# Selection and Characterization of Novel Tissue Selective E3 Ligases


October 25th, 2022

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# Outline of Presentation

- Kymera's pipeline
- Target Selection
- Kymera's E3 platform
- E3 Selection and Validation
  - LED Criteria
    - Ligandability, Expression & Degradation
  - PoC for a novel E3 ligand
- Summary and Outlook

**Founded 2016**

KYMERA

**Recognition**



**Partnerships**

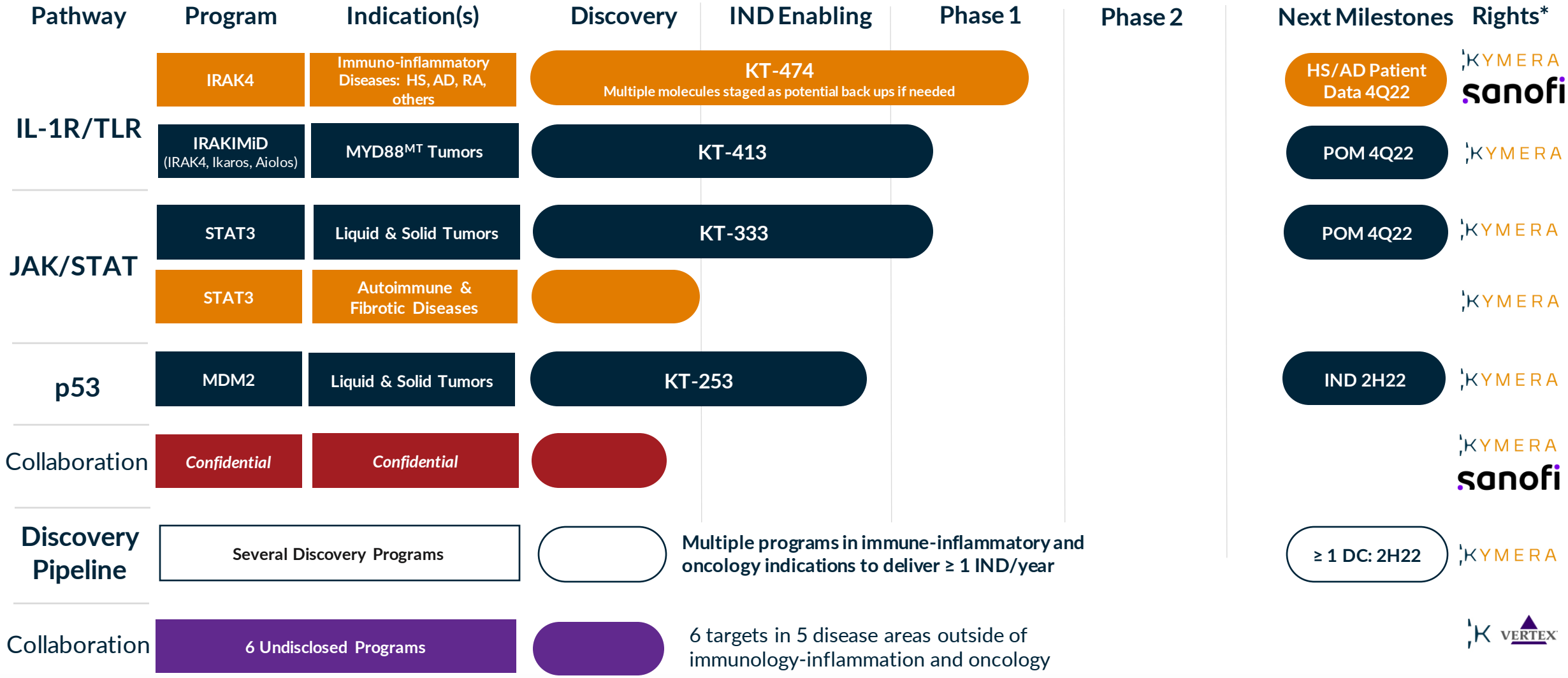
sanofi

VERTEX

A-ALPHA BIO

* POC, proof-of-concept

# Kymera's Pipeline of Novel Protein Degraders




*Option to participate equally in the development and commercialization of Sanofi-partnered programs in the US

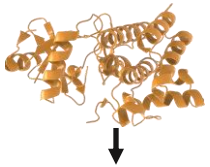
# How We Select Our Targets

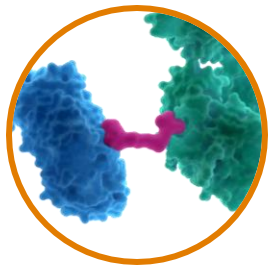
## Expanding the Druggable Proteome with TPD

### ID Drugged Targets with Clear Degrader Advantage

**Small molecule binders exist but unable to drug target fully**

e.g. IRAK4, MDM2…



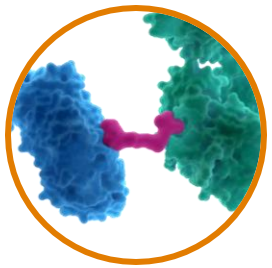


Heterobifunctional Degraders

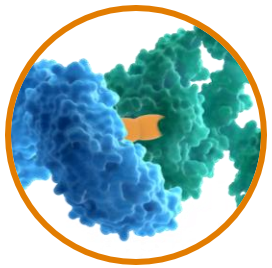
### UD Undrugged Targets

**No other technology can drug**

| Ligandable Proteins | Un-ligandable Proteins |
|---|---|
| e.g. STAT3… | e.g. MYC, β-catenin… |



Heterobifunctional Degraders

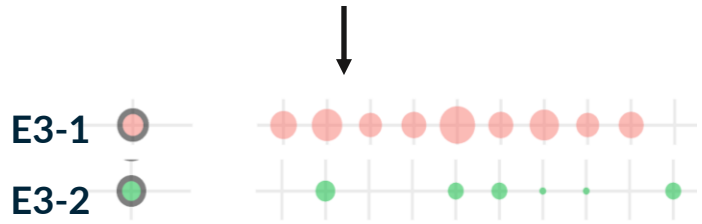


Molecular Glues

### TR Clinically Validated Targets Unlocked by E3 Ligase Differential Expression

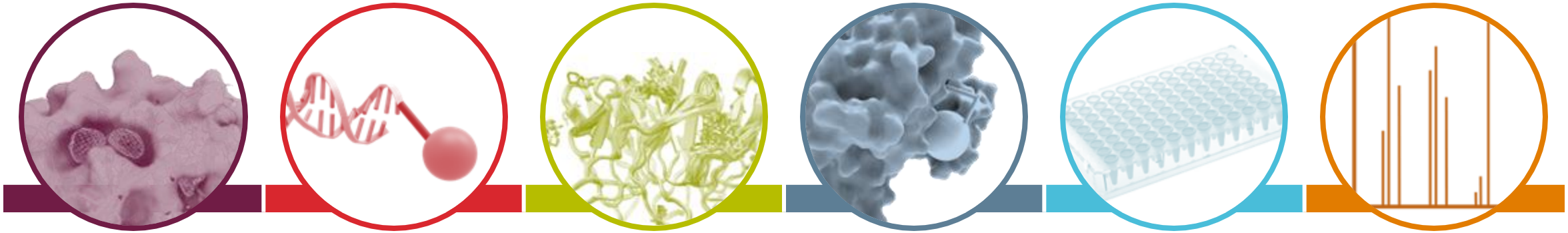
**On target unwanted pharmacology limits clinical application**

e.g. BH3, JAK's…




Tissue sparing or selective E3 ligases eliminate unwanted toxicity and allow full clinical potential

# Comprehensive Hit Finding Toolbox for Novel E3 Ligase Ligands



## Virtual Screen

**Criteria**
- Availability of structure or homology model

**Approaches**
- DB ~8 million purchasable cpds
- Cloud enables screen < 24hrs
- AI to improve enrichment

## DEL

**Criteria**
- High quality protein
- Ideal QC profile (single-species by SEC; <5% aggregation by DLS)

## Fragment-Based Screen

**Criteria**
- Availability of high quality (crystallization-grade) protein
- Robust crystallization system

**Approaches**
- SPR, NMR
- X-ray
- LC/MS (covalent)

## Cysteine Covalent Screening

**Criteria**
- Proteins have reactive cysteines

**Approaches**
- Covalent fragment screening on recombinant protein
- Whole cell covalent fragment screening

## HTS

**Criteria**
- Available high-throughput assay format

**Approaches**
- Focused library
- Diversity set

## ASMS

**Criteria**
- Availability of high-quality protein

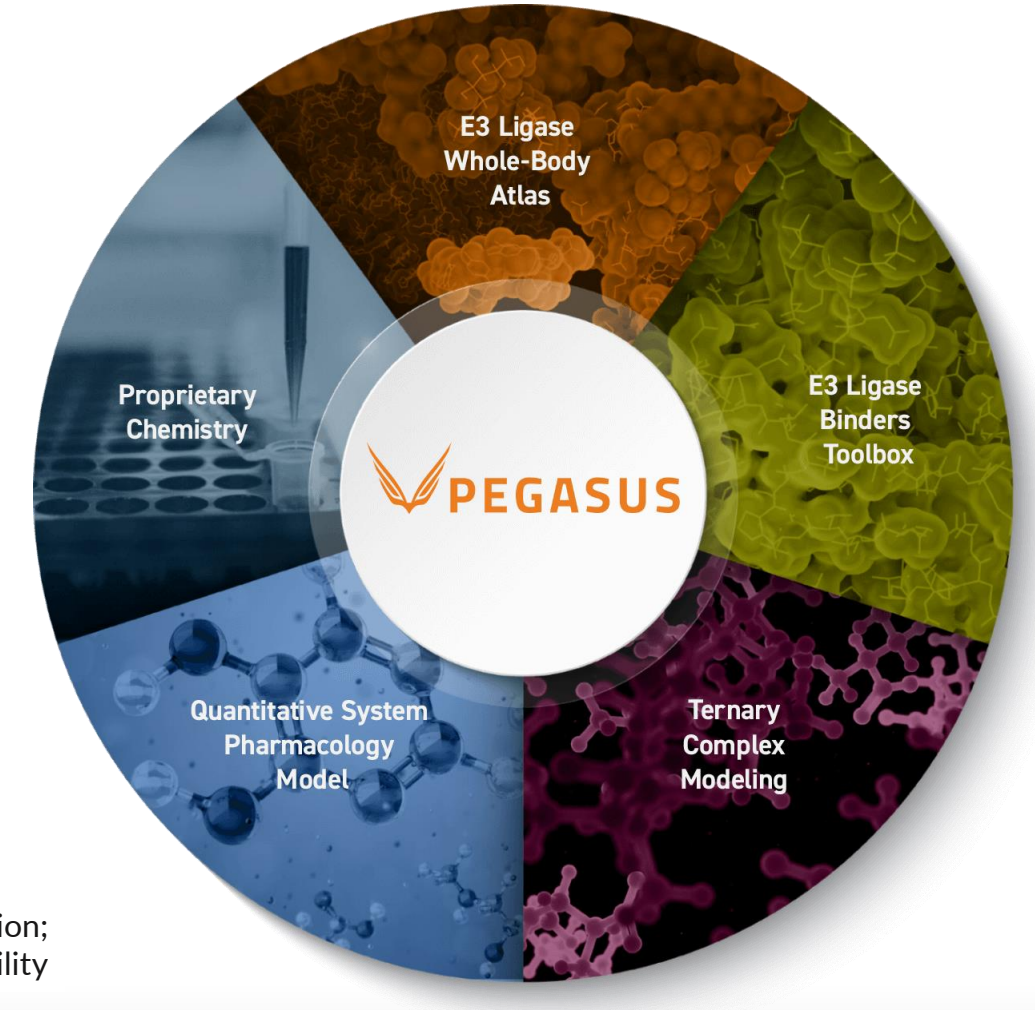
# Kymera Platform for Harnessing Novel E3 Ligase Biology

## E3 Ligase Whole-body Atlas

- RNA & protein expression profiles of ~ 600 E3s
- Disease & healthy tissues & cells (tissue distribution, absolute abundance, & subcellular localization)
- Novel E3 & POI pairing based on expression & biology

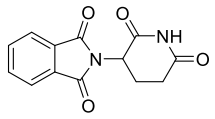
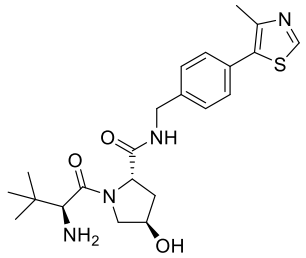
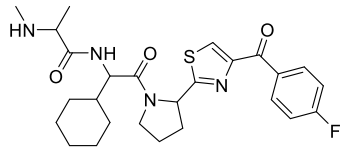
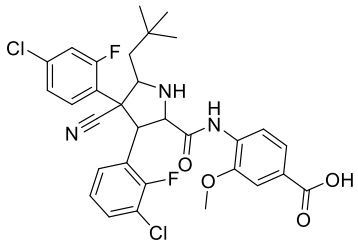
## Proprietary Chemistry

- High-quality ligands for novel E3s
- Innovative degraders for new therapeutic opportunities
- Advanced molecular design principles for improved drug properties (e.g., $DC_{50}$, $D_{max}$, $S_{H_2O}$, $P_{app}$, & $F_{oral}$)*




* $DC_{50}$, concentration at half-maximum degradation; $D_{max}$, maximum degradation; $S_{H_2O}$, aqueous solubility; $P_{app}$, cell permeability; $F_{oral}$, oral bioavailability

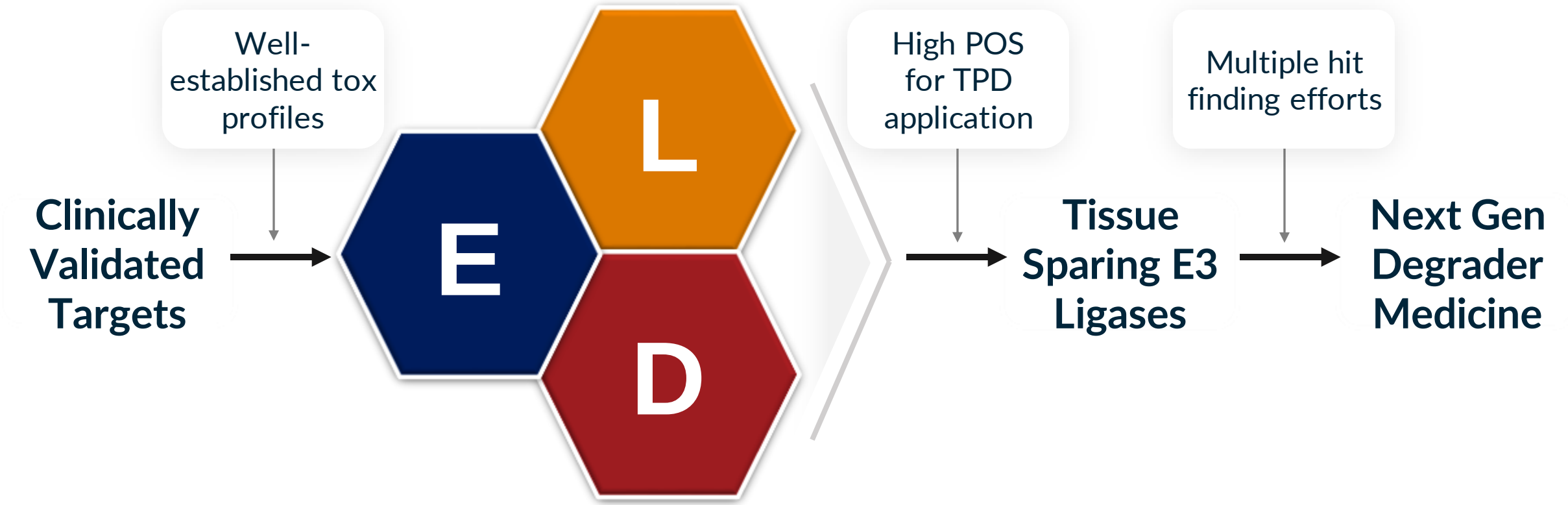
# Current E3 Landscape Today and Limitations

| E3 Ligase | Cereblon | VHL | IAP | MDM2 |
|---|---|---|---|---|
| Compounds | Thal, Pom | VH032, VL285, and derivatives | LCL161, GDC-0152 | Nutlins |
| |  |  |  |  |
| | **Thalidomide** | **VH032** | **LCL161** | **Idasanutlin** |
| MW | **258** | **431** | **500** | **616** |
| LogP | **0.02** | **0.85** | **3.78** | **4.50** |
| PSA | **109** | **84** | **91** | **112** |
| Limitations | iMiD Biology; stability/ epimerization | Peptide-based renders oral BA challenging | Auto-ubiquitination/ NF-kB modulation; cytotoxicity making interpretation of results difficult | On-target biology |

- Ubiquitous expression is both good and bad; can **increase risk** of off-target/adverse effects
- Desired properties for novel E3 ligands:
  - Low $M_W$/drug-like properties
  - No cytotoxicity/ neosubstrate effects
  - Spares normal protein homeostasis
  - Tissue sparing

# How We Select Tissue Sparing E3 Ligases

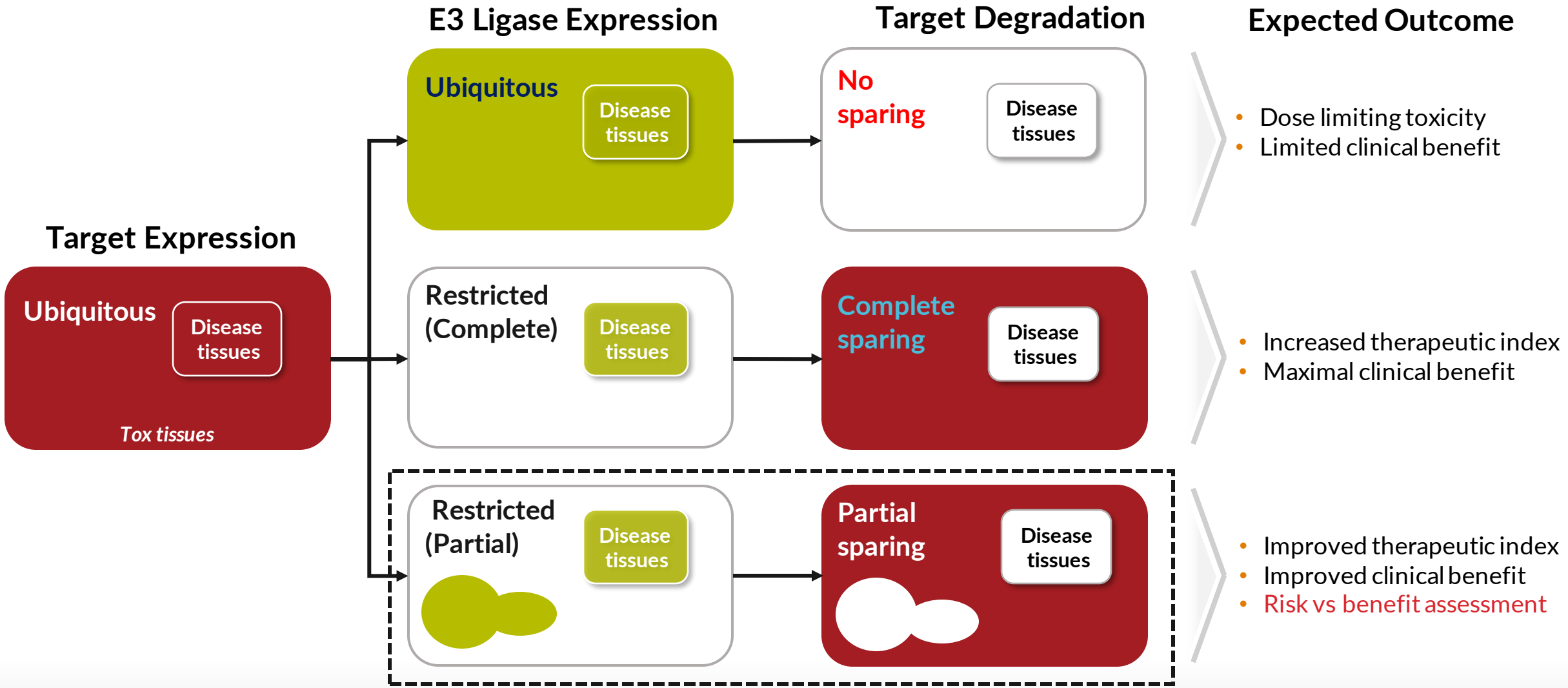
## L.E.D Criteria Serves to Identify Matching E3 Ligases




**L**igandability; **E**xpression; **D**egradation

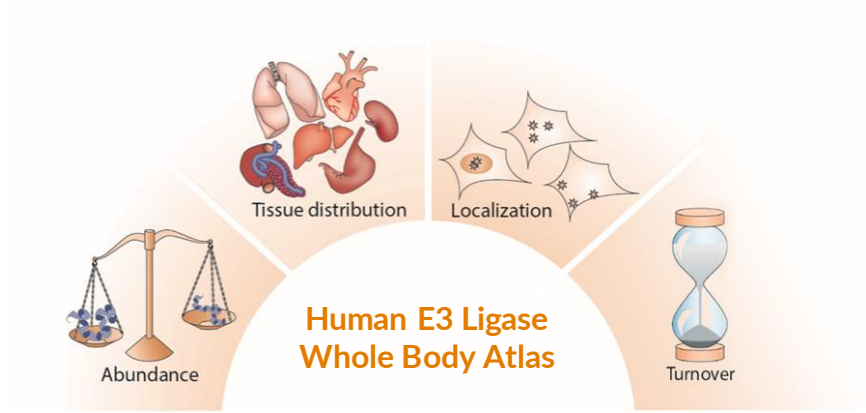
# Desired Outcome for Tissue Sparing E3 Ligases

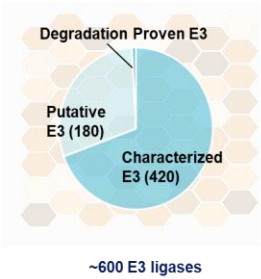
## Increase of Therapeutic Index



# Human E3 (& POI) Absolute Expression Atlas in Health & Disease

## Invest in E3s with Tissue Sparing Potential for Targets with Unmet Clinical Need




**E3 Ligase Matching TA or Target Criteria**

- Pan expressed
- Differentially expressed
- Disease-specific
- Tissue-specific
- Tissue-sparing

MaxQuantAtlas

### Relative Abundance in Health and Disease

- Tissue sparing or Ubiquitous
- Expression in disease: Broad or restricted

### Absolute Abundance

- Benchmarking expression of novel E3s vs CRBN/VHL
- E3: target stoichiometry to predict efficiency of ternary complex formation

### Subcellular Localization

- Match E3 and POI subcellular location
- ID colocalized (interacting) partners for compartment specific degradation approaches

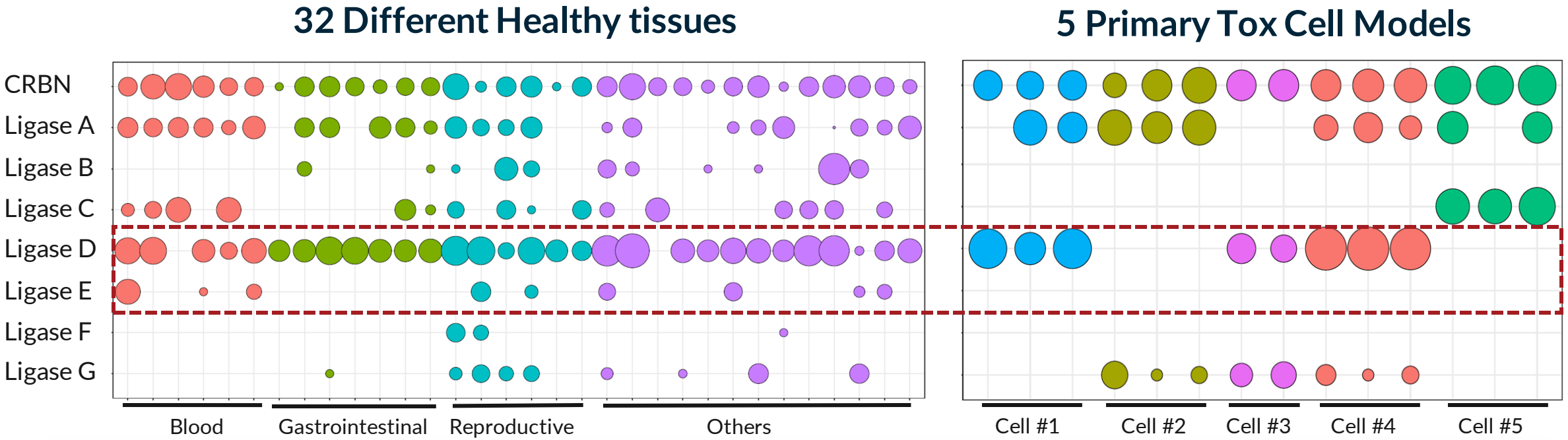
### Half-Life

- E3 and POI(s): QSP modeling and covalent hit strategies

### Advanced Uses: e.g., Targeted Delivery of Degraders

- Selected expression of differentially expressed surface expressed proteins

# Protein Expression of Select E3s in Healthy Tissues



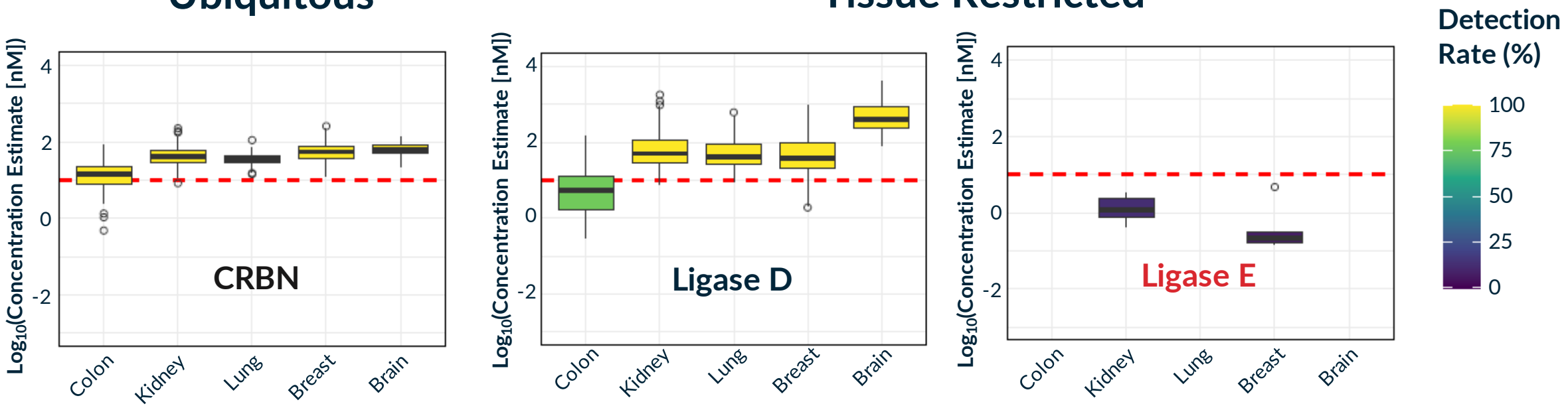

- Broadly expressed E3s with specific sparing potential (e.g. Ligase D) offer broad therapeutic opportunities
- E3 ligases with very restricted expression in normal tissues (e.g. Ligase B, E, F) may have little clinical utility, unless expression is seen to be upregulated in the disease settings.
- However, complex absence across a proteomics dataset could be due to detection limit. In these E3 cases, it is important to confirm by the bulk tissue RNAseq data (e.g. GTEX) as well as scRNAseq studies for a specific tissue.

**Proteomics Team** Data: Heathy Tissue E3 Atlas incorporating internal and published (Wang et al., *Mol Syst Biol*, 2019) deep label-free proteomics datasets.

# Disease Expression informs Clinical Utility of TR E3 Ligases

## Ubiquitous

## Tissue Restricted



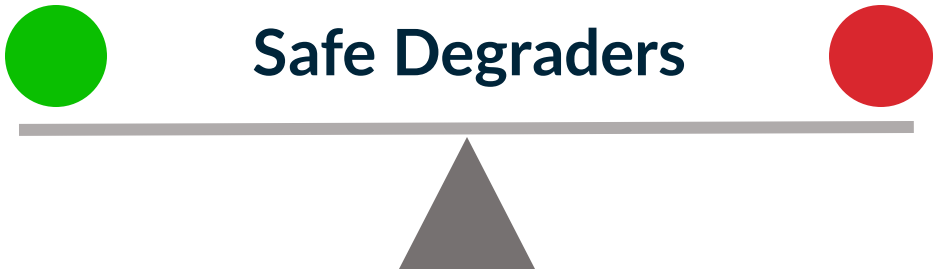

Data: Selected datasets from the Clinical Proteomics Tumor Analysis Consortium (CPTAC) and Cancer Genome Atlas Program (TCGA) reprocessed in E3 Atlas.

- For expression in the actual cancer cells (vs surrounding stroma), we always evaluate protein and RNA data from CCLE and scRNAseq data from tumor samples.

# Lessons for TPD from E3 Atlas Mining Exercises

**BENEFIT**

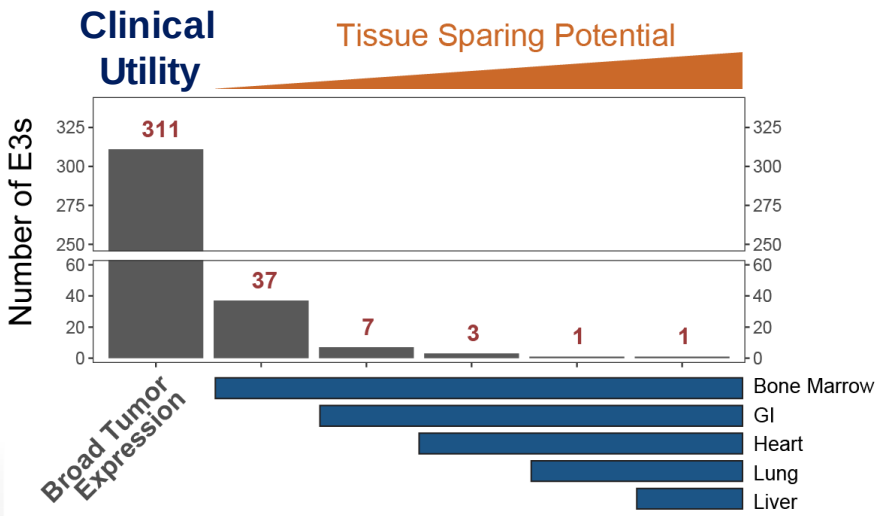
Clinical utility
Candidate E3



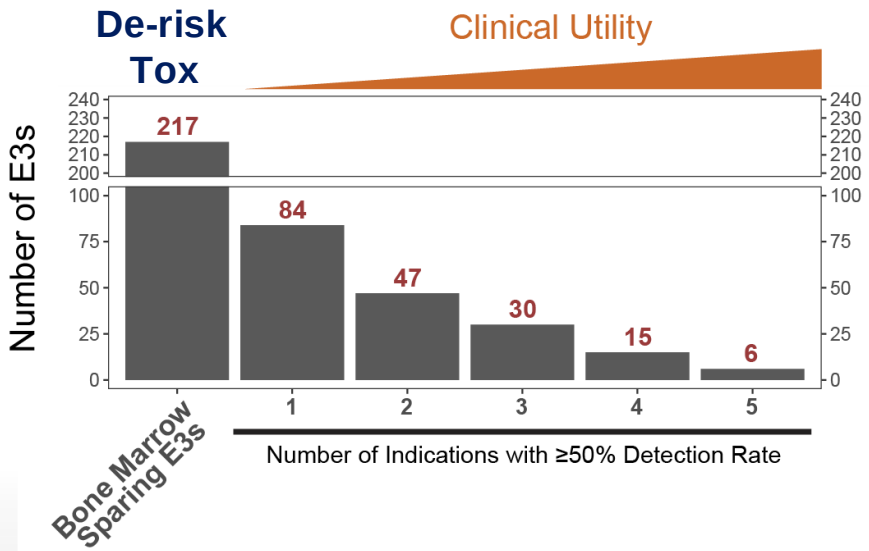

**RISK**

Clinical tox
De-risk all tissues

## 1 'Very' Restricted Expression Profiles Yield Rather Limited Candidate E3 Ligases for Drug Discovery




## 2 E3 Candidates Addressing 'all' Potential Toxicity Concerns May Have Little Clinical Utility

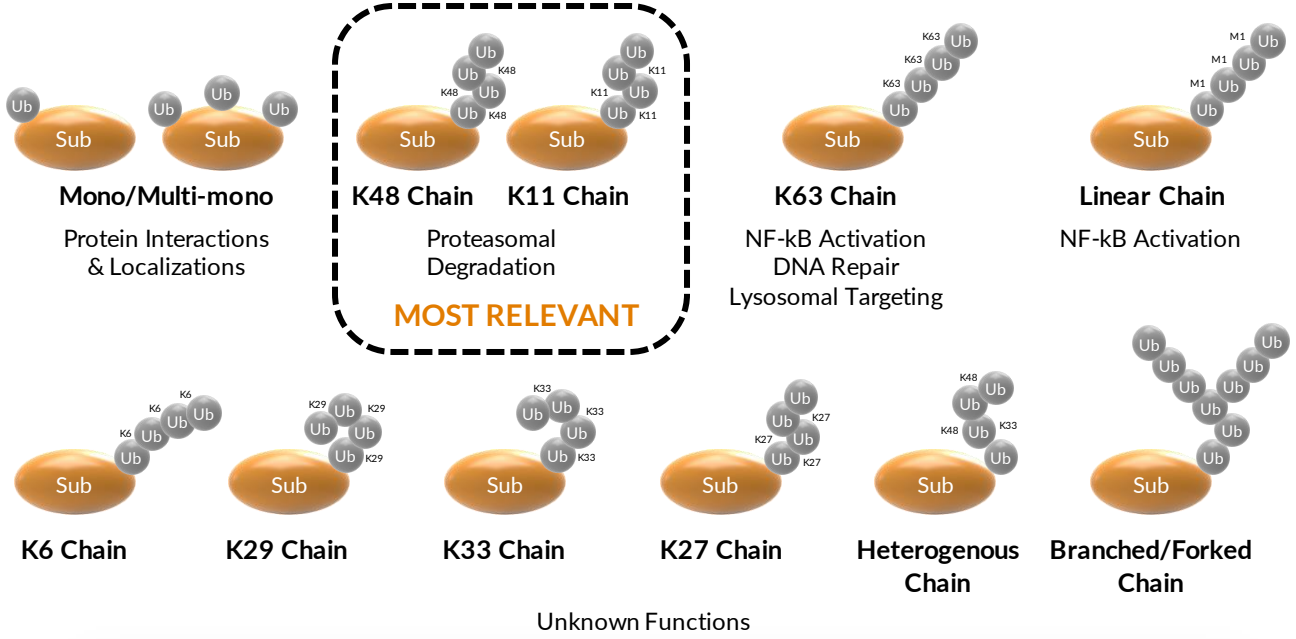


# Why We Validate Degradative Activity of E3 Ligases

## Different Types of Ubiquitin Linkages

- >600 known E3 ligases in human

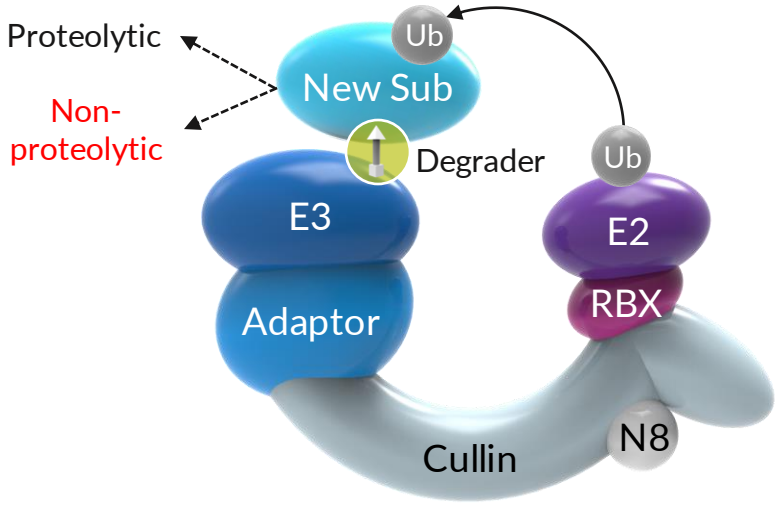
**Ubiquitin:** $NH_2$–MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG–COOH
1 6 11 27 29 33 48 63 76




- The vast majority (>95%) of human E3 ligases are RING domain based which **do not** specify ubiquitin chain linkage

## Unknown Risks for Novel E3 Ligases

- Not all E3 ligases are suitable for TPD application



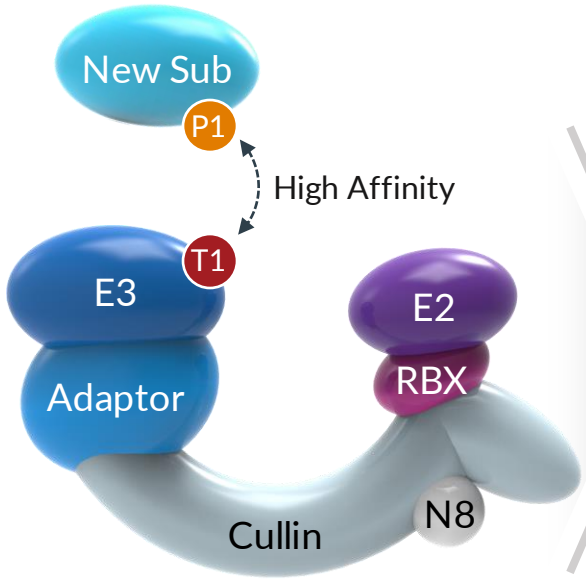

- Validation of intrinsic **degradative activity** is a must-have mitigation strategy

Adapted from: Park CW at al. *BMB Rep.* 2014

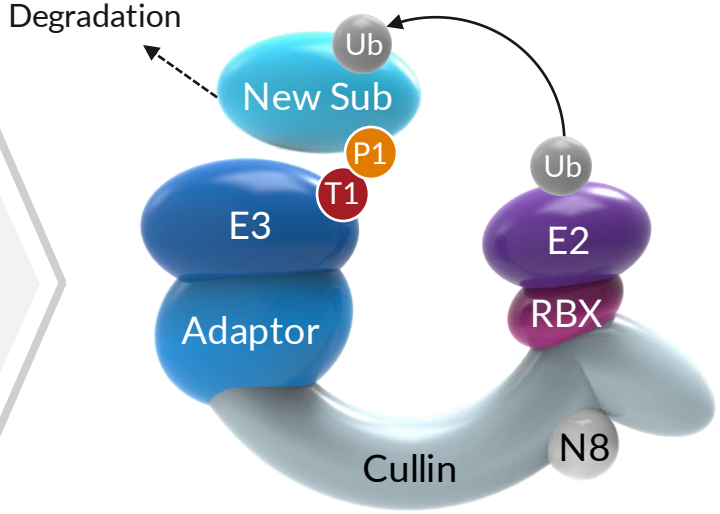
# How We Validate Degradative Activity of E3 Ligases

## New Proximity Assay Based on High Affinity Peptide-protein Pair

### Leveraging Peptide-Protein Pair

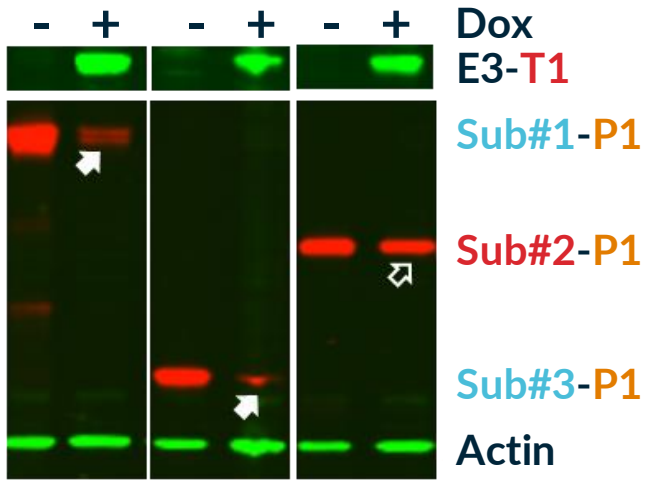



### Forced E3-Sub Interaction




### PoC of New Proximity System

Example with Ligase D

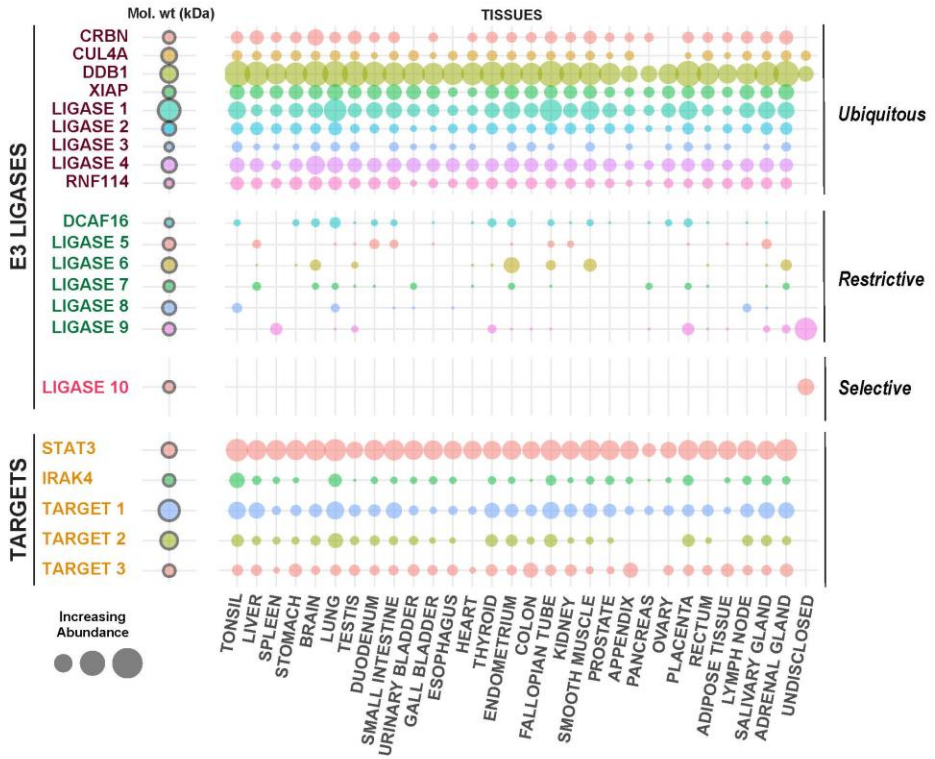



- Not all neo subs are equally degradable!
- Ubiquitination is likely to happen on subs

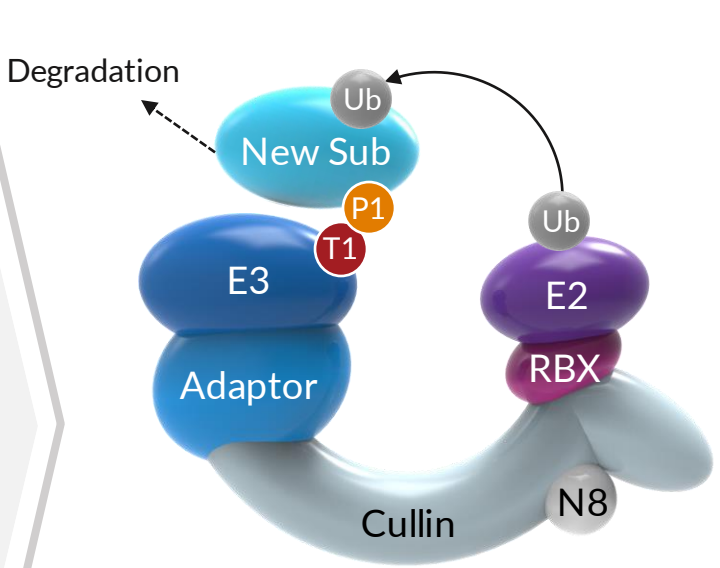
- Leveraging a **high affinity interaction** between peptide (T1) and protein (P1) to enable a forced proximity of E3 and substrates
- Enable early assessment of **intrinsic degradative activity** of novel E3 ligases in both cellular and cell-free contexts
- Small peptide size (~15a.a.) allows for assessment of "D" with **a minimal perturbation** of the natural conformation of E3 ligases
- Affinity between T1-P1 could be "**tunable**" by T1 variants; Scalable and quantitative with readily degradable reporter proteins

# D Degradation at Scale across 40 Tissue Restricted E3 Ligases

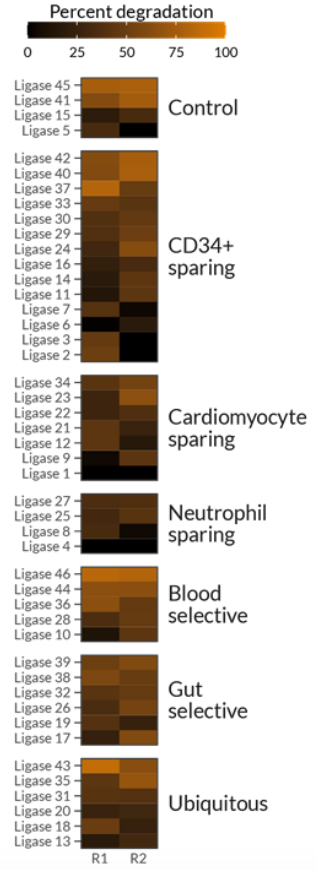
## Tissue Sparing E3 Selection




## Forced E3-Substrate Interaction




## Degradation at Scale



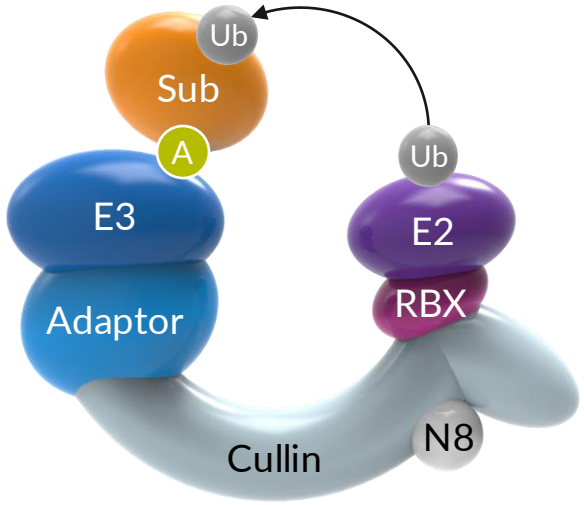

- This process allows for prioritization of E3s with robust activity across substrates
- Additional orthogonal approaches can rescue false negatives resulting from tag interference of E3 function

# Functional Ligand Discovery: Ligandable & Productive

- **Ligandability**: *likelihood* of identifying a small-molecule binder with affinity < 1 uM
- **Productivity**: *likelihood* of converting the ligand into a degrader with therapeutic potential

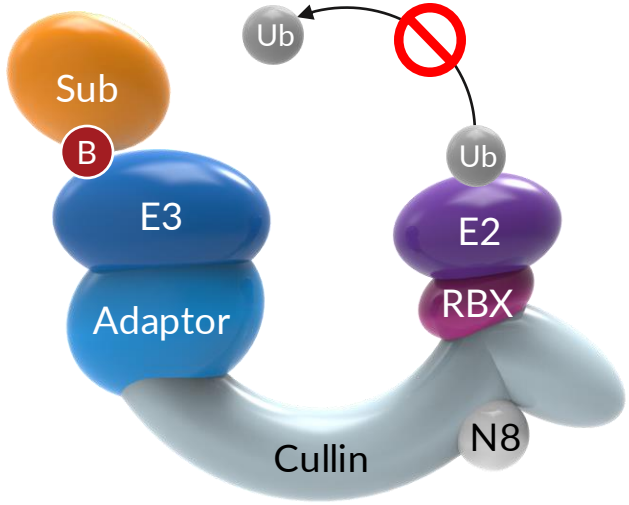
## Site A

Ligandable & Productive for TPD




## Site B

Ligandable but Non-productive




- Ligands that bind to either site A or B can lead to TCF (Ternary Complex Formation) but **only site A binders** could be converted to efficient degraders
- **Degrader competent** site(s) are identified within **substrate binding modules** to yield "**ligandable**" & "**productive**"

# What Makes an E3 Ligandable at Kymera?

**Ligandability**: *likelihood* of identifying a small-molecule binder with affinity < 1 uM

**Productivity**: *likelihood* of converting the ligand into a degrader with therapeutic potential

Ligandability assessment helps optimize resources towards **PoS**

## Qualifier

### Precedence and Datamining

- ❑ Contains ligandable domains/protein family analysis
- ❑ Known substrate(s)
- ❑ Known and validated small-molecule

### Structure-based Assessments

- ❑ Ligandability score
- ❑ Cryptic pocket available

### Experimental/Biophysical

- ❑ Identified hits from pilot screens

## Key Challenges

### Precedence and Datamining

- Data reliability, clean-up/curation
- Data integration

### Structure-based Assessments

- Requires structure of target protein or homology

### Experimental/Biophysical

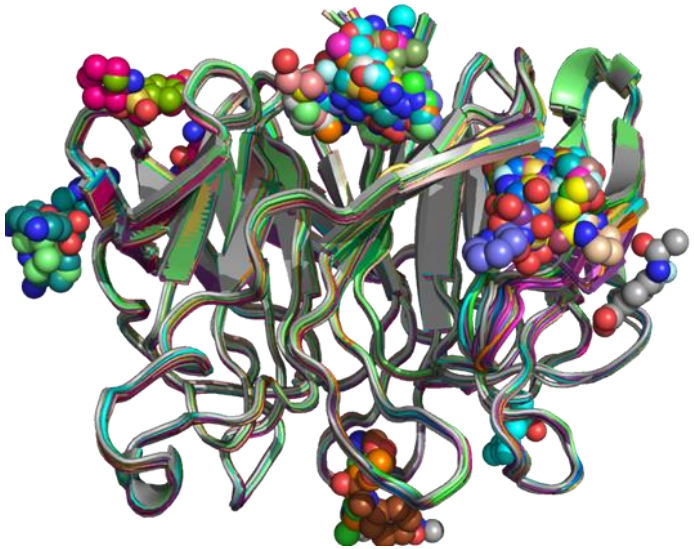
- Protein expression/stability

# Ligandability Assessment by Pilot Screens

Must be structurally enabled

## Fragment-Based Screen

### Example of FBS by X-ray Crystal

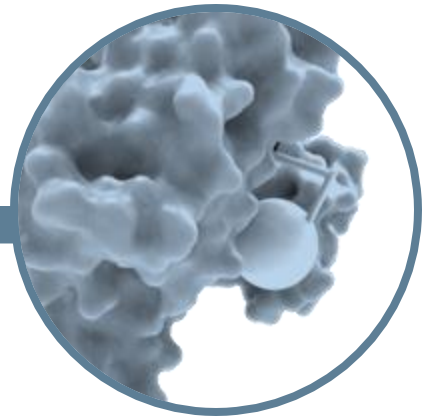


**Criteria**
- Availability of high quality protein with robust crystal system

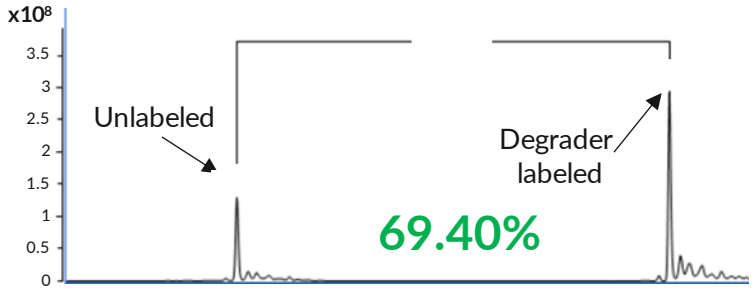
**Approaches**
- Orthogonal validation of hits by SPR and NMR by SBDD

**Advantages**
- Rapid evaluation of multiple potential ligandable sites

## Cysteine Covalent Screening



### Covalent Screen by Intact MS



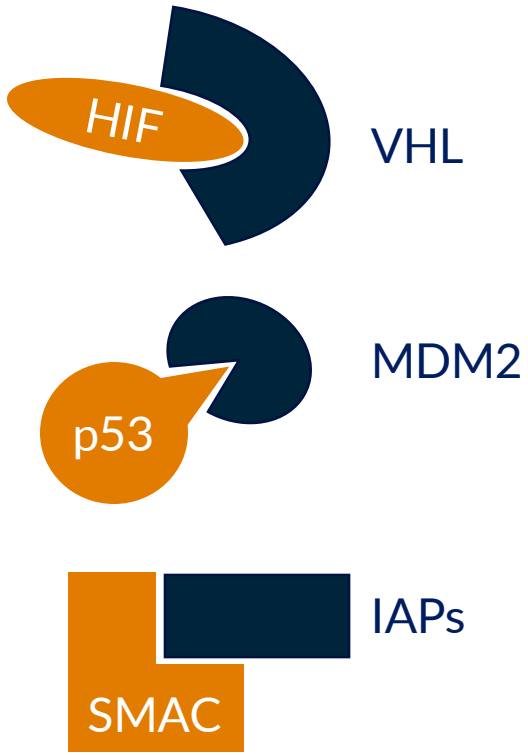

Assessment of **functional competency** by *in vitro* ub and/or COFFEE assay with functionalized (degrader-labeled) E3 ligase

**Criteria**
- Surface exposed reactive cysteines

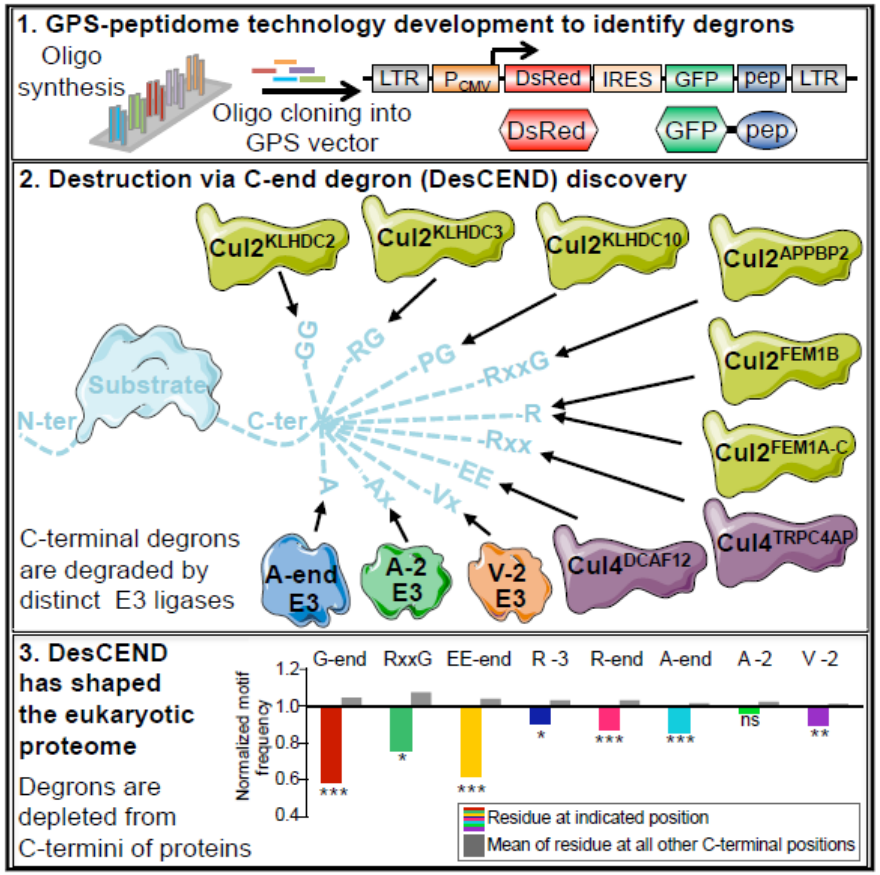
**Approaches**
- Covalent fragment screen on purified protein by intact MS

**Advantages**
- Early assessment of functionality of Cys sites by covalent degraders

# Past Precedent informs Potential Future Success




Examples of liganded E3s and their cognate interactions




*Elledge Lab: Koren et al. Cell 173:1622-1635 (2018)*
*Yen Lab: Lin et al. Mol Cell 70: 602-613 (2018)*

- The GPS system identified E3 ligases that act on defined C-terminal amino acid sequences regulating protein turnover
- These ligases with well defined, simple binding sequences represent an E3 class with peptidomimetic potential

# Novel C-end E3 Ligase Characteristics and Ligandability Assessment

| | |
|---|---|
| **E3 Ligase Type:** | C-end |
| **Known Substrates:** | Endogenous substrates |
| **Crystal Structures:** | Structure solved |
| **Expression:** | Broadly expressed |

## Precedence and Datamining

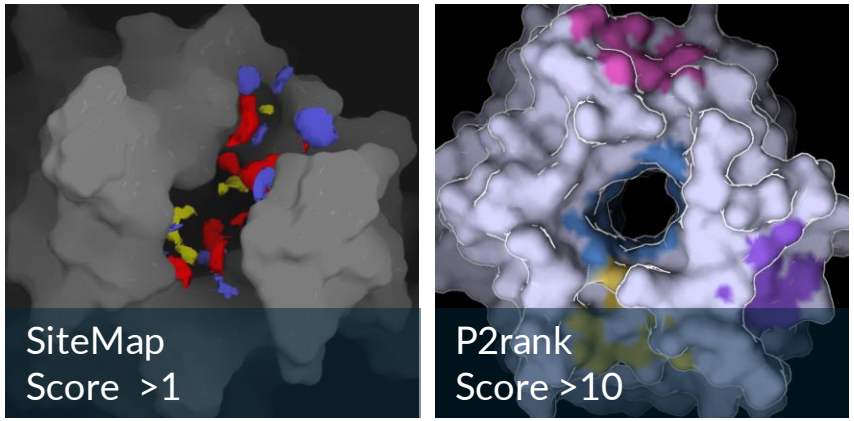
- ☑ Contains ligandable domains/protein family analysis
- ☑ Known substrate(s)
- ☐ Known and validated small-molecule

## Structure-based Assessments

- ☑ Ligandability score
- ☐ Cryptic pocket available

## Experimental/Biophysical

- ☑ Identified hits from pilot screens



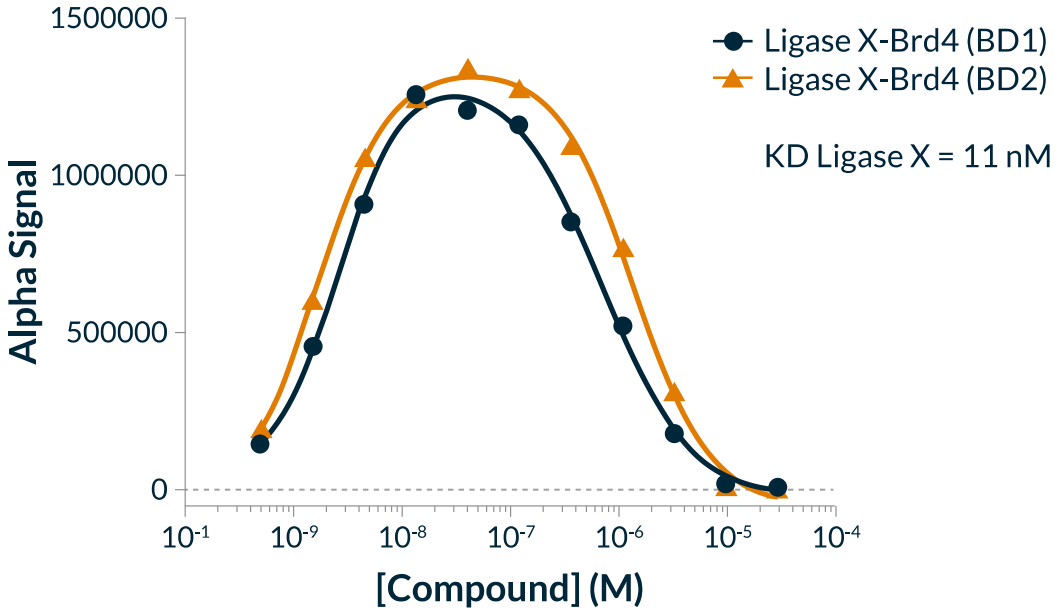

2 orthogonal *in silico* methods suggest pocket is ligandable

SBDD/Hit-finding activities initiated based on **ligandability** assessment, known substrate preference and in-house established X-ray crystallography system

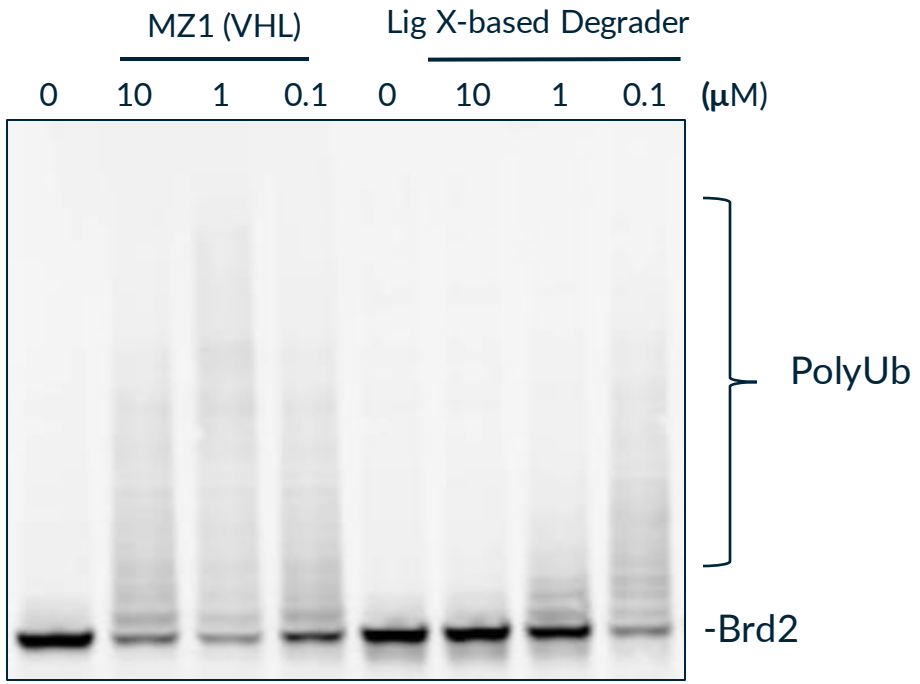
# Ligase X Peptidomimetic Degrader Promotes Ternary Complex Formation and Brd2 Ubiquitination *In vitro*

Peptidomimetic ligand of Ligase X based degrader provided **validation** but not suitable start point for hit finding

## Ternary Complex Formation - AlphaLISA



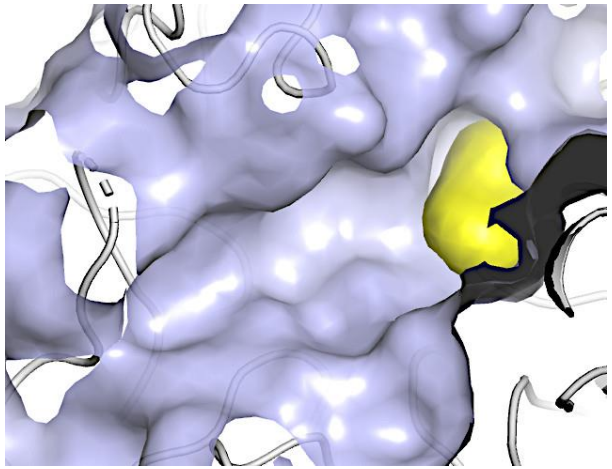

## Cell-free Brd2 Ubiquitination (OCI-LY10)



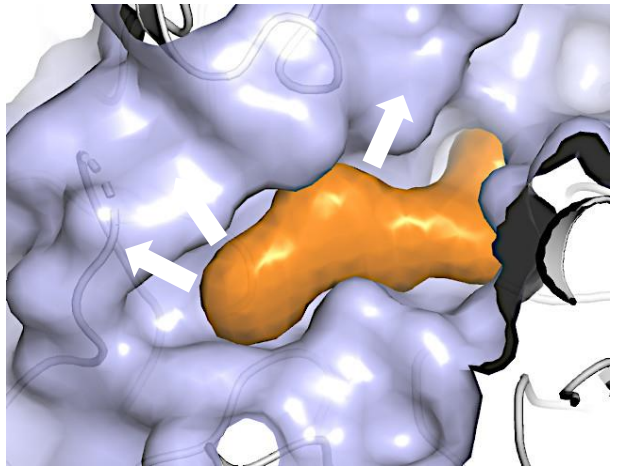
# An Early Fragment X-ray Structure Solved along with Virtual Library Evaluation Led to Very Potent Binders of this Target

| | HTRF |
|---|---|
| Fragment bound X-ray structure | $IC_{50}$ > 1 mM |
| Rational design to explore SAR | $IC_{50}$ < 30 µM |
| *In silico* library evaluation and synthesis | $IC_{50}$ < 5 µM |
| 3x | |
| Rational design to optimize library hits | $IC_{50}$ = < 0.1 µM<br>MW <400; clogP 0.7 |

| | |
|---|---|
| Total # of virtual compounds evaluated | 40K |
| Total # of crystal structures | 18 |
| Total # of compounds made | 195 |



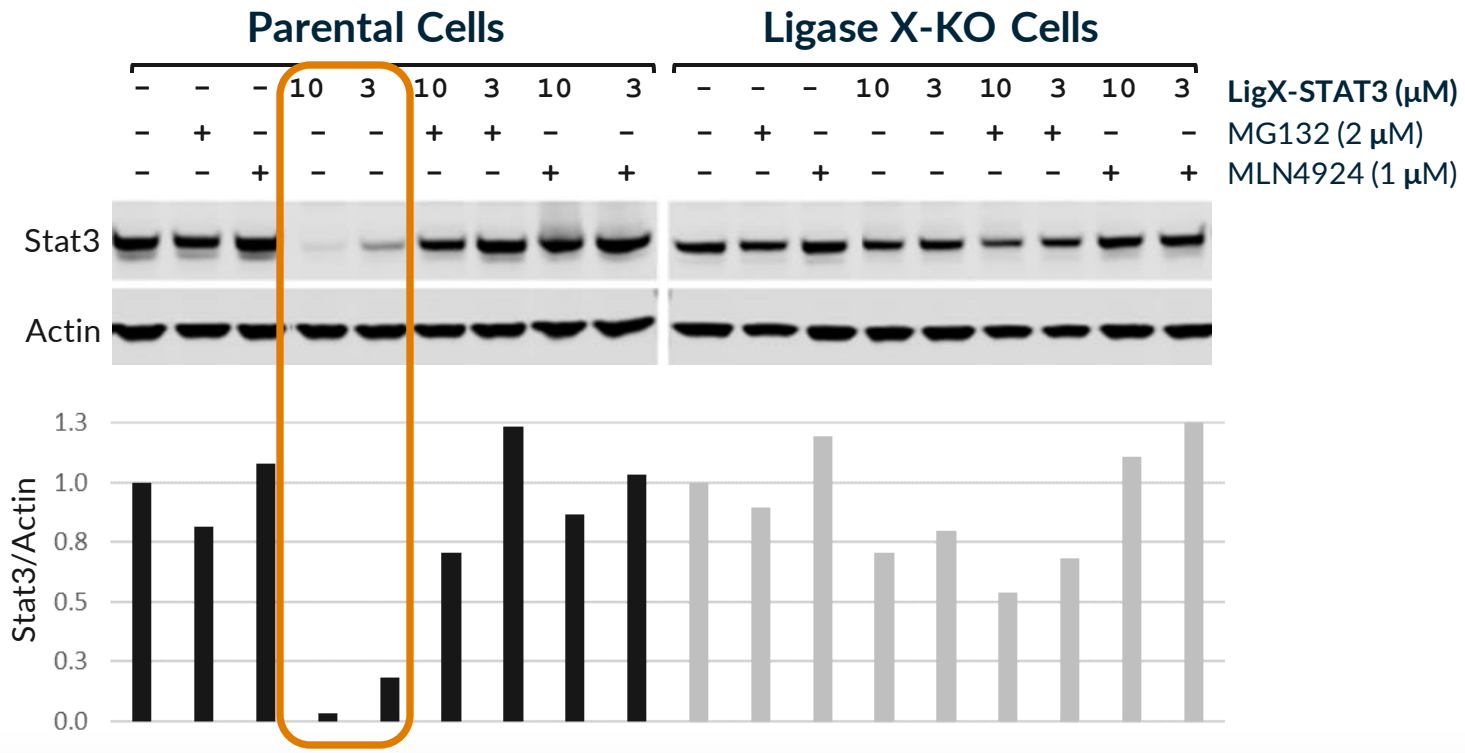
X-ray with Fragment



X-ray with Optimized Ligand

- Successfully applied SBDD to rapidly identify diverse E3 ligase ligands
- Multiple exit vectors identified and confirmed via chemistry, molecular modeling and X-ray
- Degraders synthesized for BRD4 + additional Kymera targets including STAT3 and IRAK4

# STAT3 Degrader Based on Ligase X Demonstrates Broad Degradation Across Multiple Cancer Cell Types




| Cells (Assay) | $DC_{50}$ (μM) |
|---|---|
| **A549** (HiBiT) | **0.20** |
| **Su-DHL-1** (MSD) | **0.82** |
| **Uveal Melanoma 92-1** (WB) | **<1** |
| **OVCAR-3** (WB) | **0.6** |
| **OVCAR-8** (WB) | **1.0** |

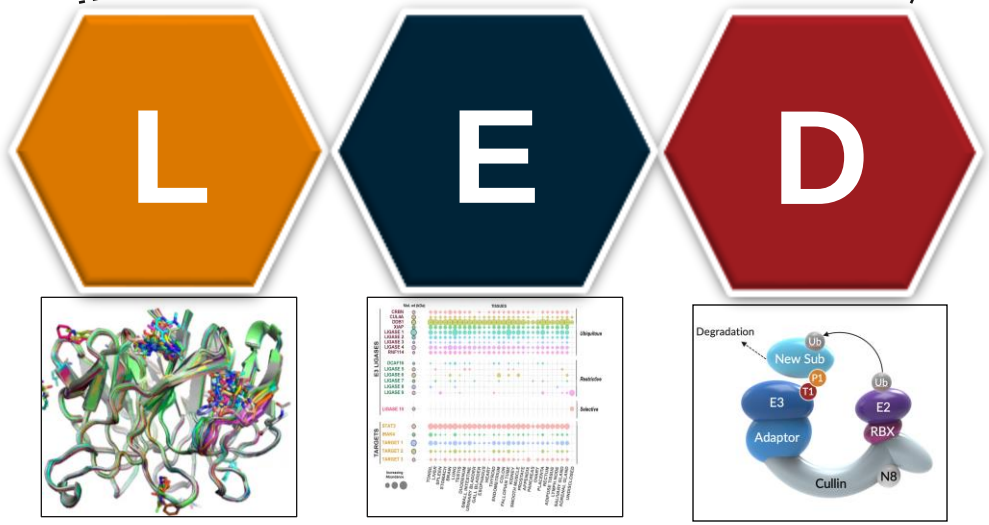
- Degrader LigX-STAT3 demonstrated dose-dependent degradation of STAT3, achieving >50% STAT3 degradation at 1 μM.
- STAT3 degradation was rescued by proteasome inhibitor MG-132 or neddylation inhibitor MLN4924, indicating UPS mediated protein degradation
- Knockout of ligase X abolished STAT3 degradation, indicating the degradation is ligase X dependent.

# Summary and Future Ambition

- TPD with **tissue sparing E3 ligases** can help maximize the therapeutic index of clinically well-validated targets by minimizing on-target toxicity
- Tissue restricted degradation can enable new **therapeutic opportunities** for these classes of targets
- The E3 Ligase Whole-Body Atlas identified multiple E3 ligases with **restricted expression** across different healthy tissues and tox cell types
- Using our L.E.D. E3 selection criteria improves the probability of success for functional ligand discovery, as exemplified by Ligase X

## Enabling TPD-based Precision Medicine


### E3 Selection Criteria




**Enabling SBDD** | **Selection via E3 Atlas** | **"D" at scale**

KYMERA
EST. 2016

# Thank You