Ubiquigent

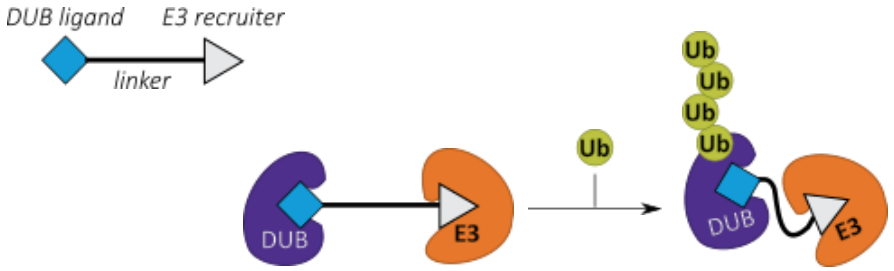
USP30 inhibitor case study

Application of the DUB platform at Ubiquigent to support the development of USP30 inhibitors

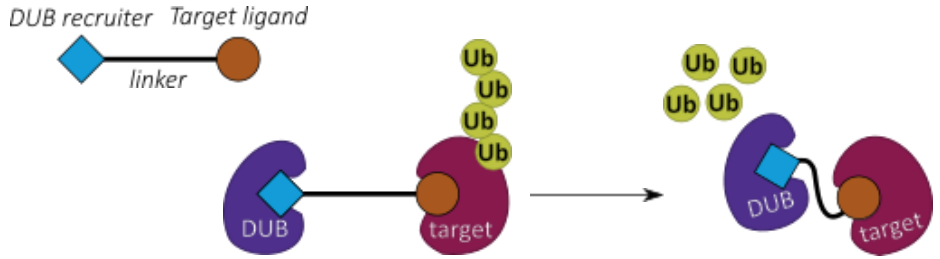
DUB modulation for therapeutic effects

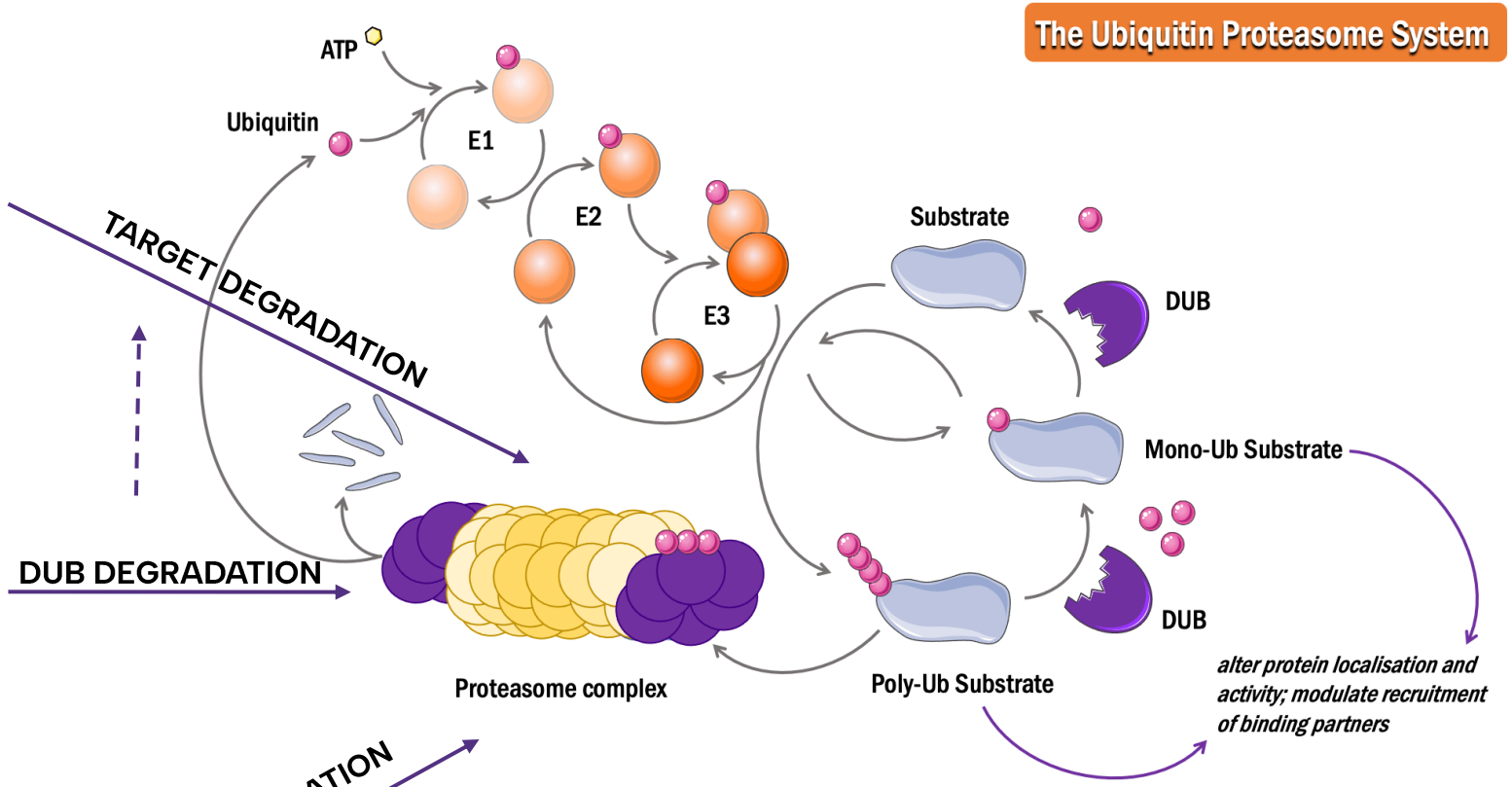
DUB inhibitor

DUB-targeting PROTAC



DUBTAC





USP30 target rationale

- Ubiquitin-specific protease 30 (USP30) is a deubiquitinating enzyme (DUB) localized in the mitochondrial outer membrane and peroxisomes owing to its unique transmembrane domain.[1]
- USP30 employs a unique catalytic triad and molecular architecture to preferentially cleave Lys6-linked ubiquitin chains.[2]
- USP30 plays an essential role in several cellular events, such as PINK1/Parkin-mediated mitophagy, pexophagy, BAX/BAK-dependent apoptosis, and IKKβ–USP30–ACLY-regulated lipogenesis/tumorigenesis.[3]
- Dysregulation of USP30 is associated with a range of physiological disorders, such as neurodegenerative disease, hepatocellular carcinoma, pulmonary disorders, and peroxisome biogenesis disorders.[3]
- Depletion of USP30 enhances the clearance of mitochondria by increasing mitophagy and also promotes Parkin-mediated cell death. Conversely, USP30 overexpression decreases PINK1/Parkin-mediated mitophagy in cells.[4]
- Accordingly, inhibition of USP30 represents a potential actionable drug target for intervening in the pathologies associated with PINK1/Parkin deficiency-induced mitophagy dysfunction, such as Parkinson's disease and pulmonary fibrosis.[5]
- Disclosed USP30 inhibitors in development include natural compounds, phenylalanine derivatives, N-cyano pyrrolidines, benzosulphonamide, and other small molecules. Mission Therapeutics USP30 inhibitor, MTX652, has successfully completed Phase I clinical assessment in healthy subjects, and is intended for the treatment of chronic kidney disease, heart failure, muscular dystrophy and idiopathic pulmonary fibrosis.[3]

[1] Clague, Michael J, and Sylvie Urbé. "Integration of cellular ubiquitin and membrane traffic systems: focus on deubiquitylases." *The FEBS journal* vol. 284,12 (2017): 1753-1766. doi:10.1111/febs.14007
[2] Gersch, Malte et al. "Mechanism and regulation of the Lys6-selective deubiquitinase USP30." *Nature structural & molecular biology* vol. 24,11 (2017): 920-930. doi:10.1038/nsmb.3475
[3] Wang, Feng et al. "USP30: Structure, Emerging Physiological Role, and Target Inhibition." *Frontiers in pharmacology* vol. 13 851654. 3 Mar. 2022, doi:10.3389/fphar.2022.851654
[4] Bingol, Baris et al. "The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy." *Nature* vol. 510,7505 (2014): 370-5. doi:10.1038/nature13418
[5] Bingol, Baris, and Morgan Sheng. "Mechanisms of mitophagy: PINK1, Parkin, USP30 and beyond." *Free radical biology & medicine* vol. 100 (2016): 210-222. doi:10.1016/j.freeradbiomed.2016.04.015

DUB inhibitor platform from Ubiquigent

- Unrivalled track-record and long-standing expertise in DUB and UPS biology
- Extensive chemistry, medicinal and structural design expertise
- Access to a range of relevant technologies throughout network of collaborators

DUB target identification and validation → **Hit discovery** → **Hit-to-lead and lead optimisation** → **Novel DUB inhibitor portfolio**

Identify and validate DUB targets

- PROTEOME*profiler*
- UBISITE*profiler*
- DUB*atlas*
- DUB*scan*

Identify DUB inhibitors

- DUB*profiler*

Access our proprietary DUB-targeting compounds

Eliminate false positives

- REDOX*profiler*

Hit confirmation

- Custom orthogonal assays

Establish potency and selectivity

- DUB*profiler*

Demonstrate target engagement and selectivity

- DUB*profiler*-Cell
- DUB*profiler*-Tissue

Determine MOA

- DUB*mechanism*

Pathway analysis

- PROTEOME*profiler*
- UBISITE*profiler*

DUB*profiler*™

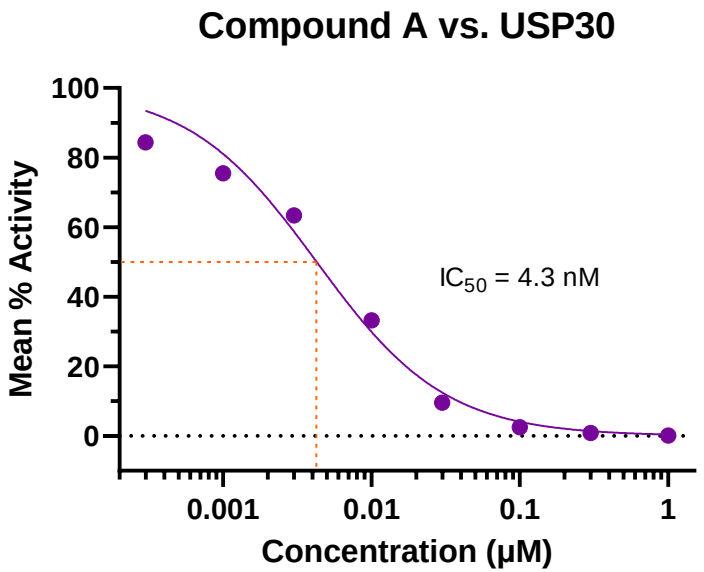
- DUB*profiler*™ is based on a robust ubiquitin-rhodamine(110)-glycine enzymatic assay, comprising a panel of DUB enzymes representative of the entire human DUB family

- DUB inhibitors can be rapidly screened across the DUB*profiler*™ panel to establish their potency and DUB enzyme selectivity

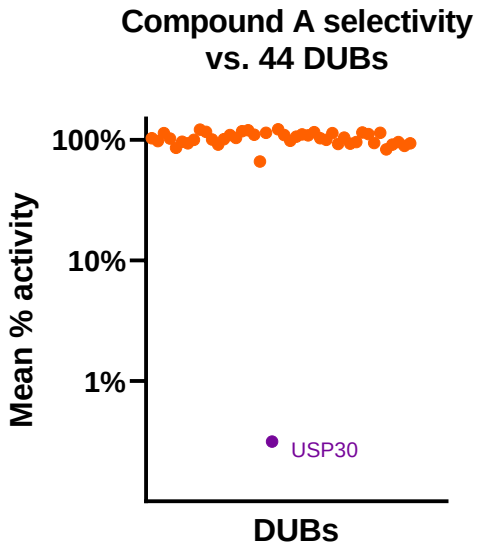
- Compound A was submitted for evaluation in DUB*profiler*™

In vitro potency and selectivity of Compound A

IC_{50} value of Compound A against USP30 in DUB*profiler*™



Compound A selectivity at ~200x IC_{50} against panel of 44 human DUB enzymes in DUB*profiler*™



DUB*profiler*–Cell™ and DUB*profiler*–Tissue™

- DUB*profiler*-Cell™ and DUB*profiler*-Tissue™ are flexible assays to determine target engagement and selectivity of DUB inhibitors in cell lysates, cell cultures, or tissue samples

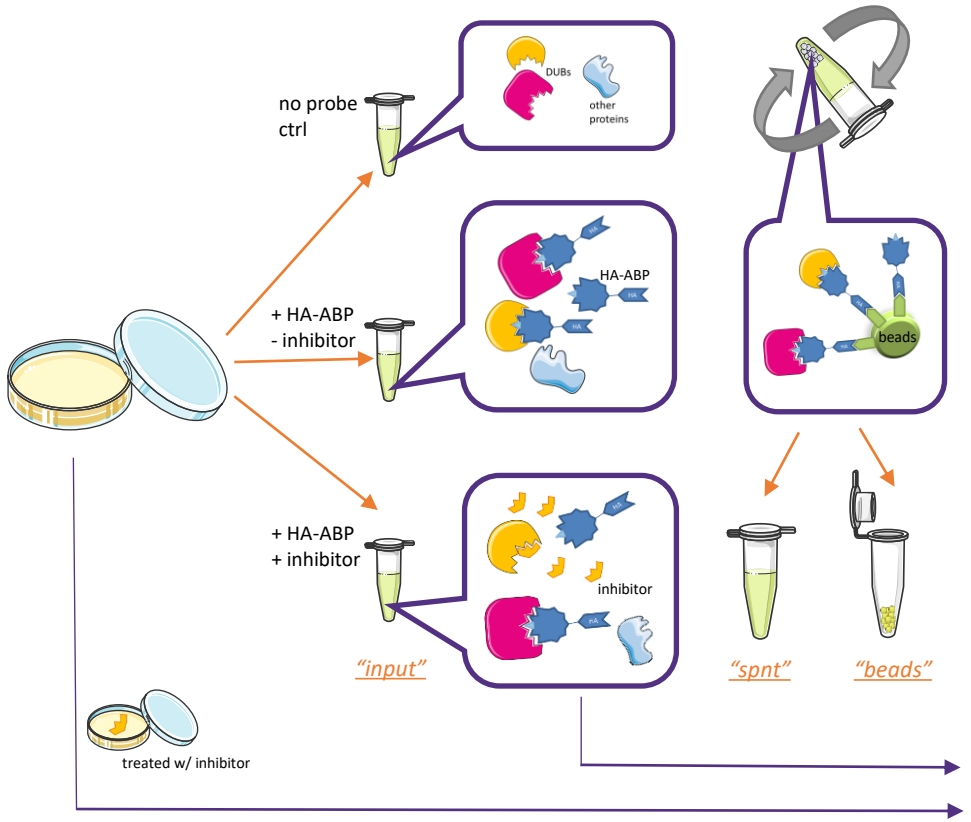
- The assays use activity-based probes (ABPs) to engage and capture active DUBs (the 'DUBome') in disease-relevant samples

- By revealing which DUBs are engaged and captured by the ABP in a given sample, target engagement of test compounds with any one or all of the detected DUBs can then be determined, to establish compound selectivity. Biomarkers associated with the mechanism of action can also be measured in the same samples

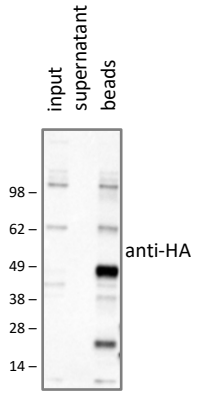
- Evaluating DUB target engagement in the context of a live cell or tissue environment can support DUB inhibitor programmes as they move towards clinical development. For example, the ability to demonstrate DUB target engagement in tissues derived from compound-dosed animals can be correlated with pharmacokinetic data and efficacy

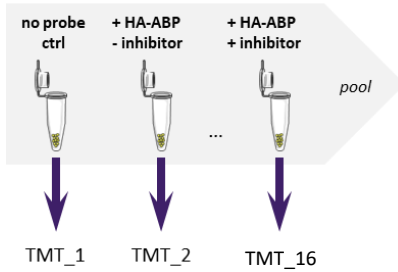
Target engagement analysis using ABPs in cell lysates and live cells

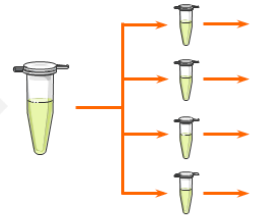
1. Cell lysis
2. Compound incubation and labelling with activity probe
3. IP with anti-HA beads
4. Control blot
5. Tryptic digest and TMT labeling
6. Sample pooling and fractionation
7. LC-MS/MS
8. Data analysis and report generation

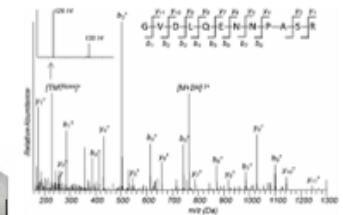


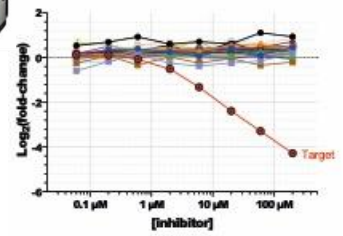
Live cell treatments for target engagement analysis

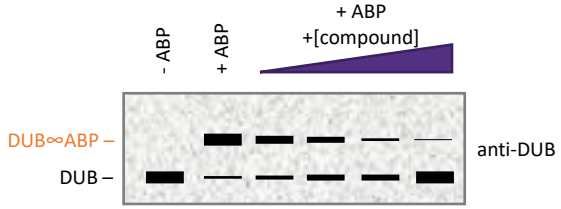




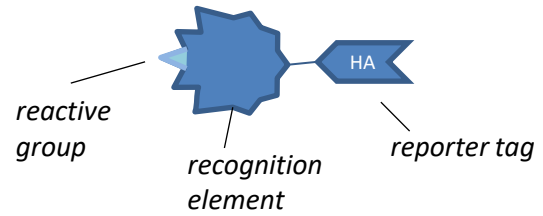




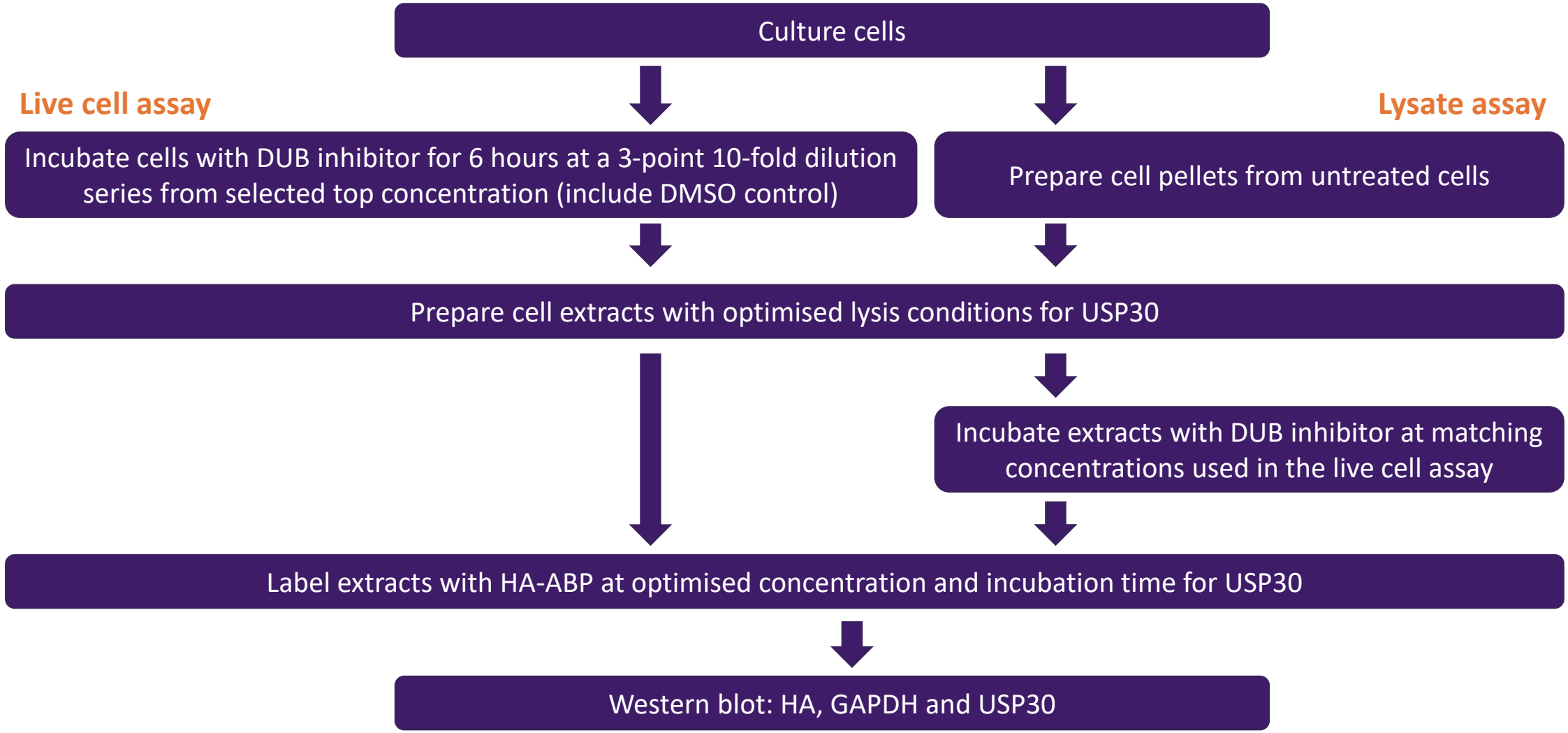




ABP

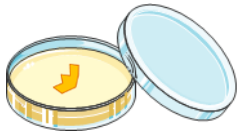


DUB*profiler*–Cell™ Workflow



Target engagement in SH-SY5Y cells with DUB*profiler*-Cell™

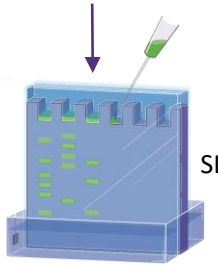
Live Cell Assay



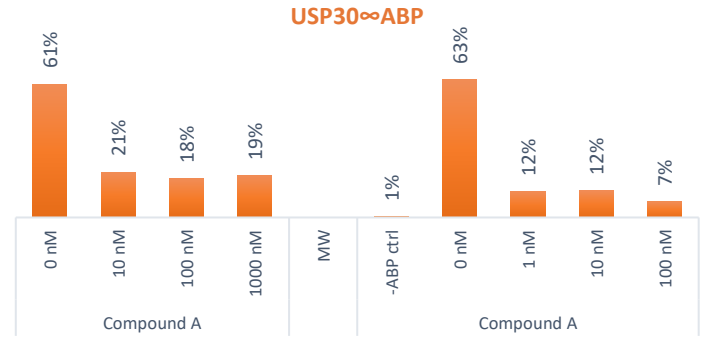
Cells treated w/ inhibitor

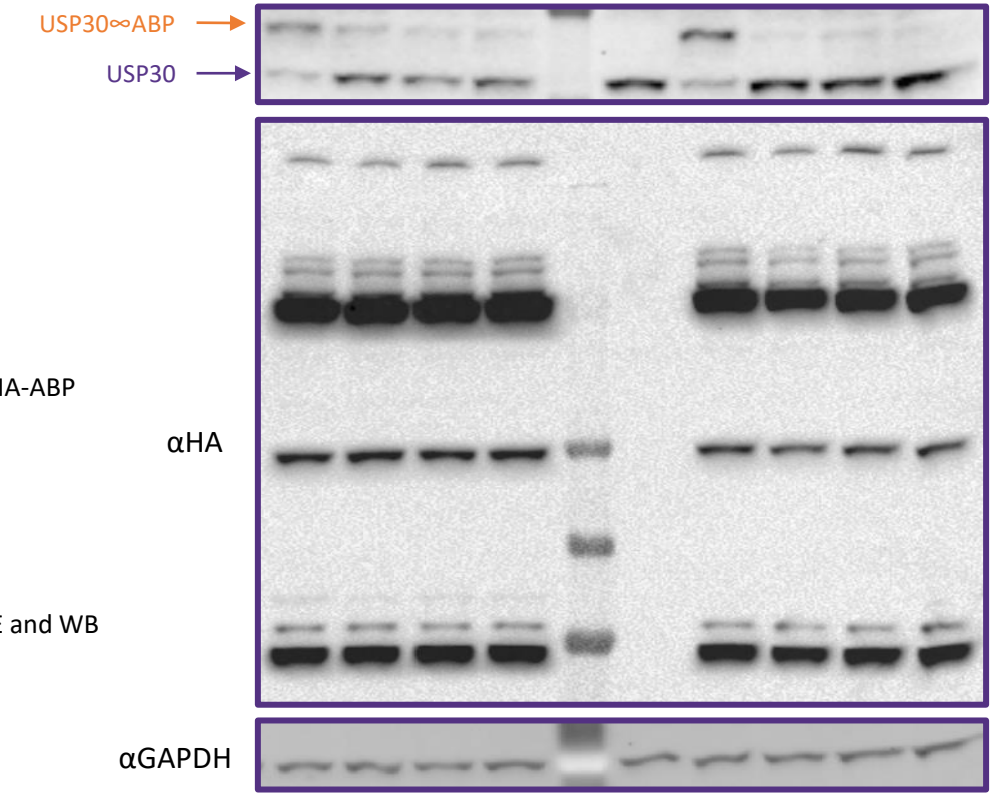
Lysis

Incubate with HA-ABP

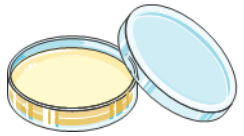


SDS-PAGE and WB





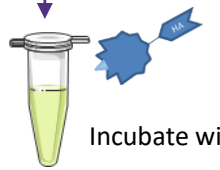
Lysate Assay



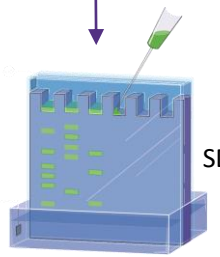
Control cells

Lysis

Pre-incubation with compound



Incubate with HA-ABP



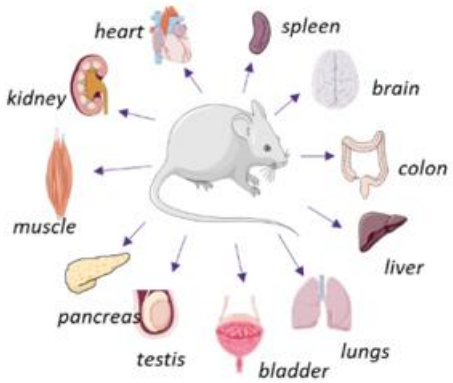
SDS-PAGE and WB

Lysate:
Compound A engages USP30 and inhibits probe binding (USP30∞ABP) in cell lysates at low nM.

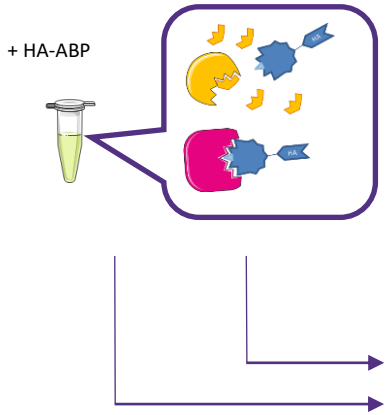
Live Cells:
Cells treated with Compound A exhibit reduced probe binding to USP30 vs. DMSO (0 nM) controls, suggesting the compound is cell permeable and engages USP30 in cells.

Target engagement analysis using ABPs in animal tissues

1. Tissue preparation & lysis



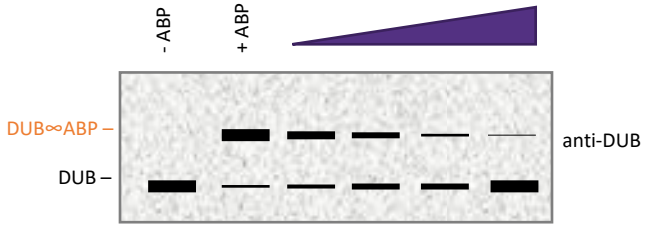
2. Incubation with ABP



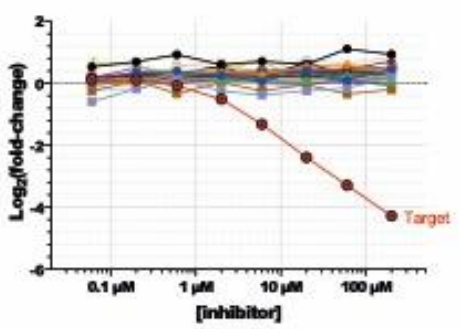
Additional ex vivo treatments to demonstrate target engagement

3. Western blotting for DUB target of interest

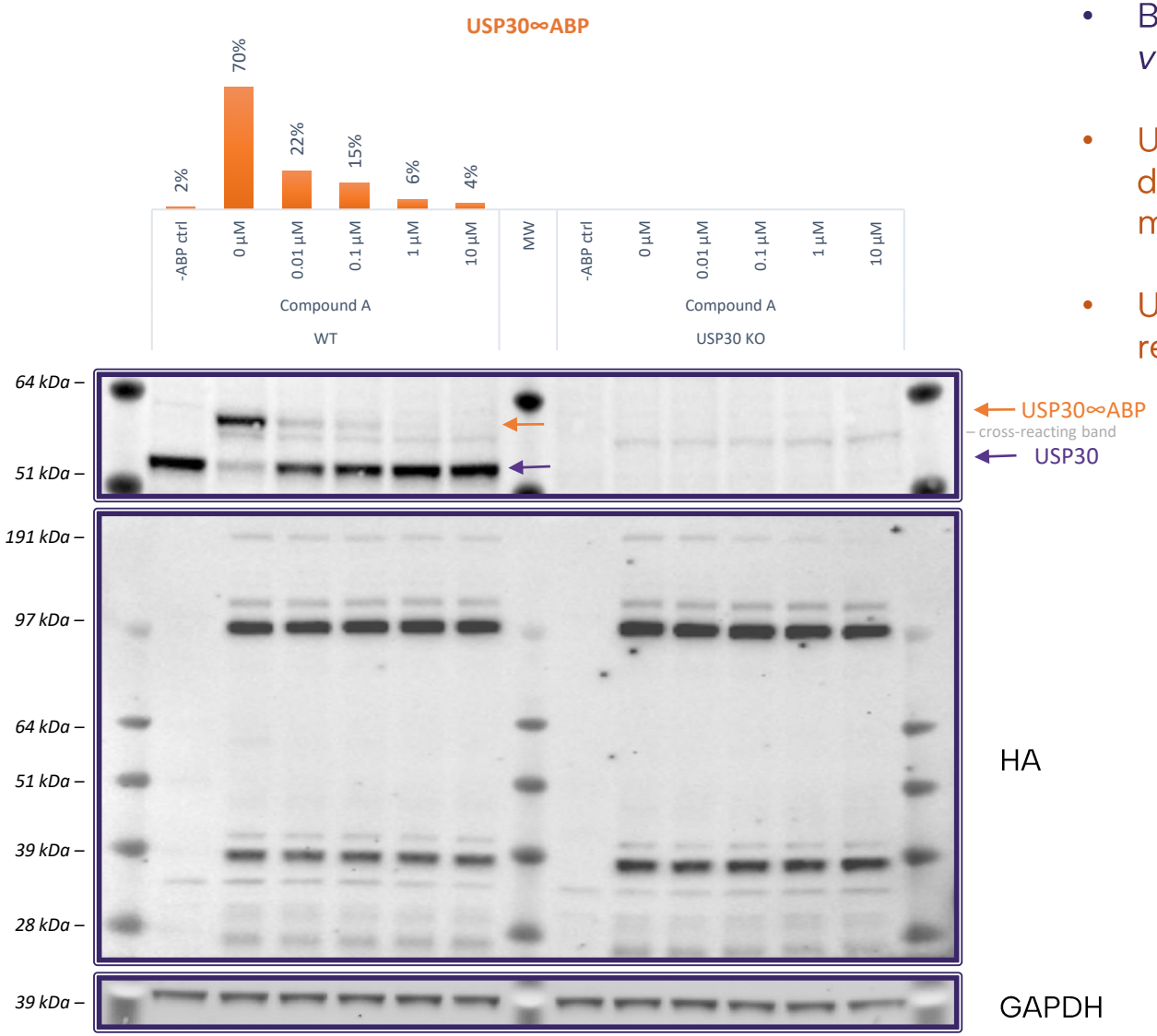
Ex vivo dose response
or *in vivo* dose escalation



4. Optional MS post-enrichment

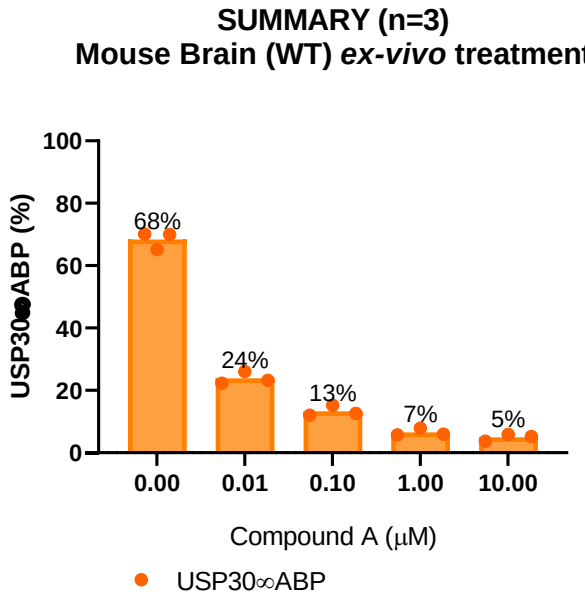


Validation of USP30 target engagement assay in mouse brain tissue from WT and USP30 KO animals



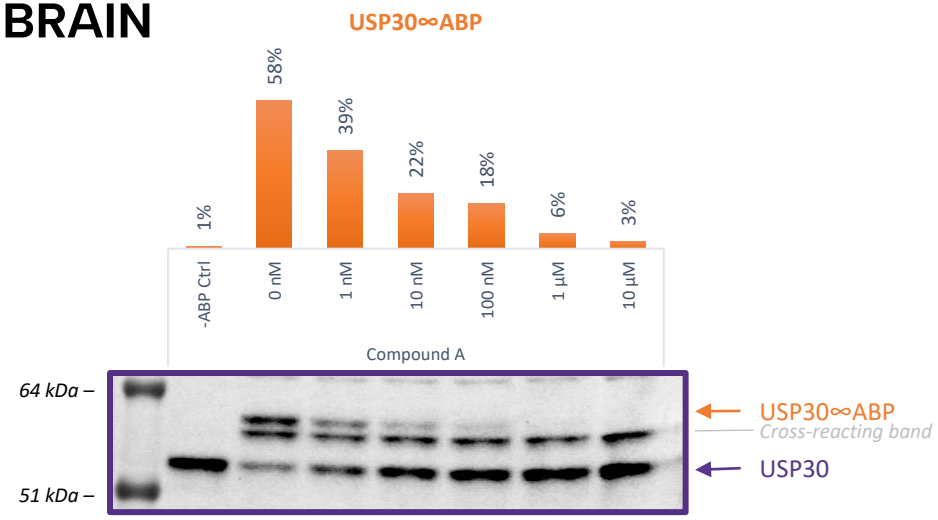
Representative data; total of 3 animals in each group

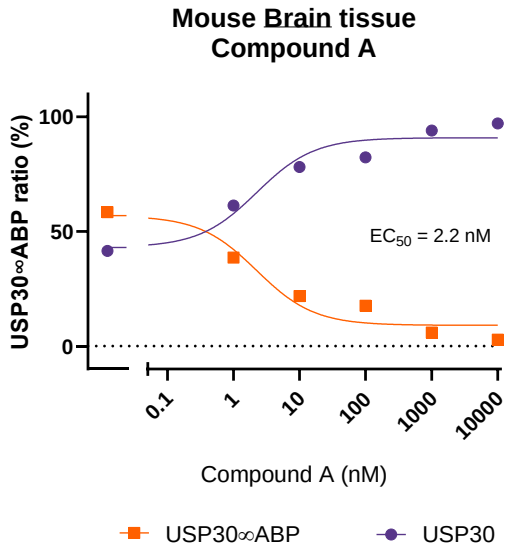
- Brain tissues from USP30 WT and USP30 KO mice were incubated *ex vivo* with the ABP in the presence or absence of Compound A
- USP30 was clearly identified in WT samples and absent in KO samples, demonstrating that the probe-bound band is USP30 (and that the middle band is a non-specific cross-reacting band)
- USP30∞ABP binding in WT samples was inhibited in a dose responsive manner by Compound A



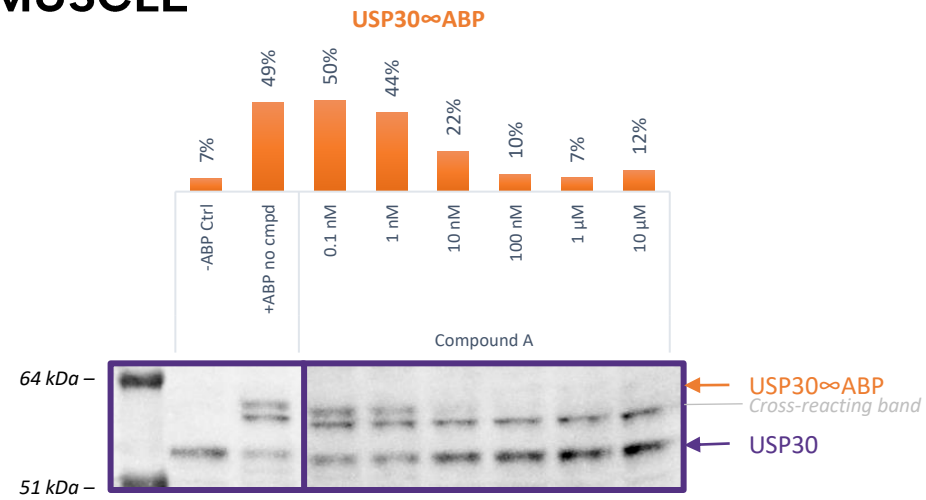
Impact of *ex vivo* compound treatment on USP30 target engagement in mouse brain and muscle

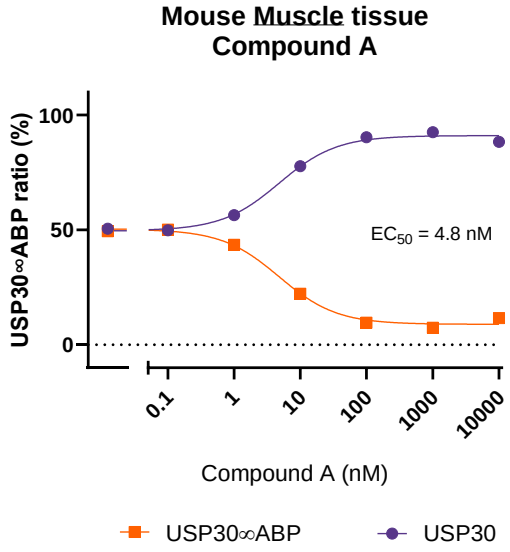
BRAIN





MUSCLE

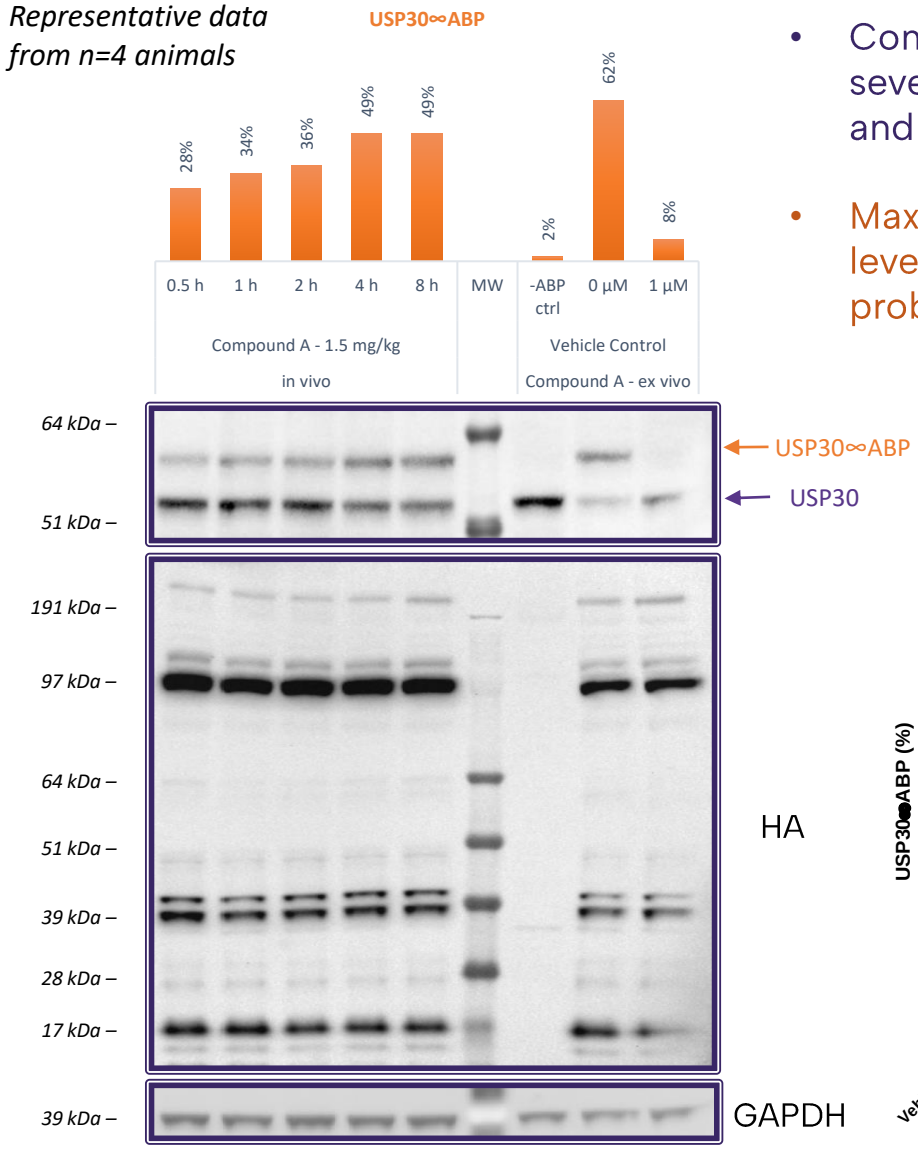




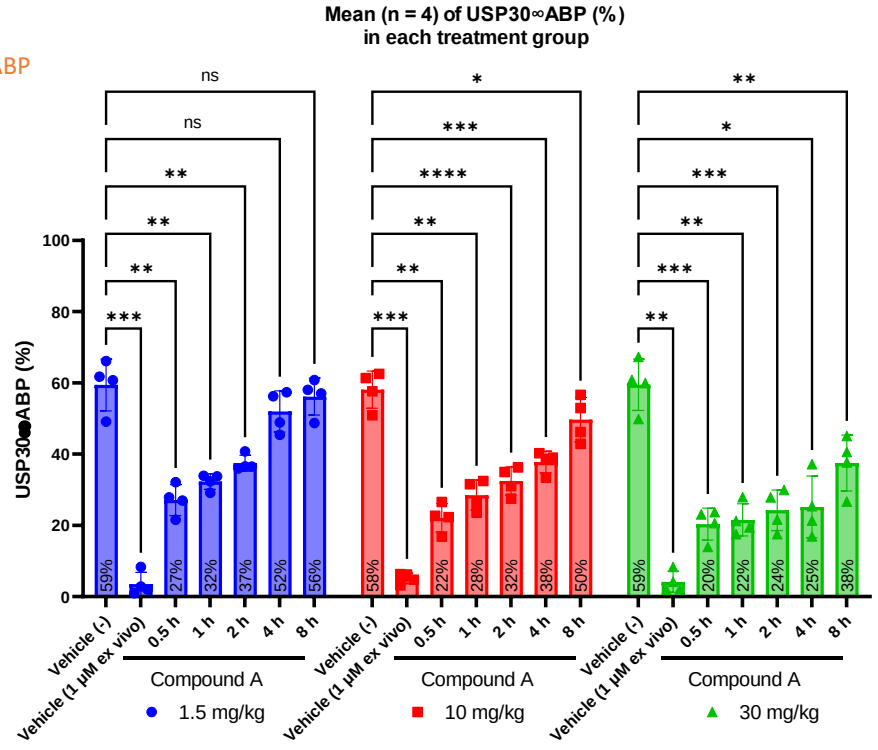
- USP30 and probe binding are detectable in mouse brain and muscle tissues
- EC_{50} values can be determined for Compound A against USP30 in *ex vivo* treatments of brain and muscle (2.2 nM and 4.8 nM, respectively)

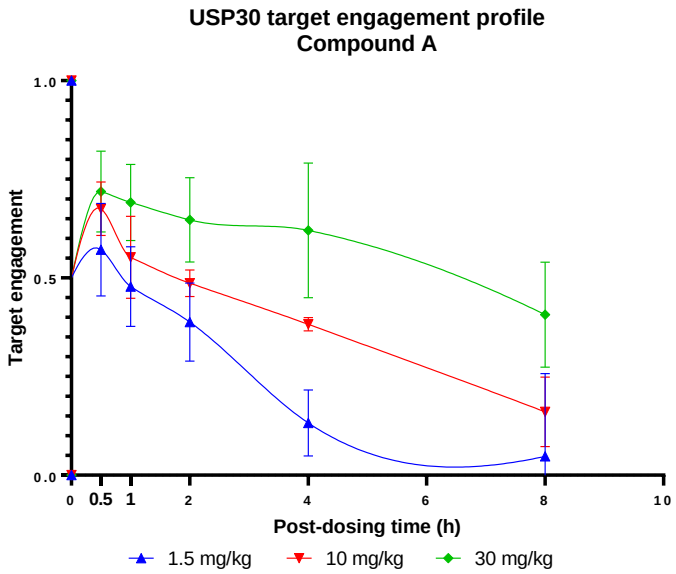
USP30 target engagement in mouse brain tissue from animals dosed with Compound A

Representative data from n=4 animals



- Compound A was administered orally at different dosages and the animals were sacrificed at several time points post-dosing to harvest multiple tissues for target engagement analysis and PK/PD studies
- Maximum target engagement was observed in mice dosed with 30 mg/kg. At higher dose levels, target engagement was sustained over time, whereas at the lowest dose (1.5 mg/kg) probe binding was restored to levels similar to the vehicle controls by 4–8h post-dosing





Summary

- Using the DUB*profiler*™, DUB*profiler*-Cell™ and DUB*profiler*-Tissue™ suite of assays, Ubiquigent was able to determine that Compound A is a highly potent, selective and cell permeable inhibitor of USP30

- The assay development (not shown) and target engagement assays performed in tissues harvested from mice confirmed that the compound was capable of target engagement at all dose levels

- The results of the target engagement assay can be compared with pharmacokinetics, biomarkers and *in vivo* efficacy endpoints from these same mice, yielding powerful insights about the mechanism of action of this compound

- Such experiments will also prove informative as this compound approaches clinical testing

For more information about how the DUB platform at Ubiquigent can accelerate your inhibitor discovery and development, please contact

services@ubiquigent.com