*scan*™ Assays: Summary Table

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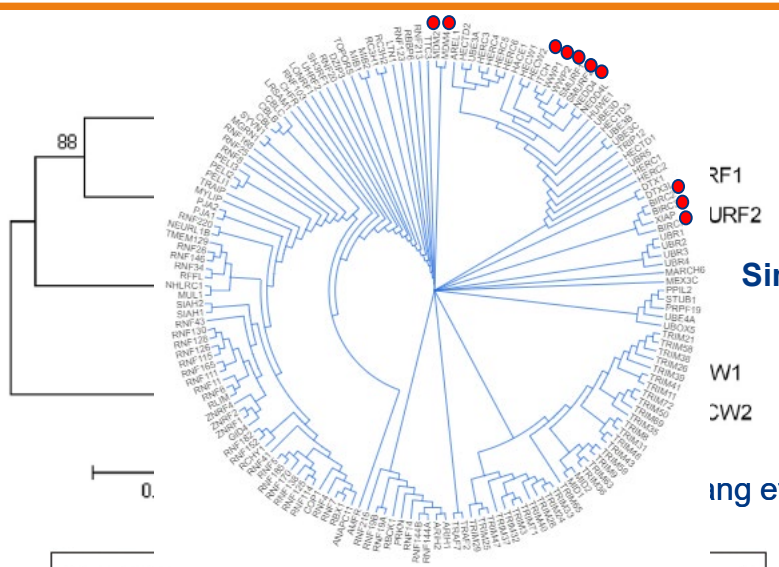
5. Hu et al., 2004, Proteomics

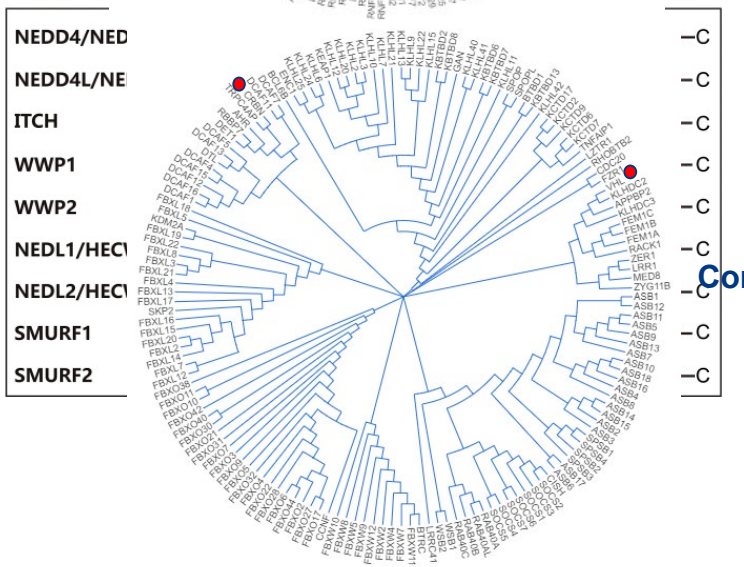
Take Home Message – E3*scan*™ Development

- We have developed robust and high throughput E3*scan* assays
- We show selective binding of HECT-NEDD4 family substrates to different members of this E3 ligase family
- These results demonstrate the capabilities of E3*scan* in screening for selective ligands across the E3 ligase family
- More E3 targets in development, not limited by available small molecule ligand



Simple E3 ligases tree

ıng et al., 2015, FEBS Letters



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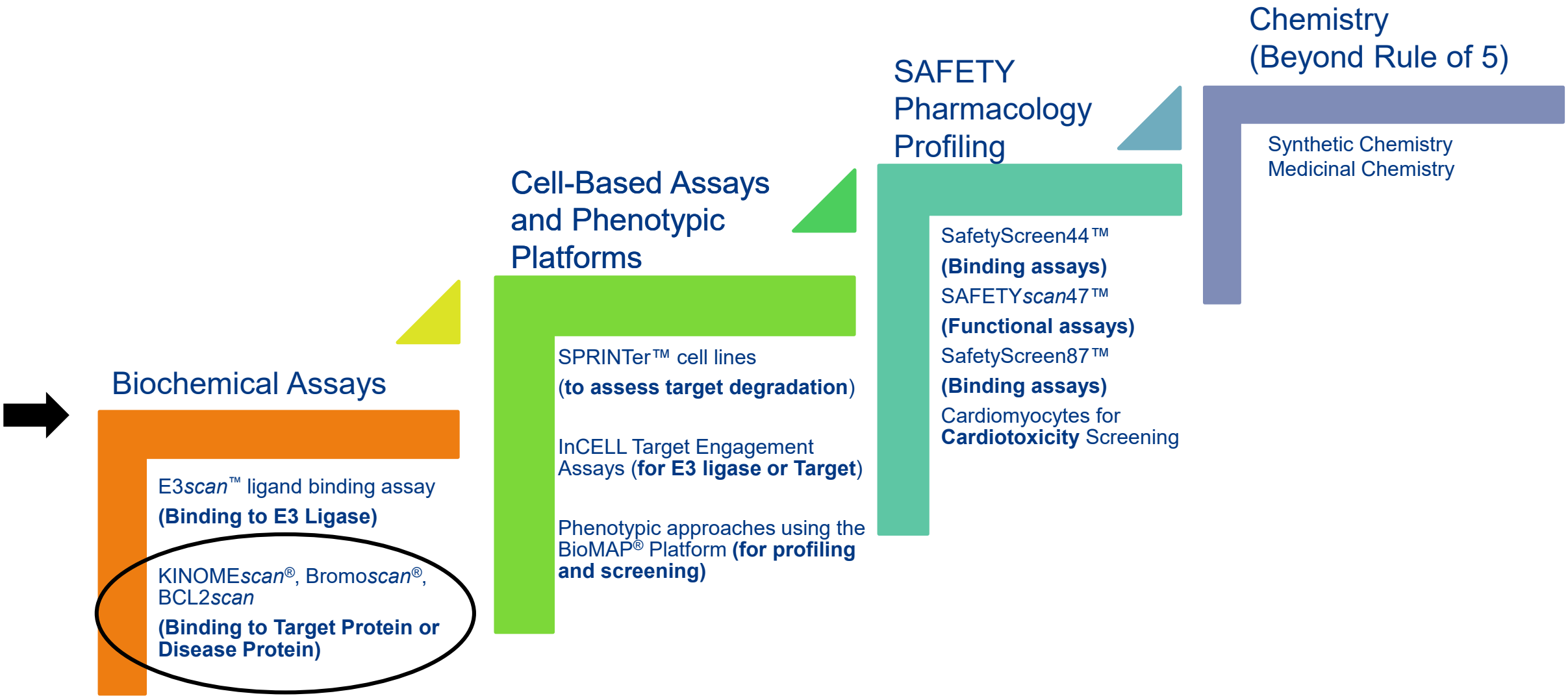
https://ubihub.thesgc.org/static/UbiHub.html

Eurofins Discovery TPD Portfolio: Assays and Analysis

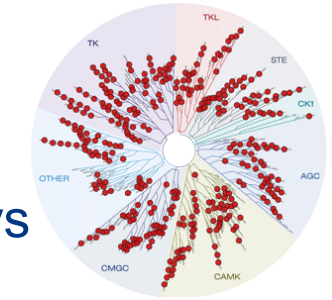


Measure Binding Affinity to Target Proteins using KINOME*scan*, BROMO*scan, and* BCL2*scan*

- 489 Kinase assays – including clinically relevant mutants
- 40 Bromodomain assays – 60% coverage of the entire family
- BCL2 assays – 5 family members
- Additional custom assay development available (not currently offered in commercial portfolio)
- Potency and selectivity
- Binding mode/Kinetics
- Turn around time: 5 days for weekly submissions, ~20 days for library screen

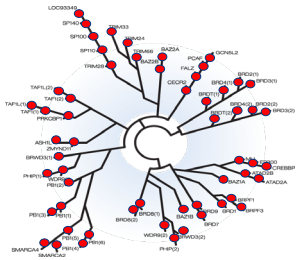
KINOME*scan*®

489 assays



BROMO*scan*®

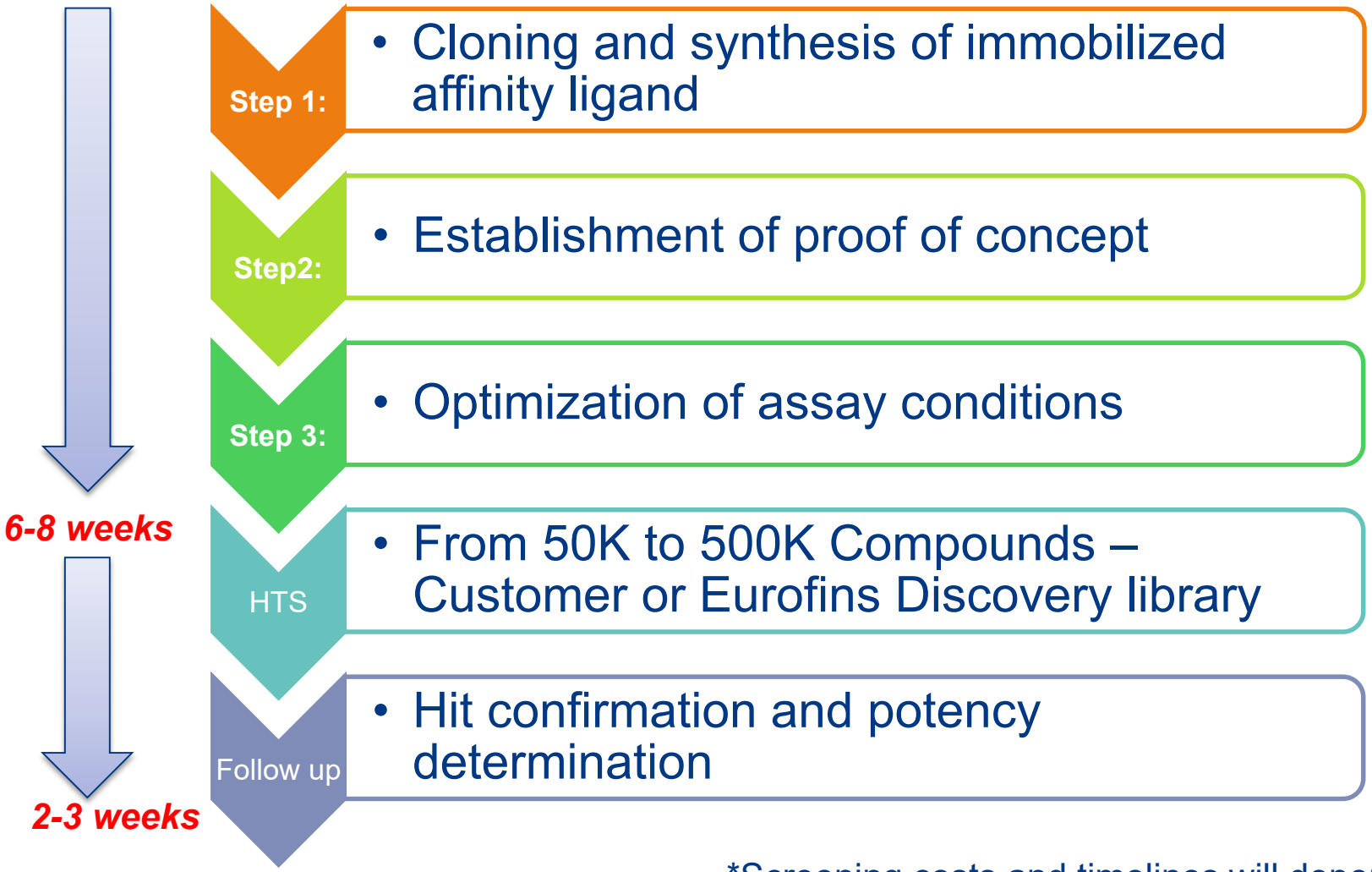
40 assays



BCL2*scan*

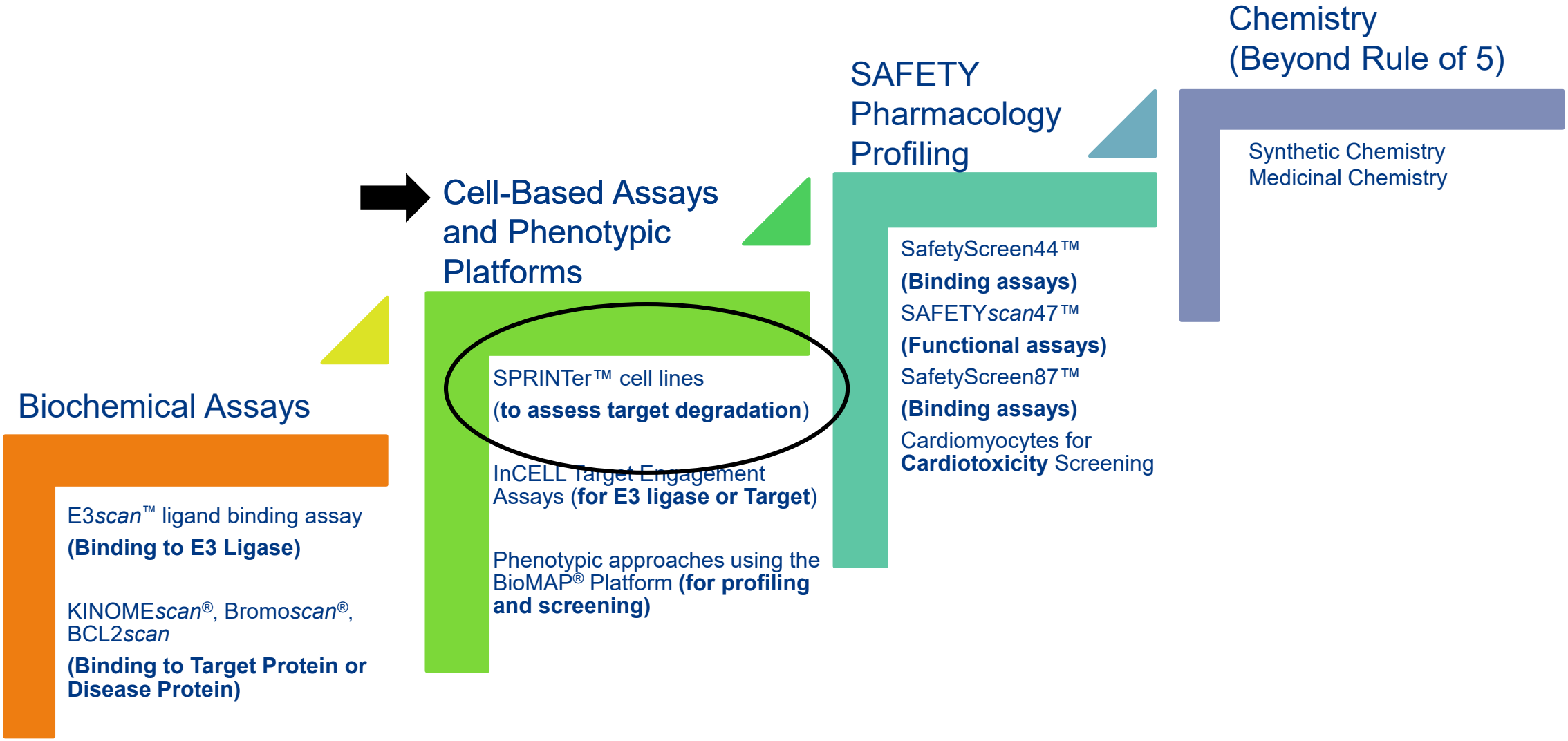
All 5 anti-apoptotic BCL2 family proteins represented

E3*scan*™ HTS Standard Flow Chart for Custom Assay Development



*Screening costs and timelines will depend on assay format and compound numbers

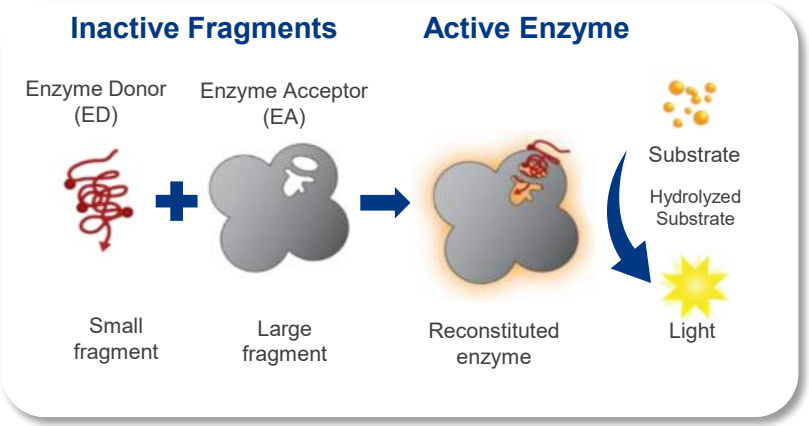
Eurofins Discovery TPD Portfolio: Assays and Analysis



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

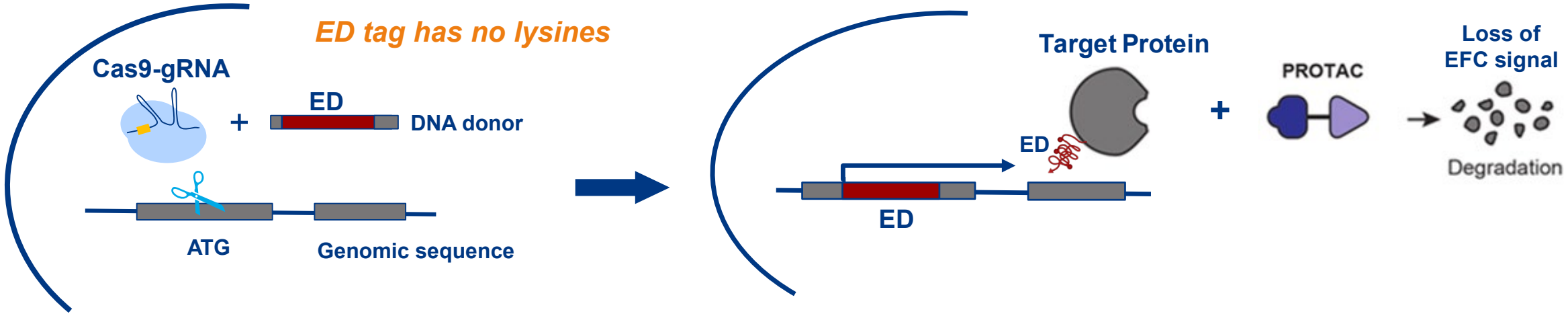


Solution:

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)

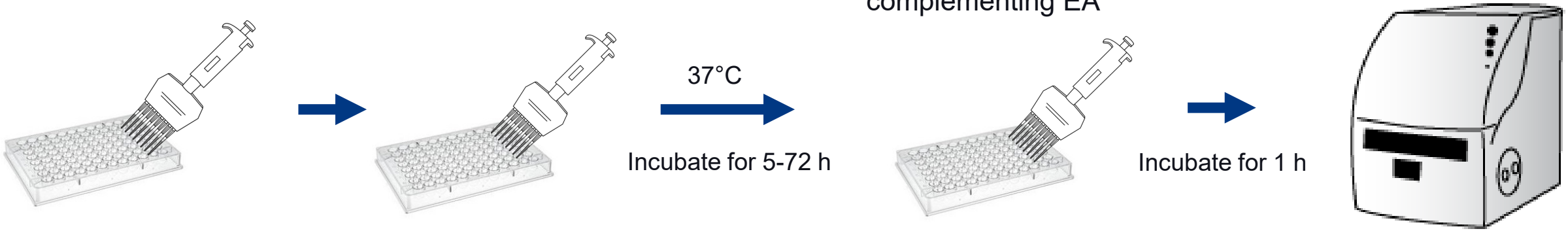
37°C

Incubate for 5-72 h

Add cell lysis reagent and detection reagent containing complementing EA

Incubate for 1 h

Read signal on plate reader



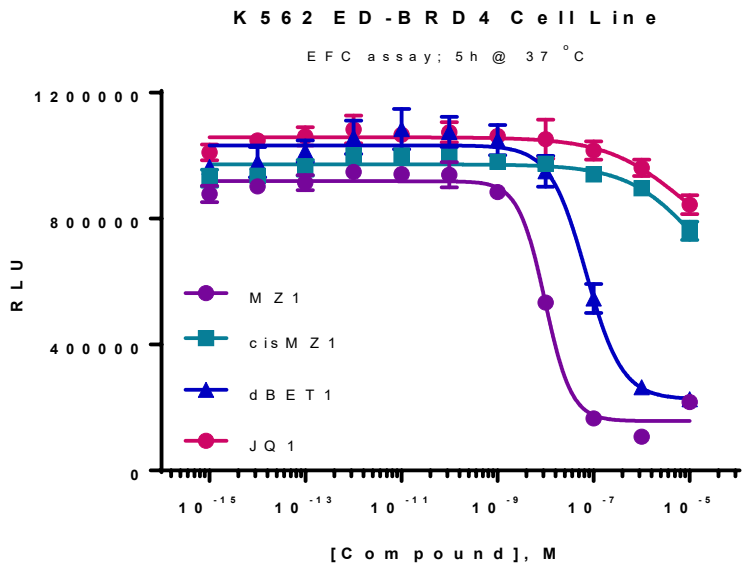
SPRINTer™ Assays Proof-of-Concept

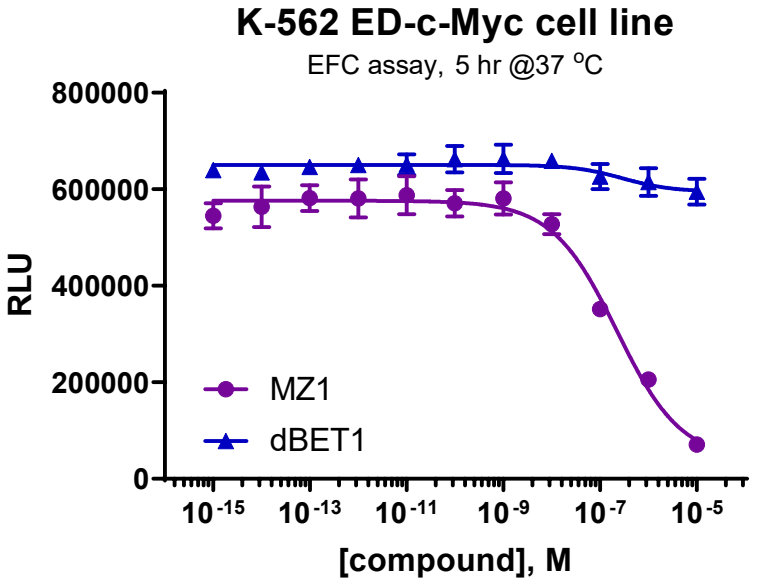
SPRINTer Protein Turnover Biosenor Cell Lines	
K562 **BRD4** Cell Line	HCT-116 **BRD4** Cell Line
K562 **c-Myc** Cell Line	HCT-116 **c-Myc** Cell Line

Tool PROTAC Molecules

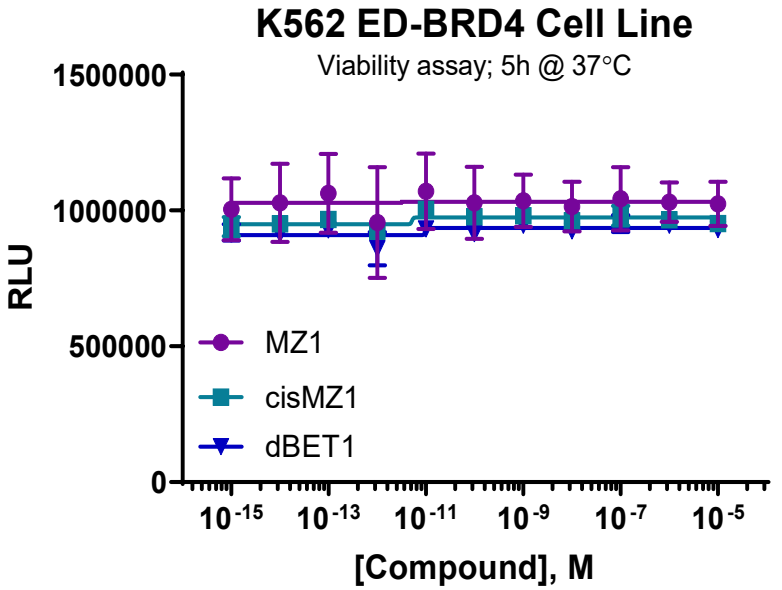
Tool Molecules	BRD4 Ligand	E3 Ligase Ligand
MZ1*	JQ1	VHL-1 (VHL)
dBET1*	JQ1	Thalidomide (cereblon)

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





EFC Assay (functional)

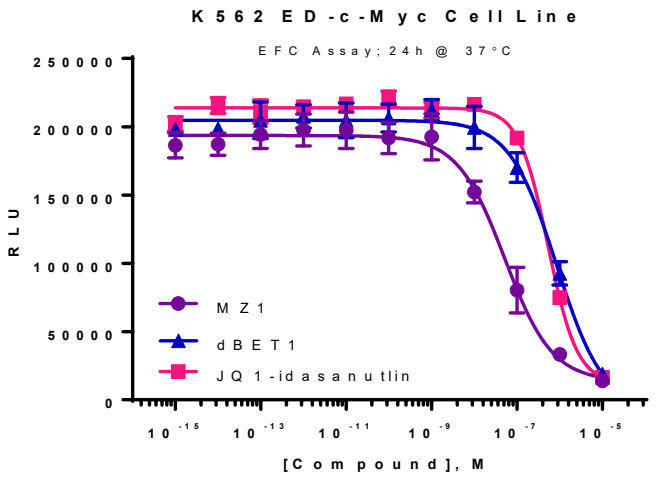


Phenotypic Assay (cell viability)

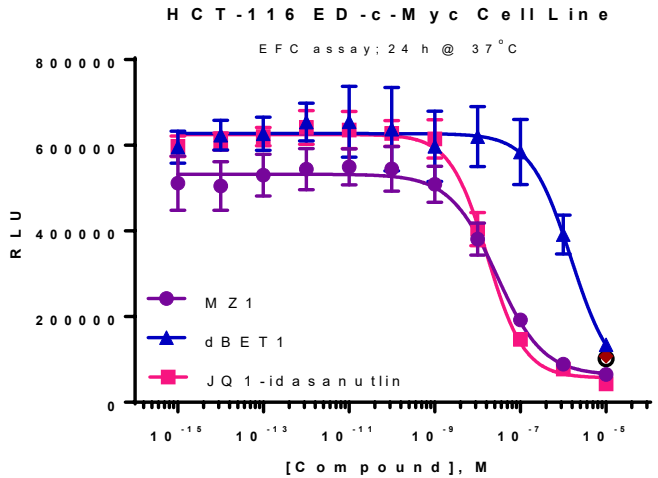
BET = Bromodomain & Extra-Terminal Motif; BRD4 = Bromodomain-containing protein 4

SPRINTer™ Assay Characterization

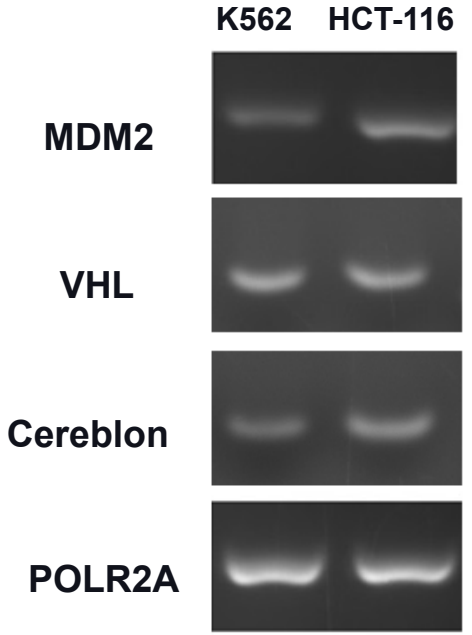
Differential PROTAC Efficacies Among Cell Models Correlates with Relative E3 Ligase Expression



MZ1 > dBET1 > JQ1-idasanutlin



MZ1 > JQ1-idasanutlin > dBET1



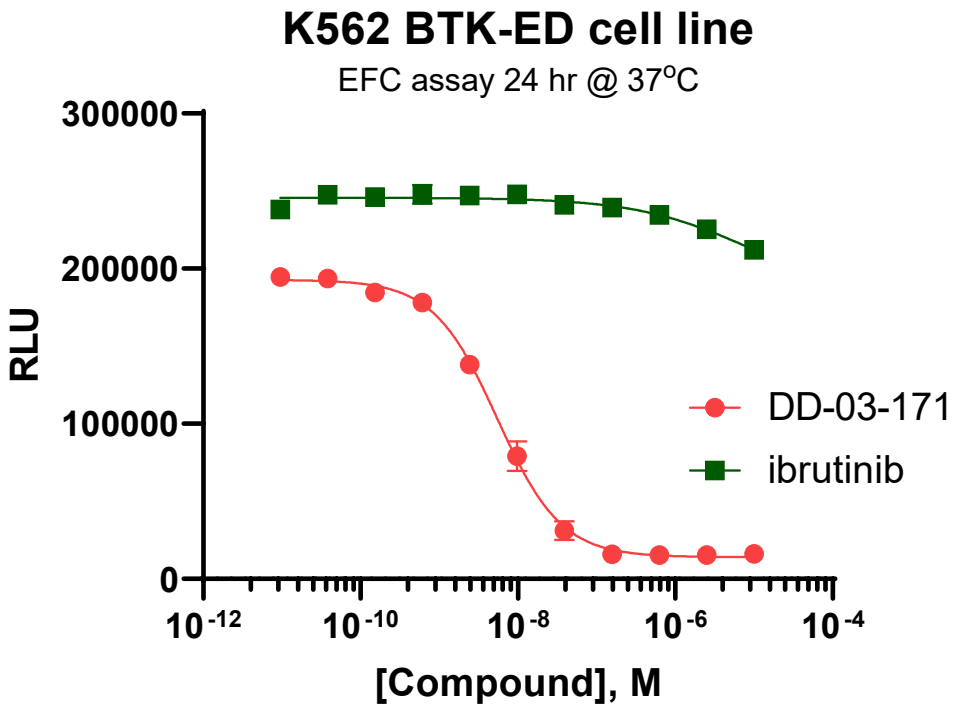
PROTAC	BRD4 Ligand	E3 Ligase Ligand
JQ1-idasanutlin	JQ1	idasanutlin (MDM2)
MZ1	JQ1	VHL-1 (VHL)
dBET1	JQ1	Thalidomide (cereblon)

Relevant cell model makes a difference

New SPRINTer ™ Cell Lines in Development

eurofins | DISCOVERY

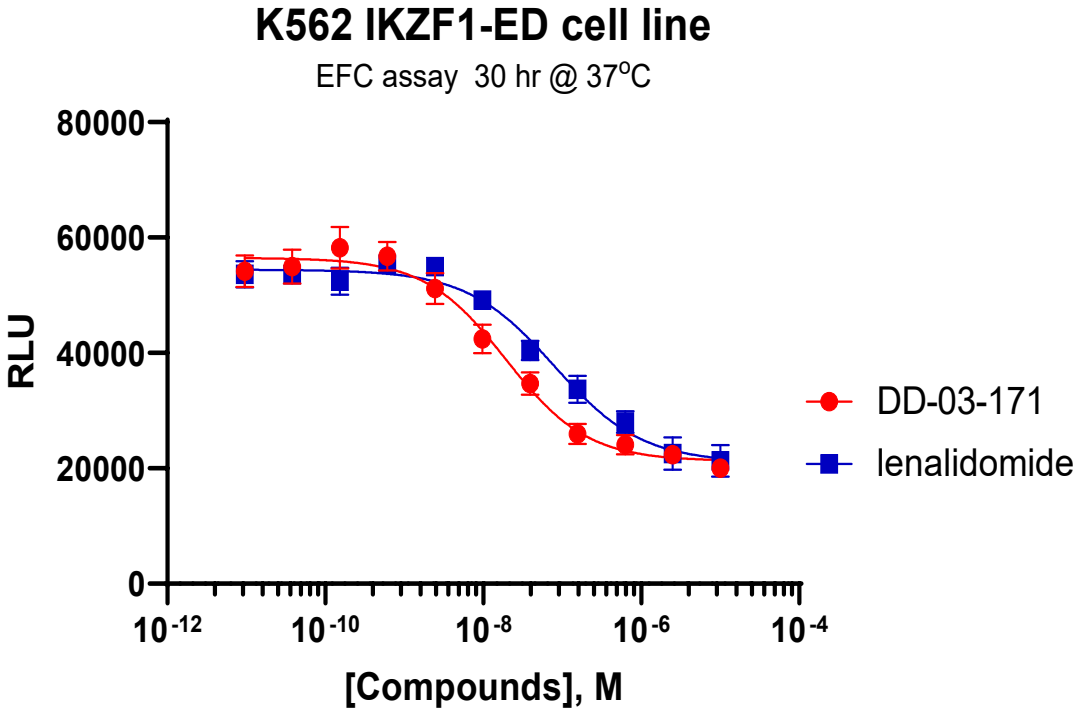
BTK: Bruton Tyrosine Kinase



Tool Molecules

Tool Molecules	BTK Ligand	E3 Ligase Ligand
Ibrutinib	Ibrutinib	-
DD-03-171	CG1746	Thalidomide (cereblon)

IKZF1: IKAROS family zinc finger 1

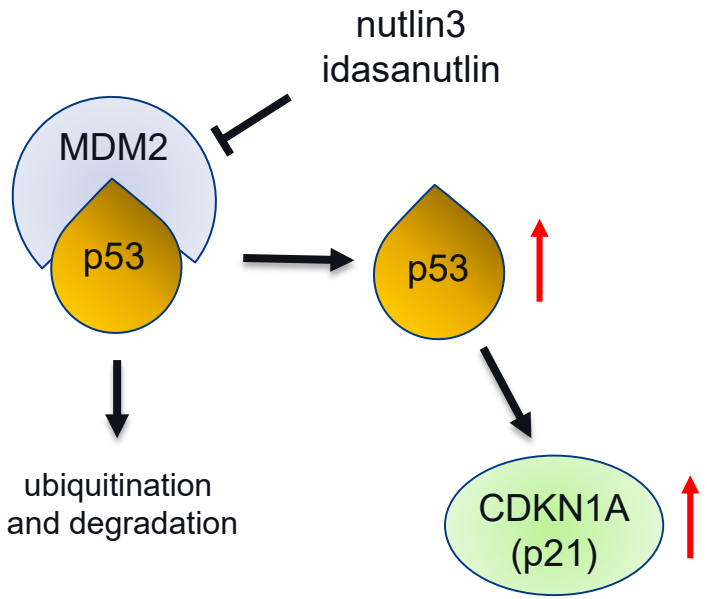


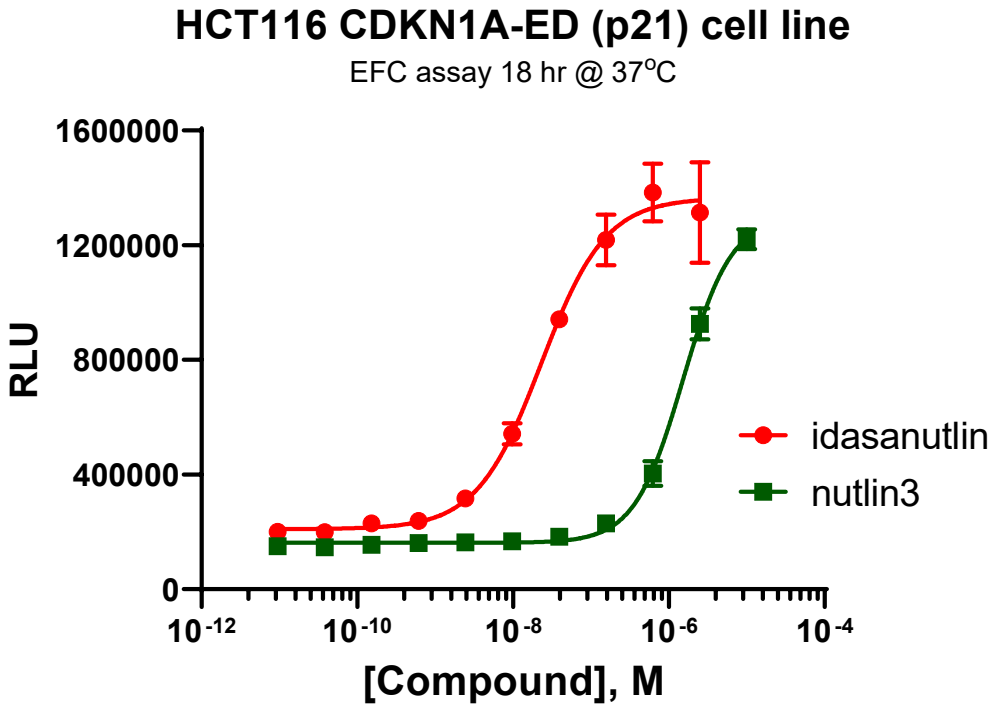
Tool Molecules

Tool Molecules	IKZF Ligand	E3 Ligase Ligand
lenalidomide	-	Lenalidomide (cereblon)
DD-03-171	CG1746	Thalidomide (cereblon)

New SPRINTer ™ Cell Lines in Development

E3 ligase inhibitors stabilize target proteins





CDKN1A (p21) Biosensor is a surrogate readout for TP53 protein stability

SPRINTer ™ Cell Lines Conclusions

Cellular EFC Biosensor Cell Lines to Quantify Protein Turnover

- **SPRINTer Cell Lines** are a mid to high TP platform to quantify changes in **endogenous** protein levels in disease relevant cell models
- **Two SPRINTer Cell lines, BRD4** and **c-Myc,** in two cell models are ready to ship
- New **SPRINTer Cell Lines** including **BTK**, **IKZF1** and **CDKN1A (p21)**, are in 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

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

Visit **discoverx.com/turnover** to learn more

Take Home Message – Eurofins capabilities for Targeted Protein Degradation (TPD)

- Eurofins Discovery **TPD Portfolio** includes a comprehensive suite of target-based, cellular, and phenotypic approaches to discover and develop prioritized TPD candidates with high therapeutic potential.
- Identify and characterize new, potent, and selective ligands that bind and reprogram E3 ligase substrate specificity for TPD using our **E3*scan*™** ligand binding assay platform.
- Develop, characterize, and validate the warhead end of novel PROTACs using our **KINOME*scan*®, BROMO*scan*®, and BCL2*scan*** ligand binding assays

Thank You!

Learn more at:

- Booth #104 and Posters
- eurofinsdiscoveryservices.com/proteindegradation
- discoverX.com/turnover (for SPRINTer™ cell lines)

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