How to cite: *Angew. Chem. Int. Ed.* **2024**, *63*, e202319579
doi.org/10.1002/anie.202319579

Protein Profiling

A Modular Turn-On Strategy to Profile E2-Specific Ubiquitination Events in Living Cells

*Caitlin J. Hill[+], Suprama Datta[+], Nicholas P. McCurtin, Hannah Z. Kimball, Molly C. Kingsley, Abraham L. Bayer, Alexander C. Martin, Qianni Peng, Eranthie Weerapana, and Rebecca A. Scheck**

Abstract: A cascade of three enzymes, E1–E2–E3, is responsible for transferring ubiquitin to target proteins, which controls many different aspects of cellular signaling. The role of the E2 has been largely overlooked, despite influencing substrate identity, chain multiplicity, and topology. Here we report a method—targeted charging of ubiquitin to E2 (tCUbE)—that can track a tagged ubiquitin through its entire enzymatic cascade in living mammalian cells. We use this approach to reveal new targets whose ubiquitination depends on UbcH5a E2 activity. We demonstrate that tCUbE can be broadly applied to multiple E2s and in different human cell lines. tCUbE is uniquely suited to examine E2–E3-substrate cascades of interest and/or piece together previously unidentified cascades, thereby illuminating entire branches of the UPS and providing critical insight that will be useful for identifying new therapeutic targets in the UPS.

Dysfunction within the ubiquitin-proteasome system (UPS) is implicated in many diseases, including cancers, neurodegenerative disorders, and infection pathogenesis.[1] A sequential cascade, involving the activities of three enzymes (E1–E2–E3), is responsible for the transfer and attachment of ubiquitin (Ub) to substrate proteins.[2] The central enzyme in this cascade, the E2 ubiquitin-conjugating enzyme, facilitates thioester exchange from the E1~Ub conjugate to form an E2~Ub thioester, which then interacts with the E3 ligase to catalyze isopeptide bond formation linking Ub to the target protein (Figure 1). The E2 plays an underappreciated yet pivotal role, working in concert with the E3 to dictate the specific substrate identity, ubiquitin chain multiplicity, and polyubiquitin chain topology.[3] Despite a growing number of reports that point to the importance of the E2,[3–4] it remains difficult to define the complete cascade of interactions that links ubiquitin to a target protein.[2a,5] In humans, two E1s, roughly 40 E2s, and more than 600 E3s have been identified.[6] Because each E2 can interact with multiple E3s, and *vice versa*, there are thousands of possible transient E2/E3 combinations, each of which could result in a specific ubiquitinated outcome. Moreover, redundancy within this network renders traditional methods, including genetic or pharmacological manipulation, poorly suited for its study.[2a,5–6] Similarly, in vitro reconstitution typically requires prior knowledge of E2/E3 pairs, or relies on promiscuous E2s. As a result, the specific E2/E3 pairs that promote particular ubiquitination events remain largely unidentified.

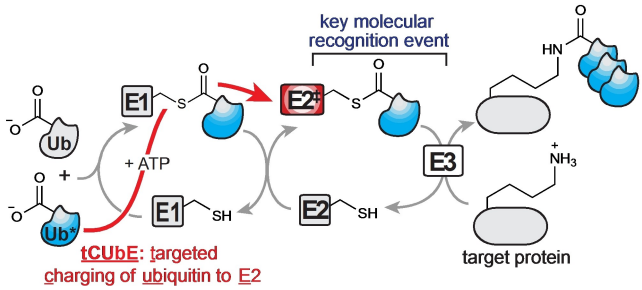

Figure 1. An overview of cellular ubiquitination, in which the small protein ubiquitin (Ub) becomes attached to substrate proteins through the sequential activities of three enzymes (E1–E2–E3). Here we describe the development of a new method called tCUbE (targeted charging of ubiquitin to E2) that can track a tagged ubiquitin (Ub*) all the way through its path.

Several recent reports have sought to reveal interactions with E2s, E3s, and–in a few cases–substrates, that are captured by engineered thioesters, electrophilic traps or photocrosslinkers.[7] While these methods advance our ability to reveal interactions within the UPS, most cannot label substrates[7a–d] and many cannot profile the activity of RING E3s that do not possess active site cysteines.[7b–e] Additionally, they all require chemical manipulation of recombinant proteins that are generated in vitro and then introduced into live cells or lysates. Capturing such interactions in live cells has remained an ongoing challenge. Herein we present a new method that allows a tagged ubiquitin to be followed all the way through its enzymatic

[*] Dr. C. J. Hill,[+] Dr. S. Datta,[+] N. P. McCurtin, H. Z. Kimball, Dr. M. C. Kingsley, A. L. Bayer, A. C. Martin, Prof. R. A. Scheck
Department of Chemistry
Tufts University
Medford MA 02155 USA
E-mail: rebecca.scheck@tufts.edu

Q. Peng, Prof. E. Weerapana
Department of Chemistry
Boston College
Chestnut Hill MA 02467 USA

[+] Equal contribution.

cascade, called **t**argeted **c**harging of **ub**iquitin to **E**2 (tCUbE) (Figures 1 & 2). tCUbE uses chemically induced dimerization to target a unique, tagged ubiquitin (Ub*) to a single, specific E2 ($E2^+$), thereby allowing direct and unambiguous detection of ubiquitination events mediated by that $E2^+$. tCUbE is designed to be easily applied in living mammalian cells, allowing interactions with all parts of the endogenous UPS without knockdown or inactivation of native enzymes. Here we establish that tCUbE is able to report on the activity of a specific $E2^+$ in living mammalian cells and can be used to broadly profile E2 activity using proteomics. We also demonstrate the utility of tCUbE using multiple E2s and human cell lines.

tCUbE relies on a chemically-induced interaction of two engineered modular protein partners expressed in mammalian cells: an N-terminal HA-tagged ubiquitin (Ub*) and a FLAG-tagged E2 of interest ($E2^+$). These variants also include complementary binding (targeting FK506 binding protein (FKBP) and FKBP-rapamycin binding (FRB)) and split intein (*Sce* VMA^N and VMA^C) domains, both used in the original reports of conditional protein splicing,[8] that facilitate their association and subsequent dissociation, respectively (Figure 2A,B). Importantly, Ub* retains a free C-terminus so that it can be activated by endogenous E1 enzymes, interact with other components of the ubiquitination cascade, and ultimately modify substrate proteins. In our initial studies, we chose to use UbcH5a as a model $E2^+$ as it is commonly used for in vitro ubiquitination assays and is known to accept a wide range of potential substrates.[9]

The tCUbE assay is initiated by association of Ub* and $E2^+$ upon chemical induction by rapalog (Figure 2A, step 1). To avoid off-target interactions with mTOR, we use a rapamycin derivative, rapalog, that induces dimerization with FKBP for rapalog-binding, but not wild-type, FRB (rb-FRB).[10] Upon rapalog-induced association, Ub* is activated by endogenous E1 enzymes and can then be transferred to $E2^+$ through native transthioesterification, yielding a charged $E2^+$~Ub* thioester. Rapalog-induced association of Ub* and $E2^+$ also initiates intein self-splicing, thereby liberating the charged $E2^+$~Ub* thioester from the ternary complex formed between FKBP, rb-FRB, and rapalog (Figure 2A, step 2). Unlike many split inteins, the complementary halves of the *Sce* VMA intein have low intrinsic affinities for one another in the absence of the FKBP-FRB targeting domains.[8a,11] Thus, once free from these domains, Ub* can then be transferred from the $E2^+$~Ub* thioester to endogenous E3s and/or substrates (Figure 2A, step 3). Products that arise from the targeted interaction of Ub* and $E2^+$ are detected via HA epitope (on Ub*) following immunoprecipitation and/or western blot. We used in vitro ubiquitination experiments with recombinantly expressed Ub* and $E2^+$ (UbcH5a) variants, to confirm that Ub* can be activated by recombinant E1s to form charged and, subsequently, spliced $E2^+$~Ub* thioesters (Figure S1).

To assess if tCUbE captures $E2^+$ activity in living cells, Ub* and $E2^+$ (UbcH5a) were transiently co-expressed in HEK-293T cells. We found that rapalog treatment led to a substantial increase in high molecular weight HA signal when cells were co-transfected with both Ub* and $E2^+$

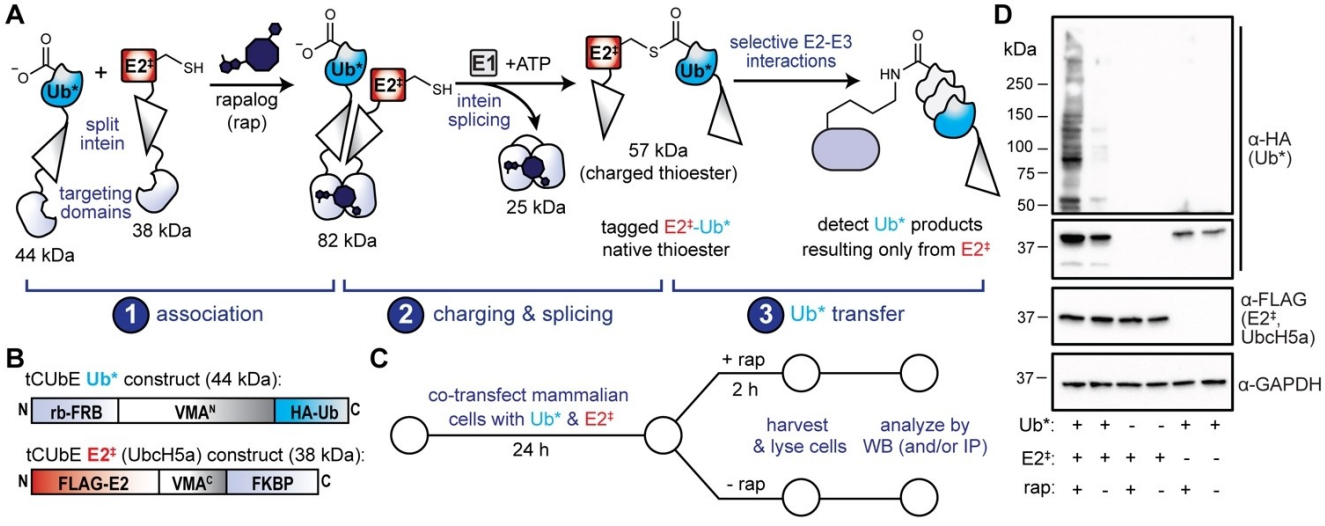

Figure 2. Targeted charging of ubiquitin to E2 (tCUbE). (A) tCUbE relies on the targeted interaction of Ub* with an E2 of interest ($E2^+$). First, treatment with rapalog induces association of Ub* and $E2^+$ (step 1). Next, Ub* is charged onto $E2^+$ through the activity of native E1 enzymes. The association of Ub* and $E2^+$ also leads to proximity-induced self-splicing of the intein domains (step 2). Finally, transfer of Ub* to endogenous substrate proteins occurs through selective interactions of the $E2^+$~Ub* thioester with endogenous E3s (step 3). (B) tCUbE variants each include complementary targeting (FKBP and FRB) and split intein (Sce VMA^N and VMA^C) domains. The Ub* variant contains an HA epitope, while $E2^+$ variants include an N-terminal FLAG epitope. (C) tCUbE is designed to be performed in living mammalian cells, using standard techniques. (D) HEK-293T cells were co-transfected with Ub* and $E2^+$ (UbcH5a). After treatment with rapalog for 2 h, the cells were harvested, lysed, and analyzed by western blot, probing with α-HA and α-FLAG antibodies to detect Ub* and $E2^+$, respectively. Higher molecular weight (>50 kDa) HA signal indicating the formation of Ub*-conjugates is observed when co-transfected HEK-293T cells are treated with rapalog. Uncropped blot images for Figure 2D are shown in Supporting Information under the Supporting Figures section.

(Figure 2C,D, Figure S2). Notably, on its own, Ub* appears somewhat recalcitrant to the endogenous ubiquitin machinery (Figure S3). However, in the presence of E2^{+} and rapalog, Ub* incorporation into higher molecular weight signal is observed. Thus, formation of high molecular weight Ub*-species is dependent on the presence of all three assay components: Ub*, E2^{+}, and rapalog. This behavior offers a benefit by providing relatively low background and high E2 specificity during tCUbE.

Building on these findings, we next considered the potential role of deubiquitinase (DUB) activity in interfering with tCUbE signal by removing Ub* products and potentially recycling spliced Ub* onto other proteins, independent of E2^{+} activity.[2a,12] To avoid this, we designed a DUB-resistant variant of Ub* (Ub*^{DUBr}) that contains a Leu to Pro point mutation in the Ub* C-terminal tail.[13] Use of Ub*^{DUBr} in tCUbE increased both the intensity of HA signal at high molecular weights and the resolution of distinct bands, while maintaining rapalog-dependence (Figure 3A). We note there is higher background in the rapalog-untreated sample when Ub*^{DUBr} is used, which we attribute to DUB resistance that is not observed for the original Ub*. However, this background was only observed when cells expressing Ub*^{DUBr} were also transfected with E2^{+}, suggesting that any background signal is due to E2^{+} activity, not endogenous incorporation of Ub*^{DUBr} (Figure S3). Additionally, there is still strong rapalog-dependence and, by inhibiting the removal of Ub* species, our ability to detect potentially transient ubiquitination events is substantially enhanced. Following this result, Ub*^{DUBr} was used in all subsequent experiments.

Next, we sought to confirm that the tCUbE design functions as intended. Specifically, we focused on two critical design elements: (1) association of Ub* and E2^{+} (Figure 2A, step 2) and (2) intein-mediated release (Figure 2A, step 3). To evaluate the effect of rapalog-mediated association on tCUbE, we designed a non-binding (nb)-Ub*^{DUBr} variant containing a wt-FRB domain that cannot bind to rapalog in place of rb-FRB.[10b,c] When cells were co-transfected with nb-Ub*^{DUBr} and E2^{+} and treated with rapalog, we observed only very low background signal that was comparable to the untreated sample with the original rb-FRB (Ub*^{DUBr}) variant (Figure 3B). To investigate the contribution of intein splicing, both an intein-splicing-impaired E2^{+} variant (E2^{+int}) and one lacking the VMA^{c} domain (E2$^{+\Delta VMA}$) were designed and used in tCUbE experiments (Figure S4).[14] The greatest extent of high MW HA signal, corresponding to Ub* (or Ub*^{DUBr}) transfer to substrates, was observed for the original E2^{+}. This finding suggests that intein splicing is important for efficient Ub* transfer.

When cells were co-transfected with Ub* and E2^{+int}, a strong ~82 kDa band accumulates in rapalog-treated samples, and this band was also present when E2$^{+\Delta VMA}$ was used (Figure S4). This same band was observed for rapalog-treated samples with the original E2^{+} (Figure 2D), and can be observed on the FLAG blot when the intein-impaired E2 (E2^{+int}) or a double mutant that is both catalytically inactive and intein-impaired (E2$^{+cat-int}$) is used. Together these

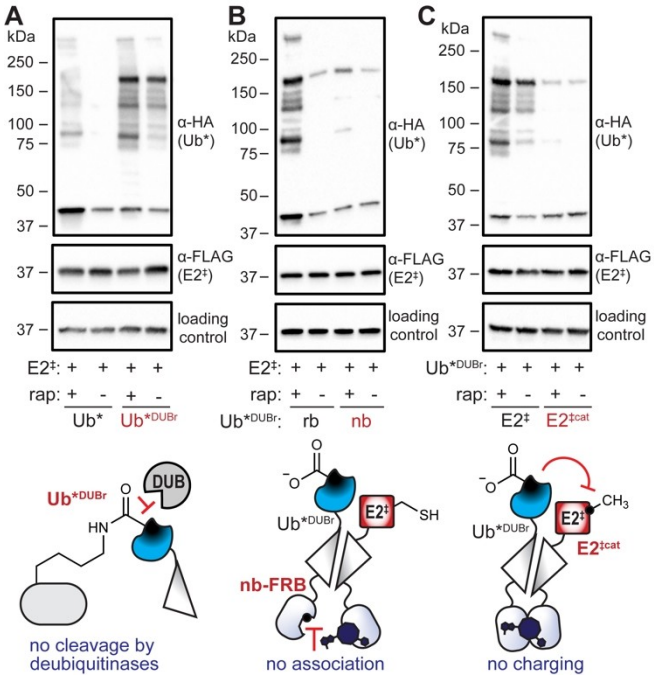

Figure 3. tCUbE optimization and validation. (A) HEK-293T cells were co-transfected with E2^{+} (UbcH5a) and either Ub* or a deubiquitinase-resistant variant (Ub*^{DUBr}), then treated with or without rapalog. The use of Ub*^{DUBr} preserves the Ub*-conjugates generated by tCUbE while maintaining rapalog-dependence, as indicated by the increase in higher molecular weight signal when probing against HA. In all panels, GAPDH is used as the loading control. (B) tCUbE performed in HEK-293Tcells co-transfected with E2^{+} (UbcH5a) and Ub*^{DUBr} variants possessing the rapalog-binding FRB domain (rb-Ub*^{DUBr}) or a non-binding variant (nb-Ub*^{DUBr}). These results demonstrate that association of E2^{+} and Ub* is required for tCUbE, as indicated by the lack of higher molecular weight HA signal when rapalog is unable to induce association. (C) tCUbE performed in HEK-293T cells co-transfected with Ub*^{DUBr} and either E2^{+} (UbcH5a) or a catalytically inactive variant (E2^{+cat}), then treated with or without rapalog. No Ub*-conjugates are observed when the catalytically inactive E2^{+} is used, thus demonstrating that E2^{+} activity drives Ub* transfer via tCUbE. Uncropped blot images for Figure 3A, B, and C are shown in Supporting Information under the Supporting Figures section.

results suggest this band is the rapalog-mediated ternary complex that forms between Ub*^{DUBr} and E2^{+} during tCUbE (Figure S4). While one would expect this ternary complex to be disrupted under the reducing and denaturing conditions required for SDS-PAGE, we found that this band was recalcitrant to complete reduction and denaturation under standard conditions. However, with longer incubations and higher DTT concentrations, it could be outcompeted (Figure S5). These results suggest that this band is the associated Ub*-rapalog-E2^{+} ternary complex. Taken together, these results confirm that rapalog-mediated association of E2^{+} and Ub* is responsible for E2^{+} charging and that intein splicing enhances the extent of Ub* transfer in tCUbE.

Importantly, we confirmed that Ub* transfer is driven by the catalytic activity of E2^{+}. To do so, we cloned a catalytically-inactive E2^{+} variant of UbcH5a (E2^{+cat}) by

mutating the catalytic Cys to Ala.[2] When cells are co-transfected with Ub*^{DUBr} and E2^{+cat}, high molecular weight HA signal was negligible, even in rapalog-treated samples (Figure 3C). This indicates that even when Ub*^{DUBr} and E2^{+cat} form a ternary complex with rapalog, transfer of Ub*^{DUBr} cannot occur without a catalytic Cys in E2^{+}. These results confirm that E2^{+} activity is responsible for Ub* transfer in tCUbE performed in HEK-293T cells.

Additionally, we performed further studies to evaluate tCUbE performance. We found that the targeted transfer of Ub* to higher MW conjugates exhibits a time and dose-dependent response to rapalog treatment (Figure S6). It was also compatible with the use of carfilzomib, a common proteasome inhibitor (Figure S7). Furthermore, despite the enhanced HA signal for cells transfected with tCUbE variants and treated with rapalog, there was no gross impact on overall cellular ubiquitination observable by western blot (Figure S8). We also expanded this methodology to other commonly used cell lines and found that tCUbE using Ub*^{DUBr} and E2^{+} (UbcH5a) or E2^{+cat} worked comparably in HeLa and U2OS cells as it did in HEK-293T cells (Figure S9).

Having established that tCUbE successfully reports on UbcH5a activity in multiple human cell lines, our next goal was to test the potential of tCUbE to profile E2 (UbcH5a) activity in a cell-based proteomics workflow. The E2 UbcH5a is known for its broad substrate scope, making it a popular choice for in vitro ubiquitination experiments. However, its endogenous selectivity remains unknown. To profile UbcH5a–mediated ubiquitination in a proof-of-concept experiment, paired tCUbE assays were performed in HEK-293T cells that were co-transfected with Ub*^{DUBr} and either E2^{+} (UbcH5a) or E2^{+cat} (catalytically inactive UbcH5a). The resulting Ub*-conjugates were isolated by immunoprecipitation against HA and were subjected to tryptic digest prior to tandem mass spectrometry analysis. We identified 20 proteins with an average of $\geq$two-fold enrichment when comparing spectral counts for tCUbE samples generated with active versus inactive UbcH5a (Figure S10). For all targets except TRIM25, this study provides the first evidence that UbcH5a contributes to their ubiquitination in human cells.[15]

Included on the list of hits were both major HSP90 isoforms, HECT and RING E3s, a DUB (USP9X), and several ribosomal proteins. A subset was validated following tCUbE in HEK-293T cells and subsequent immunoprecipitation against HA. The enriched samples were analyzed by western blot, probing for the selected targets, including BRUCE, HERC2, TRIM25, HSP90, and USP9X (Figure 4A,B). Using this approach, each of the selected targets were found to be immunoprecipitated by HA only when the active UbcH5a E2^{+} was present during tCUbE. We also found that one of the hits–HSP90–could not be immunoprecipitated by HA when HA–Ub, which lacks tCUbE targeting and splicing domains, was used in place of Ub*^{DUBr}, providing additional support for the specificity of tCUbE (Figure S11). Together, these findings suggest that tCUbE is a viable method to identify UPS targets.

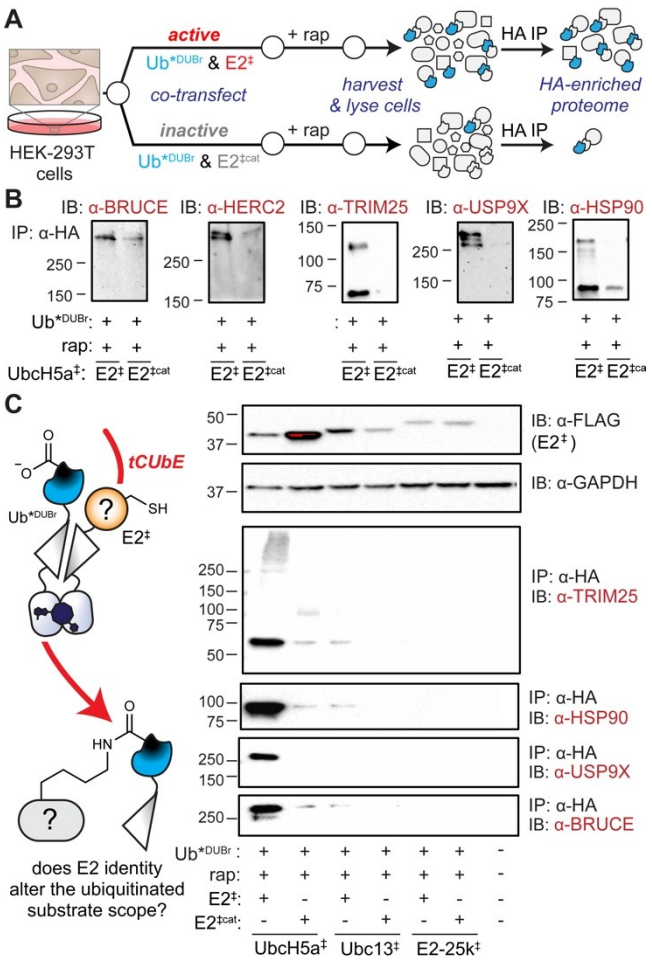

Figure 4. tCUbE reveals specific targets resulting from activity of a particular E2. (A) To validate hits suggested by a proof-of-concept proteomics experiment, tCUbE samples were enriched by immunoprecipitation against HA; these samples were then analyzed by western blot, probing against the indicated hit protein of interest. (B) Bands observed for active E2^{+} only, or the appearance of a high molecular weight band with active E2^{+} demonstrate that the substrate is ubiquitinated by UbcH5a. (C) To further validate if the proteins identified are specific to the E2 of choice, tCUbE was performed across three different pairs of active and inactive E2^{+}'s, including UbcH5a^{+}, Ubc13^{+} and E2-25K^{+}. Enriched samples after immunoprecipitation against HA were analyzed by western blot, probing for the proteins of interest across all three E2s and untransfected cells. Clear and intense bands were observed for active UbcH5a only confirming the specificity of tCUbE variant towards ubiquitination of the targets identified from proteomics analysis performed for UbcH5a. Uncropped blot images for Figure 4B and C are shown in Supporting Information under the Supporting Figures section.

Given our success in using tCUbE to profile UbcH5a substrates, we next sought to expand the methodology to other E2s. We prepared three additional E2^{+} variants, including catalytically active or inactive versions of Ubc13, E2-25K, and Rad6B. We found that, like UbcH5a, these E2s led to a prominent increase in higher molecular weight HA signal that was only observed when the active E2^{+}s were treated with rapalog (Figure S12, Figure S13). While UbcH5a is known for its broad substrate scope in vitro, these

other E2 s are known for more specific roles..[4a,16] Thus, we next sought to assess the specificity of tCUbE by searching for validated UbcH5a^{+} hits when tCUbE was accomplished with other E2^{+}s. To do so, we performed tCUbE with 3 different pairs of active and inactive E2^{+}'s (UbcH5a^{+}, Ubc13^{+} and E2-25K^{+}). After immunoprecipitation against HA, the enriched samples were then analyzed by western blot, probing against selected targets, including TRIM25, HSP90, USP9X and BRUCE. This experiment not only confirmed that these proteins are indeed targets of UbcH5a activity in HEK-293T cells, but also revealed that they are not targets for other E2s, like Ubc13 and E2-25K (Figure 4C). These results further demonstrate that tCUbE can reliably report on ubiquitination events that are dependent on a specific E2.

Having established the compatibility of tCUbE with proteomics-based target identification workflows, our next goal was to evaluate the rapalog-dependent enrichment of specific substrates during tCUbE using label-free quantitative proteomics. To do so, we performed tCUbE assays in HEK-293T cells co-transfected with Ub*^{DUBr} and either E2^{+} (UbcH5a) or E2^{+cat} (catalytically inactive UbcH5a), both with and without rapalog treatment. After enrichment for Ub*^{DUBr}-containing conjugates using HA immunoprecipitation, the resulting samples were analyzed using bottom-up proteomics (Figure 5). Label-free quantitative analysis evaluating fold-change differences revealed statistically significant enrichment of discrete sets of cellular proteins when comparing across samples (Figure 5A,B). We identified 227 high-confidence candidates across all four conditions. Nearly half (49.3 %) were discovered when comparing active (E2^{+}) to inactive (E2^{+cat}) tCUbE, both with rapalog induction (sample A and X, respectively) (Figure 5B,C; Figure S14).

Of the 180 unique proteins that were enriched across all conditions, 78 (~43.3 %) were specific to the profile of active (E2^{+}) vs. inactive (E2^{+cat}) tCUbE upon rapalog induction (Figure 5C; Figure S14). Additionally, only 4 unique proteins were enriched when comparing active (E2^{+}) tCUbE with or without rapalog (sample A or B, respectively). There was also only minimal overlap (11 proteins) of the rapalog-treated active (E2^{+}) vs. inactive (E2^{+cat}) set and the active (E2^{+}) rapalog treated vs. untreated set. This suggests that background hits due to rapalog-mediated association are minimal, and that most tCUbE hits report on *bona fide* UbcH5a activity.

To further evaluate the UbcH5a tCUbE profile, we generated a heatmap comparing significantly enriched hits denoted by π-score,[17] which revealed candidate proteins specific to active E2 upon rapalog induction (Figure 5D, Figure S14). Several UPS-related proteins are among this list, including phospholipase A2 activating protein (PLAP), ubiquitin carboxyl-terminal hydrolase 17 like protein (U17 L3), a putative polycomb group protein (ASXL1), and SUMO-activating enzyme subunit-1 (SAE1).Further inspection of the extracted ion chromatograms (EICs) for unique peptides specific to these hits showed clear elevated abundances for tCUbE performed with active E2^{+} and rapalog treatment (Figure 5E). Similarly, hits identified from our pilot study, including the E3 ligase TRIM25 (TRI25) and heat shock protein HSP90 A (HS90 A), also exhibited elevated abundances in the rapalog-treated, active E2^{+} tCUbE samples (Figure 5E, Figure S14). The quantitative ratio (estimated by $\log_2$FC) of peptides identified from these protein hits showed higher enrichment in cells with active E2^{+} and rapalog induction, though they fell below the threshold used to establish hits in our follow-up study. Taken together, these data indicate that tCUbE will become a powerful tool for broadly profiling E2 activity.

Here we have established that tCUbE is a viable, general, and useful method that can be used to profile E2-dependent effects on ubiquitination in living cells. tCUbE makes use of engineered protein constructs and standard cell culture techniques and does not require the chemical and/or semisynthesis of engineered E2~Ub thioesters or ubiquitin probes, making it straightforward to apply for researchers across many disciplines. While there are other strategies that have been used to provide information about E3-substrate interactions,[18] there are very few that can report on E2 activity. Indeed, methods that can be used to reveal E2 function typically fall short of being able to label substrates[7a–d] and many cannot capture RING E3 ligases..[7b–e] Thus, the major strength of tCUbE is that it offers a significant advance by reporting on the full complement of E2 activity within a living cell.

Still, a few aspects of the tCUbE design warrant further investigation. Additional studies are needed to optimize the extent of *trans*-splicing that may be a limiting feature for tCUbE efficiency in the current design. Similarly, more studies are needed to determine both the binding affinities and dissociation kinetics for the post-splicing split intein domains, which could have important implications for the future refinement and optimization of tCUbE. Additionally, more investigation is required to fully disentangle the influence of the remaining intein 'scar' on Ub*–a necessary byproduct of tCUbE–on the resulting ubiquitin conjugation multiplicity and/or pattern.

In summary, we have demonstrated that tCUbE can be applied to different E2s and in multiple human cell lines. We established that tCUbE can reveal interactions with multiple classes of E3 ligases, including RING E3 ligases that facilitate ubiquitin transfer indirectly and are often overlooked by other methods.[7b–d] In our future work, we plan to apply tCUbE for use in quantitative proteomic workflows using isobaric tags, along with standard protein synthesis, proteasome, or deubiquitinase inhibitors. This, combined with the use of standard and deubiquitinase-resistant Ub* variants, will offer an excellent platform for profiling the UPS, including deubiquitinase activity. We also plan to use tCUbE to create a complete map of the UPS by profiling the substrates of each of the 40 E2s that have so far been characterized. Thus, we anticipate that tCUbE is uniquely poised to offer the most nuanced picture of the UPS to date.

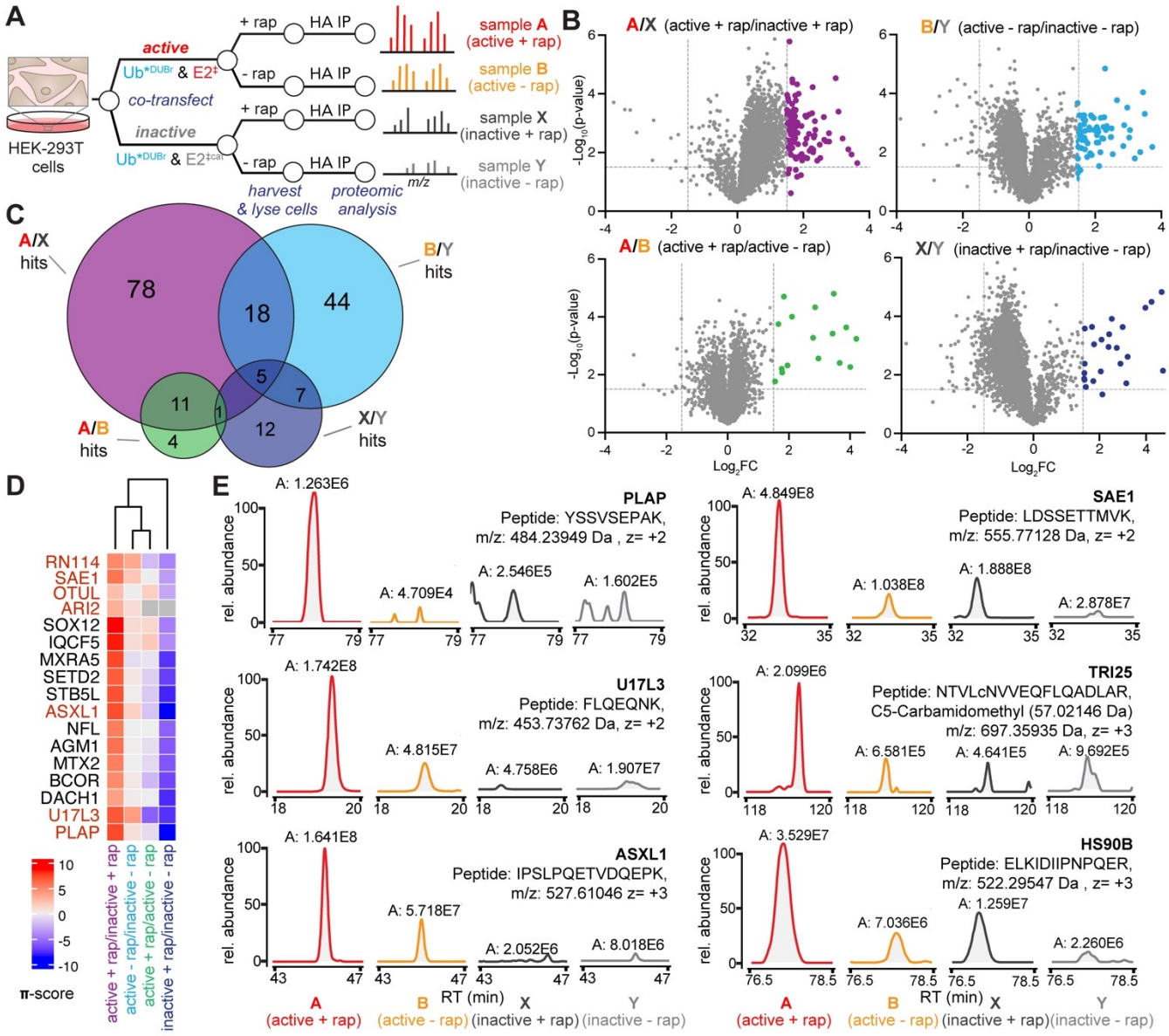

Figure 5. Label-free quantification of E2-specific targets identified by tCUbE. (A) Cell-based tCUbE-MS workflow showing HEK-293T cells co-transfected with Ub*^{DUBr} and either E2‡ (active UbcH5a) or E2$^{\ddagger cat}$ (catalytically inactive UbcH5a) in the presence or absence of rapalog, followed by label-free quantitative proteomic analysis. (B) Volcano plots showing enriched proteins (highlighted; log_2fold change (FC) $\geq$1.5 and -log_{10}p value $\geq$1.5), comparing tCUbE performed with active or inactive UbcH5a and with or without rapalog induction ($n=3$). Log_2FC ratios are plotted along the x-axis and-Log_{10} p-values are plotted along y-axis. (C) Venn diagram depicting the discrete sets of identified proteins enriched in tCUbE. The largest number (43.3%) of non-overlapping hits were observed when comparing rapalog-treated active vs. inactive UbcH5a samples (purple). (D) A heatmap quantitatively comparing the high-confidence hits across all four conditions revealed candidate proteins that are specific to tCUbE (missing values = grey). (E) Extracted ion chromatograms (EICs) of specific precursor ions attributed to the indicated hits show elevated abundances ("A") in tCUbE samples from rapalog-treated cells expressing active UbcH5a.

Acknowledgements

This work was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number R01GM134097. We gratefully acknowledge J. Kritzer and members of the Scheck lab for helpful comments during the preparation of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are openly available in MassIVE at https://massive.ucsd.edu, reference number MSV000093101.

Keywords: ubiquitin proteasome system · post-translational modifications · protein profiling · conditional protein splicing · chemical methods

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Manuscript received: December 18, 2023
Accepted manuscript online: January 30, 2024
Version of record online: February 19, 2024