Conditional degron tags (CDTs) are a powerful tool for target validation that combines the kinetics and reversible action of pharmacological agents with the generalizability of genetic manipulation. However, successful design of a CDT fusion protein often requires a prolonged, ad hoc cycle of construct design, failure, and re-design. To address this limitation, we report here a system to rapidly compare the activity of five unique CDTs: AID/AID2, IKZF3d, dTAG, HaloTag, and SMASH. We demonstrate the utility of this system against 16 unique protein targets. We find that expression and degradation are highly dependent on the specific CDT, the construct design, and the target. None of the CDTs leads to efficient expression and/or degradation across all targets; however, our systematic approach enables the identification of at least one optimal CDT fusion for each target. To enable the adoption of CDT strategies more broadly, we have made these reagents, and a detailed protocol, available as a community resource.

A major focus in biomedical research is the discovery of novel therapeutic targets. Many targets arise from genome-wide association studies or large-scale functional experiments and are often poorly characterized, bringing exciting opportunities and concomitant challenges. When the underlying biological functions of a novel protein of interest (POI) are unknown, the consequences of its perturbation are also unpredictable. These unknowns are relevant for estimating both therapeutic efficacy and potential normal tissue toxicity. Unfortunately, the generation of tool compounds to enable proof-of-concept efficacy and safety studies requires significant time and resources and such compounds are not available early in target validation studies, precisely when the need to address these questions is most important.

Genetic suppression strategies, including CRISPR/Cas9 and shRNA are valuable and generalizable tools for target validation; however, these modalities suffer from off-target effects (especially for shRNA), slow mechanisms of action, irreversible perturbation of the POI (CRISPR), and an inability to titrate the extent of POI inhibition. As such, these technologies often do not recapitulate the phenotypic consequences seen with a small molecule or biologic inhibitor. To address these concerns, chemical genetic systems for post-translational protein control have been developed, which we refer to as conditional degron tags (CDTs). These systems employ protein tags rendering a fused-protein partner sensitive to tunable protein degradation upon treatment with a “degrader drug.” Importantly, CDTs can impart rapid degradation kinetics, reversible recovery of protein levels after drug removal, and orthogonal and/or known off-target effects of the degrader drug.

Although CDTs show promise as powerful tools for early target validation, their generalizability across many POIs has not been...
evaluated systematically. Anecdotally, we have found highly variable results when applying these different CDTs to cancer-relevant targets, which significantly impacted our ability to use CDTs for our studies. Two possibilities are presented: either certain CDTs can be broadly used against various POI with robust activity and should be prioritized, or each POI requires a unique strategy, thus requiring a systematic assessment of multiple CDT strategies.

Here, we evaluate the robustness and generalizability of five different CDTs fused to either the N- or C-terminus of 16 different POIs and expressed as V5 fusions under a strong (SFFV) or a weak (PGK) promoter. Importantly, we find that none of the CDTs analyzed performed optimally across all targets. Instead, we find that expression levels and drug-induced degradation varied widely and in an unpredictable manner. Still, our systematic analysis of different CDT fusions allows the identification of potently degraded CDTs for each POI. Finally, we compare multiple CDTs in functional assays for two POIs, and in each case, we identify at least one CDT fusion protein that phenocopied both expression of the wild-type protein and its genetic inactivation. Together, these results indicate that parallel testing of multiple CDTs enables the rapid and successful development of POI-CDT fusions for target validation studies.

**Results**

**Generation of a systematic CDT panel of lentiviral vectors** We focused on five CDTs (Fig. 1a): auxin-inducible degron (AID)8,9, dTAG10,11, IKZF3 degron (aa130–189, IKZF3d)12,13, HaloTag14,15, and small molecule-assisted shut-off (SMASh)16. Except for the SMASh tag, each CDT achieves degradation by “reprogramming” an E3 ubiquitin ligase with a small molecule to recognize the CDT-tagged protein. In contrast, SMASh-tagged fusion proteins are processed by the proteolytic self-cleavage of a degron to yield the untagged, wild-type POI; here, treatment with protease inhibitors allows the expression of the degron-fused POI—which is rapidly degraded—and the gradual disappearance of the untagged POI. We generated a panel of 20 lentiviral

| Conditional Degron Tag (CDT) | Size | Mechanism | Drug and top dose tested | Ref. |
|-------------------------------|------|-----------|---------------------------|------|
| Auxin Inducible Degron (AID) | 7 kDa | Molecular glue for the Tir1 E3 ligase | Indole-3-acetic acid (IAA, 500 μM) | 8, 9 |
| dTAG                          | 12 kDa | PROTAC for the CRBN or VHL E3 ligases | dTAG13 or dTAG V1 (1 μM) | 10, 11 |
| IKZF3d                        | 7 kDa | Molecular glue for the CRBN E3 ligase | Pomalidomide (Pom, 1 μM) | 12, 13 |
| HaloTag                       | 34 kDa | PROTAC for the VHL E3 ligase | HaloPROTAC3 (1 μM) | 14, 15 |
| SMASh                         | 7* kDa | Prevents self-cleavage of unstable degron | Asunaprevir (1 μM) | 16 |

Fig. 1 | A vector panel to systematically evaluate conditional degron tag (CDT) fusion protein expression, degradation, and function. a Summary table of the five CDTs evaluated in this work. Each CDT, except for small-molecule-assisted shut-off (SMASh), functions through reprogramming an E3 ubiquitin ligase with a small molecule to recognize the degron fusion protein. SMASh fusion proteins self-cleave a degron tag; the addition of the small molecule blocks this self-cleave. The SMASh tag itself is 34 kDa, but after self-cleave, the stably-expressed (V5) tag is only 7 kDa. b Plasmid design for systematically evaluating CDT fusions to a protein of interest (POI). We employed a P2A cleavage site to separate an antibiotic-resistance gene (PuroR) and the fusion protein. A V5 epitope tag enables detection and quantitation of the fusion protein by western blot. A rigid linker (EAAK₅, Link) was also incorporated between the degron tag and the cloning site in all constructs except AID and SMASH, where the CDT is separated from the POI by the V5 tag. These expression cassettes were cloned into a lentiviral vector for stable integration into the genome. c Validation of the CDT panel using NanoLuciferase. Nano-Luciferase fused to the indicated CDT was expressed using the PGK or SFFV plasmids. Here, expression levels are reported as the anti-V5 IB band intensity normalized to the anti-Vinculin band intensity and then scaled to the total expression of all ten CDTs evaluated to highlight the relative expression differences between the different CDTs. Degradation is reported as the percent reduction in the anti-V5 IB band intensity after drug treatment. See Supplementary Fig. 2a for the full IB for both PGK and SFFV constructs.
vectors to enable testing any POI with these different CDTs fused at either terminus, expressed under the control of a weak (PGK) or strong (SFFV) promoter (Fig. 1b). We hypothesized that the resulting panel of expression vectors would enable the more rapid discovery of at least one degradable construct for any given POI.

To validate the vector design, we cloned NanoLuciferase (NanoLuc) into each vector in-frame with the CDT. Polyclonal HEK-293T cells stably expressing each NanoLuc-CDT construct were generated via lentiviral transduction under conditions favoring single integration to allow for the comparison of expression levels. To assess AID tags, we co-expressed the Oryza sativa E3 ubiquitin ligase TIR1, as reported previously. To efficiently assess degradation across different doses (as previously reported), we used 10 nM to 1 μM for all drugs except indole-3-acetic acid (IAA), which was tested at 5 to 500 μM and time points (6–72 h), we developed an anti-V5 in-cell western (ICW, Supplementary Fig. 1a–d). Except for minor viability defects observed for HaloPROTA C3 at 1 μM, these doses were not toxic to HEK-293T cells (Supplementary Fig. 1a). We evaluated the baseline expression of each NanoLuc-CDT fusion via anti-V5 immunoblot (IB, Fig. 1c and Supplementary Fig. 2a). We noted different expression levels for each construct and generally higher expression from the SFFV promoter (e.g., IKZF3d, and SMASH generally provide the best dynamic range between baseline expression levels and efficiency of degradation across 12 (75%) of the targets analyzed (Fig. 2c, d). Although IKZF3d constructs were degraded robustly across the same number of targets, the poor expression of IKZF3d fusion proteins decreases the overall dynamic range. The HaloTag system enabled degradation for a smaller number of targets, and, as noted above, only a few targets were degraded efficiently by the AID/AID2 systems. Nevertheless, several POI were only efficiently degraded by these less robust technologies (e.g., XPR1 with AID2-C). Notably, while none of the CDTs tested worked across all targets, different CDTs performed in this regard, we compared the kinetics of degradation for each CDT across multiple targets. While each CDT has its own particular strengths and weaknesses, we consistently identify at least one CDT fusion construct for each POI that was efficiently expressed and degraded (>95%) in the presence of the relative degrader drug (Fig. 2c, d).

Kinetics of degradation and recovery across CDT fusion proteins

A key feature of CDTs is the ability to modulate the expression of a given target with fast and reversible kinetics. To determine how different CDTs performed in this regard, we compared the kinetics of degradation for each CDT across multiple targets. While each CDT has an exemplary POI with complete degradation after 24 h of drug exposure, the specific POI had a large effect on the kinetics of degradation (Fig. 3 and Supplementary Fig. 9, 10). For example, GFP and RFluc fusion proteins were degraded rapidly by most CDTs within 6 h of treatment, while maximal degradation of the transmembrane protein XPR1 was only observed after 48 h (e.g., XPR1-dTAG, Fig. 3b).

These findings suggest that certain substrates might have fast re-synthesis rates that must be overcome by the optimal CDT, engage more slowly with the degradation machinery, and/or require additional processing before protein levels decrease (e.g., extraction from the plasma membrane). In general, we observed slower kinetics of degradation for the SMASH tag fusion proteins, consistent with its
unique mechanism of action (Supplementary Figs 9, 10). Between different targets, the kinetics ranged from clearance of both the untagged POI and the degron-tagged POI within 24 h (VPS4A, Supplementary Fig. 10c) to 5 days (PRMT5 and WSB2, Supplementary Fig. 10b, d) to retention of the degron-tagged POI for up to 10 days (MCL1-SMASh-C, Supplementary Fig. 9d).

We next evaluated the reversibility of each technology by performing drug washout experiments for the NanoLuc-, RFLuc-, and PRMT5-CDTs fusions (Fig. 3c and Supplementary Fig. 10e, f). Twenty-four hours after drug treatment, media were replaced with drug-free media and protein levels were monitored for up to 96 h. Except for NanoLuc-dTAG-C, the levels of most CDT fusion proteins recovered within 24 h. Altogether, these results indicate that the kinetics of degradation and recovery are highly variable, unpredictable, and dependent both on the CDT and the POI analyzed and thus, should be determined empirically for each POI fusion.
Finally, we assessed the expression and induced degradation in multiple cellular contexts and determined the degree to which a CDT fusion retains the physiological activity of the POI. We observed that for PRMT5-CDT fusion proteins, most cell lines showed similar patterns of expression and degradation (Supplementary Fig. 11a). However, XPR1-CDT fusion proteins were more variable across different cell lines (Fig. 4a and Supplementary Fig. 11b, c). For example, XPR1-dTAG-N fusion proteins were expressed and degraded in HEK-293T and SNGM cell lines, but barely detectable and not degraded in IGROV1 (Fig. 4a and Supplementary Fig. 11b), possibly due to the important role of XPR1 in maintaining the viability of this cell line.29, or

Functional assessment of XPR1- and MCL1-CDTs

However, XPR1-CDT fusion proteins were more variable across different cell lines (Fig. 4a and Supplementary Fig. 11b, c). For example, XPR1-dTAG-N fusion proteins were expressed and degraded in HEK-293T and SNGM cell lines, but barely detectable and not degraded in IGROV1 (Fig. 4a and Supplementary Fig. 11b), possibly due to the important role of XPR1 in maintaining the viability of this cell line.29, or
to differences in protein homeostasis mechanisms across cellular contexts. We also assessed the performance of each CDT in murine contexts using the NIH-3T3 cell line. As previously reported, the IKZF3d system was inefficiently degraded in this context while each other CDT maintained the performance observed in HEK-293T (Supplementary Fig. 11d).

Consistent with prior studies, we found that each CDT technology did not impair the enzymatic activity of RFluc (Supplementary Fig. 12a and refs. 9, 10, 12, 15, 16, 35–38). Thus, we evaluated the ability of two CDT fusion proteins—XPR1 and MCL1—to phenocopy both full activity at baseline and its loss of function upon drug treatment.

The activity of XPR1, the only annotated cellular phosphate exporter in the human genome29,30, can be assessed using 32P-labeled phosphate pulse-chase experiments to quantify cellular phosphate export. XPR1 inactivation (XPR1-KO) significantly decreases cellular phosphate export (Fig. 4b and Supplementary Fig. 12b–d). This phenotype can be rescued by re-expression of wild-type XPR1 (XPR1-WT) but not by hypomorphic alleles (e.g., XPR1 with the L21SS mutation)39. With this robust model in hand, we tested the re-expression of five CDT constructs in an XPR1-KO background. Here, we found that all CDT constructs fully restored phosphate efflux activity, often at higher levels relative to XPR1-WT, likely due to high expression (Supplementary Figs. 5b, 12c). Importantly, phosphate efflux activity was significantly decreased in each CDT fusion after treatment with the drug, with HaloTag-N displaying the largest dynamic range and fully recapitulating the XPR1-KO phenotype (Fig. 4b).

As a second exemplary case, we tested the ability of CDT fusion proteins to phenocopy the activity of the antiapoptotic protein MCL1. MCL1 plays a key role in suppressing the activation of the intrinsic apoptotic cascade through its interactions with BH3 proapoptotic proteins (e.g., BIM, PUMA, or NOXA). Genetic inactivation of MCL1 (MCL1 KO) sensitizes cells to the BCL-2, BCL-xL, and BCL-W inhibitor Navitoclax40. We tested two MCL1-CDT fusions for their ability to protect cells against Navitoclax treatment—phenocopying endogenous MCL1—and to sensitize cells to Navitoclax upon degradation—phenocopying MCL1 inactivation. Expression of MCL1-SMASh-N failed to rescue the MCL1 KO phenotype when expressed from a weak (PGK) promoter (Fig. 4c, d) but provided a large dynamic range of Navitoclax sensitivity between DMSO and Asunaprevir treatment when expressed from a strong (SFFV) promoter. In contrast, the expression of MCL1-dTAG-C from the stronger promoter impeded complete degradation and cells retained resistance to Navitoclax (i.e. MCL1 activity) even upon s treatment (Fig. 4c, d). However, when expressed at lower levels by the weaker promoter, there was a profound difference in Navitoclax sensitivity between DMSO and dTAG−1 treatment. These results highlight the importance of carefully evaluating expression and functionality levels for each CDT fusion to yield a useful tool for further target validation experiments.

Discussion

There is substantial interest in the scientific community to leverage CDTs for target validation studies, and multiple potent CDTs have been developed over the past few years. Their applicability, however, remains complicated by the fact that CDTs do not work consistently across protein targets. In our experience, each target requires iterative and lengthy cycles of cloning and testing to identify an ideal CDT strategy that leads to sufficient activity and degradation of the CDT-POI fusion to phenocopy the action of a drug.

Here, we asked whether one CDT platform typically outperforms the others, or if testing multiple strategies is necessary to develop a functional CDT. To address this question, we generated a panel of lentiviral vectors to systematically compare the efficiency of five unique CDTs fused to either the N- or C-terminus of 16 unique POIs representing different protein classes. We found that total protein expression and drug-induced degradation was highly dependent on both the technology used and the specific construct design on a target-by-target basis. In comparing efficiencies across CDTs (Fig. 2b), we noticed that the most consistently degraded CDTs were the dTAG, IKZF3d, and SMASH systems. However, it should be noted that dTAG fusions were often expressed at higher levels than SMASH and IKZF3d fusions (Supplementary Fig. 7a). This finding is important as the levels of the CDT fusion can matter both in terms of its degradation potency and in terms of its activity—that is, the ability of the CDT fusion protein to phenocopy the function of the endogenous POI. In addition, having good baseline expression with >95% degradation upon treatment provides a larger dynamic range to study POI activity, which makes for a more robust tool.

Notably, although dTAG and IKZF3d systems generally performed best, certain targets could only be degraded efficiently by other CDTs. For example, WSB2 and MCL1 were degraded efficiently (>95%) only by the SMASH-N CDT, whereas the phosphate exporter XPR1, XPR1-HaloTag-N was the only CDT fusion that could be sufficiently degraded to phenocopy XPR1 inactivation. Interestingly, AID fusions were expressed at lower levels and degraded less efficiently than other CDTs across almost all POIs, with marginal improvements using the AID2 system.

We extended our studies to the kinetics of degradation of the various CDTs. Apart from the SMASH fusions, which appeared to have slower kinetics across targets, other CDT fusions showed great variability on a target-dependent basis. It is important to note that in the SMASH system, the removal of the POI is dependent on two variables (1) the half-life of the POI (which will determine how quickly the untreated, cleaved form will disappear) and (2) the kinetics of degradation of the degron-tagged POI, a much higher molecular weight protein with unknown functional properties, which we found to often have very long and/or incomplete kinetics of degradation (Supplementary Figs. 9, 10). Overall, we observed fast kinetics of recovery across CDT fusions, further supporting the notion that CDTs are powerful systems for cell-based assessment of conditional and reversible removal of any given protein.

Finally, we illustrate the importance of assessing the functional activity of POI-CDT fusions when employing a CDT strategy. Certain tags may not be amenable to particular cellular contexts: for example, IKZF3d tags are not degraded efficiently in murine models (Supplementary Fig. 11d) and the degrader drug—pomalidomide—degrades additional targets beyond just the tagged-POI41. In addition, the AID tag additionally requires co-expression of the TIRI E3 ligase. These considerations can be predicted a priori, but the optimal CDT for ensuring a large dynamic range of POI activity is more difficult to predict. We present two case studies—XPI and MCL1—to show how the CDT fusion strategy could be hampered if the activity or expression levels are insufficient to phenocopy those of the endogenous protein, or if

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degradation is insufficient to phenocopy loss of the protein (Fig. 5a). While our studies were in the context of exogenous overexpression, we expect that insertion of a CDT at the endogenous locus for a given gene will likely have similar impacts on the expression of the gene product, although endogenous regulation may lessen the dramatic differences in expression that we observed. Indeed, here too, assessing multiple CDT fusions in parallel ensures higher chances of success.

Taken together, our data help to inform the development of a CDT strategy for target validation studies (Fig. 5b). We provide here a lentiviral vector system and a detailed Standard Operating Protocol (Supplementary Note 1) to quickly generate and test multiple CDTs in parallel to facilitate this process and highlight both successful and unsuccessful examples, the latter of which often go unpublished. Up-front testing of the five CDTs presented here enables the efficient identification of properly expressed and

Fig. 4 | Validation of functional activity for XPR1- and MCL1-CDT fusion proteins. a Comparison of expression levels for XPR1 degron fusion proteins in various cell lines. Expression was determined by quantifying the western blot V5 band intensity and normalizing it to a Vinculin loading control. b Phosphate efflux activity of XPR1 degron fusion proteins. 293T cells endogenously express XPR1, which was inactivated using CRISPR/Cas9 (XPR1-KO) followed by re-expression of wild-type XPR1 (WT), a hypomorphic allele (L218S), or the indicated SFFV-driven degron fusion proteins. Three days after the addition of degrader drug (1 μM Pomalidomide, 1 μM dTAGV−1, or 1 μM HaloPROTAC3), phosphate efflux was measured by “loading cells” for 45 min with 32PO4−3, washing away any extracellular 32P, and then incubating the cells for 60 min and measuring the percentage of 32P in the conditioned medium compared to cellular lysates. The bar height represents the mean of technical triplicates (shown as points), and the results are representative of two independent experiments. c MCL1-CDT fusions are expressed and degraded in A375 cells. Cells expressing the indicated MCL1-CDT proteins were treated with 1 μM Asunaprevir or 1 μMd TAGV−1 prior to evaluating protein levels by western blot. d Evaluation of MCL1 degron fusions to protect cells from Navitoclax-induced cell death. Endogenous MCL1 was inactivated in A375 cells expressing the MCL1-CDT fusions shown in e and then pretreated with the indicated degrader drugs for 5 days (Asunaprevir) or 1 day (dTAGV−1). At “time 0”, the cells were treated with 625 nM Navitoclax and cell growth was evaluated with live-cell imaging, and confluency was evaluated through image analysis. Error bars represent the mean of N = 3 technical replicates and are representative of N = 2 independent experiments.
degraded CDT fusions without burdensome and time-intensive iterations. If resources are limited, our work suggests the following ranking, from most to least robust: dTAG, SMASH, IKZF3d, HaloTag, and AID/AID2. This ranking takes into consideration degradation efficiency, baseline expression, absence of additional protein products (commonly seen with HaloTag-N), bio-orthogonality, and kinetics of degradation.

Future work will focus on developing CDT technologies for in vivo work. In Supplementary Table 1, we summarize prior pharmacokinetic (PK) studies for some of the small molecules analyzed here (dTAG-13, dTAG-1–4, Pomalidomide, and Asunaprevir). We could not find similar data for the 5-Ph-IAA or HaloPROTAC3, although these compounds have been previously used in vivo. Indeed, as a systematic approach to developing CDT fusions for target validation studies, a proposed timeline for evaluating the expression, degradation, and function of CDT fusions. Although 20 constructs can all be tested in parallel using the vectors and protocols presented here, priority can be given to particular CDTs if required (see Discussion). In most cases, a degraded and functionally relevant CDT fusion protein can be developed within 11 weeks. *Construct cloning via Contract Research Organization; **Functional testing is highly target-dependent and 3 weeks is an estimation.

**Methods**

**Statistics and reproducibility**

Details on statistical tests used in this study are contained in the methods for the relevant experiments. Statistical significance was calculated based on the distribution of biological replicates as opposed to technical or experimental replicates. All experiments were repeated at least three times to ensure reproducibility. No statistical method was used to predetermine the sample size. No data were excluded from the analyses. The experiments were not randomized. The Investigators were not blinded to allocation during experiments and outcome assessment.

**Construct design and cloning**

All vectors included a p6K or an SFFV promoter driving the expression of a puromycin selection cassette, a p2a self-cleaving peptide, and the specific CDT (as shown in Fig. 1b). A rigid linker separated the CDT from the POI in the dTAG, the IKZF3d, and the HaloTag constructs. The HaloTag sequence was obtained from Craig Crews’s lab. The SMASHTag ORF was obtained from Addgene. The dTAG ORF was obtained from Nathaniel Gray’s lab. The IKZF3 minidegron sequence was obtained from Ben Ebert’s lab. The AID and TIR1 ORF were obtained from Dr Johannes Zuber. A BamHI unique cloning site was included at the N or C terminal of the V5 and sequences. All constructs were generated at Epoch Life Sciences and validated in-house by cloning the NanoLuc ORF. All constructs and vector maps are available on Addgene (185760-185779). The deposited vectors express GFP-CDT fusion; the GFP insert can be excised via EcoRI/BamHI digestion for directional cloning of different ORFs.

**Compounds**

The compounds used in this study can be found in Table 1. dTAG-1 was either purchased commercially or synthesized in-house (see Supplementary Note 2). In a head-to-head comparison, there was no difference in the activity of dTAG-1 from these different sources.
Table 1 | Compounds used in this study

| Compound            | Supplier         | Cat#  |
|---------------------|------------------|-------|
| dTAG-13             | Tocris           | 6605  |
| dTAG-1              | Tocris           | 6914  |
| 3-Indoleacetic acid (IAA) | Sigma-Aldrich       | 12886-5 G |
| 5-Phenyl IAA        | MedChemExpress   | HY-134653 |
| Asunaprevir         | Ambeed Inc.      | A542126 |
| Pomaldiomide        | Tocris           | 6302  |
| HaloPROTAC3         | Promega          | CS2072A01 |
| Navitoclax          | Selleck Chemicals| S1001 |

Table 2 | Antibodies used in this study

| Antigen     | Supplier       | Cat#          | WB Dilution |
|-------------|----------------|---------------|-------------|
| V5          | Cell Signaling | D3H8Q         | 1:200 (ICW) 1:2000 (WB) |
| CellTag 800 | LI-COR         | #926-41090    | 1:500 (ICW)  |
| Anti-Mouse 780 | LI-COR       | #926-68070    | 1:1000 (ICW) 1:50000 (WB) |
| Anti-Rabbit 800 | LI-COR      | #926-32211    | 1:1000 (ICW) 1:5000 (WB) |
| Vinculin    | Sigma          | V9131         | 1:5000 (WB) |
| KIDINS220   | Protein Tech   | 21856-1-AP    | 1:2000 (WB) |
| XPR1        | Sigma          | HPA016557     | 1:2000 (WB) |

Stable cell line generation
In total, 1 × 10^6 cells were plated per well of a 24-well plate. About 200 μl degron virus was added to each well with polybrene (Santa Cruz Biotechnology) to a final concentration of 8 μg/mL and cells were returned to the incubator. 24 h after infection, the cells were selected with the relevant antibiotic.

sgRNA
MCL1 – 5’ AGGCCGCTTGGAGACCTTACGA 3’
XPR1 – 5’ TCTGCAGCAGATTAGACTG 3’

Immunoblotting (IB)
Cells were collected and lysed in RIPA buffer supplemented with protease and phosphatase inhibitors (Roche). Protein concentrations were quantified, and equal amounts of protein were diluted with sample buffer, boiled, and loaded on Bis-Tris gels (NuPAGE). Gels were dry-transferred to nitrocellulose membrane (iBlot system, Life Technologies) and then probed with the indicated antibodies diluted in Intercept blocking buffer (Li-COR) overnight. Bands were detected using a Li-COR Odyssey CLX instrument, and bands were quantified using Image Studio.

In-cell western (ICW)
About 20,000–40,000 cells were plated in 100 μL of growth media in black-walled 96-well plates with transparent bottom. Twenty-four hours later, a Tecan D300e Digital Dispenser was used to dispense drugs. To fix cells, 100 μl ice-cold methanol (Sigma-Aldrich) was slowly added to the side of the wells and the plates were incubated at room temperature for 10 min with gentle shaking. The methanol was removed, and the plates were washed five times with 200 μl of 0.1% NP40 buffer in PBS. About 50 μl of anti-V5 antibody was added, and the plates were then incubated overnight at 4 °C with gentle rocking. The next day, the plates were washed five times with 200 μl of 0.1% NP40 in PBS, then incubated for 1 h at room temperature in 50 μl of anti-Rabbit secondary and 50 μl of CellTag (Licor #926-41090). The plates were then washed three times with 200 μl 0.1% NP40 in PBS, the liquid was removed, and the plate was imaged on an Li-COR Odyssey imaging system. Quantification was performed using Image Studio.

Washout experiments
Cells were plated in a 10 cm dish and treated with the indicated compound. Twenty-four hours after treatment, cells were washed with PBS, trypsinized, and replated in a drug-free medium for the indicated times in six-well plates. POI-CDT levels were determined by IB.

In-cell luciferase assays
To determine luciferase activity of RFluc-CDT fusion proteins, cells growing in 96-well plates were treated with growth medium + 150 μg/mL 5′-Luciferin (Thermo Fisher) and luminescence was measured immediately (Envision Plate reader, Perkin Elmer). The culture medium was then replaced and cells were allowed to grow.

Transient transfection and degradation
Twenty-four hours after plating, HEK-293T cells were transiently transfected with differing amounts of the indicated vectors. Twenty-four hours later, the cells were replated, and 24 h after that, the cells were treated with DMSO or 1 μM dTAG-1. Twenty-four hours after treatment, PRMT5-CDT levels were assessed by IB.

XRBD protein purification
We kindly thank Jean Luc Battini for providing the sequence for the XRBD-mFc construct published previously29,30. The plasmid encoding XRBD (strain NZB) was synthesized and cloned into pcdNA3.4 by Thermo Fisher Scientific GENEART GmbH. XRBD protein was

All compounds were dissolved in DMSO and were typically used at a final DMSO concentration of <0.1%.

Antibodies
Table 2 contains the dilutions and catalog numbers of all antibodies used in this study.

Cell culture
HEK-293T was procured from ATCC (CRL-3216). All other cell lines were procured by the Cancer Cell Line Encyclopedia and distributed for our use. The original sources of the cell lines were ATCC (MIA-PACA2, CRL-1420; A375, CRL-1619; HCT116, CCL-247; and ES2, CRL-1978), MilliPore Sigma (IGROV1, SCC203), or the Japanese Cell Resources Bank (SNGM, IFO50313). Cell lines were grown according to the manufacturer’s instructions in Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies) supplemented with 10% FBS (HEK-293T, MIA-PACA2, A375, and HCT116) or RPMI 1640 supplemented with 10% FBS (HEK-293T, SNGM, IGROV1, and ES2). HEK-293T expressing pLX-TRC313-TIR1β were generated by the lentiviral introduction (see below) of the TIR1 open reading frame and selection with 300 μg/mL of hygromycin. AID2 fusions were assayed by Western blot analysis (SB). Six weeks after infection, the cells were selected with the relevant antibiotic.

Lentivirus production
In a 24-multiwell plate, 1 × 10^5 HEK-293T cells per well were plated in 0.5 mL of media. Twenty-four hours after plating, transfection mixtures were made containing 50 μl Opti-MEM (Life Technologies), 250 ng packaging plasmid (psPAX2), 250 ng CDT plasmid, 25 ng envelope plasmid (pMD2.G), and 1.5 μl TransIT-LT1 (Minus Bio). Transfection mixtures were incubated for 30 min at room temperature and added dropwise to cells. Twenty-four hours after transfection, the media was aspirated and replaced. Viral collections were then performed 24 and 48 h after media replacement.
expressed in CHO cells and purified using Protein A affinity chromatography by Thermo Fisher Scientific GENEART GmbH.

XRBD flow cytometry
Cells were lifted from culture vessels using TrypLE Express (Thermo Fisher cat#12604013) and then diluted in PBS + 2% FBS. About 300,000 cells were transferred to a U-bottom 96-well plate in 50 µL followed by the addition of 50 µL of a 100 mM XRBD staining solution. Cells were incubated at 37°C for 40 min, washed once, and then incubated with an anti-mouse secondary antibody conjugated to AlexaFluor488 (ThermoFisher cat A-11004). Cells were then incubated on ice for 40 min, washed four times in PBS + 2% FBS, and then analyzed on a CytoFlex LX instrument. At least 10,000 single cell events were recorded for each condition (see Supplementary Fig. 12b for representative gating strategy).

Phosphate uptake and efflux assays
To determine phosphate efflux, HEK-293T cells stably expressing the indicated CDT fusions were plated in poly-L-lysine coated 24-well plates and treated with the indicated compound for 72 h. Cells were first “pulsed” using “no phosphate” RPMI 1640 supplemented with 10 µc/mL 32P (Perkin Elmer NEX053001MC) and incubated at room temperature for 60 min. The cells were then washed with “no phosphate” RPMI1640. The efflux rate of phosphate efflux was then measured by incubating cells in high-phosphate RPMI (i.e., standard RPMI 1640) for another 45 min. The conditioned medium was collected, and the amount of 32P in the lysate and conditioned medium was measured via a scintillation counter. The extent of phosphate efflux was determined by dividing the 32P measured in the conditioned medium by the total 32P measured for that sample (in cell lysates and in the conditioned medium).

Viability assays to assess Navitoclax sensitivity with MCL1-CDTs
A375 cells were pretreated with 1µM Asunaprevir for 4 days prior to re-plating the cells in a 96-well plate (with continued exposure to Asunaprevir and co-treatment with 1µM dTAg$^\text{CDT}$). Twenty-four hours later, the cells were treated with either DMSO or 625 nM Navitoclax, as indicated. Cell confluency was then assessed using live-cell imaging every 4 h using an Essen Incucyte S3 Live-Cell Analysis Instrument.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All data associated with this manuscript are available in the Supplementary Information file or as Source Data, which is provided with this manuscript. All constructs and vector maps are available on Addgene (#185760–185779). Source data are provided with this paper.

References
1. Meyers, R. M. et al. Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells. Nat. Genet. 49, 1797–1784 (2017).
2. Dempster, J. M. et al. Extracting biological insights from the project Achilles genome-scale CRISPR screens in cancer cell lines. Preprint at bioRxiv https://doi.org/10.1101/702243 (2019).
3. Kaelin, W. G. Jr Common pitfalls in preclinical cancer target validation. Nat. Rev. Cancer 17, 425–440 (2017).
4. Arrowsmith, C. H. et al. The promise and peril of chemical probes. Nat. Chem. Biol. 11, 536–541 (2015).
5. Tsherniak, A. et al. Defining a cancer dependency map. Cell 170, 564–576.e16 (2017).
6. McDonald, E. R. 3rd et al. Project DRIVE: a compendium of cancer dependencies and synthetic lethal relationships uncovered by large-scale, deep RNAi screening. Cell 170, 577–592.e10 (2017).
7. Wu, T. et al. Targeted protein degradation as a powerful research tool in basic biology and drug target discovery. Nat. Struct. Mol. Biol. 27, 605–614 (2020).
8. Yesibolatova, A. et al. The auxin-inducible degron 2 technology provides sharp degradation control in yeast, mammalian cells, and mice. Nat. Commun. 11, 1–13 (2020).
9. Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, T. & Kanemaki, M. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. Nat. Methods 6, 917–922 (2009).
10. Nabet, B. et al. The dTAG system for immediate and target-specific protein degradation. Nat. Chem. Biol. 14, 431–441 (2018).
11. Nabet, B. et al. Rapid and direct control of target protein levels with VHL-recruiting dTAG molecules. Nat. Commun. 11, 4687 (2020).
12. Koduri, V. et al. Peptidic degron for IMiD-induced degradation of heterologous proteins. Proc. Natl Acad. Sci. USA 116, 2539–2544 (2019).
13. Sievers, Q. L. et al. Defining the human C2H2 zinc finger degrone targeted by thalidomide analogs through CRBN. Science 362, eaat0572 (2018).
14. Buckley, D. L. et al. HaloPROTACs: use of small molecule PROTACs to induce degradation of HaloTag fusion proteins. ACS Chem. Biol. 10, 1831–1837 (2015).
15. Tovell, H. et al. Rapid and reversible knockdown of endogenously tagged endosomal proteins via an optimized HaloPROTAC degrader. ACS Chem. Biol. 14, 882–892 (2019).
16. Chung, H. K. et al. Tunable and reversible drug control of protein production via a self-exciing degron. Nat. Chem. Biol. 11, 713–720 (2015).
17. England, C. G., Ehlerdng, E. B. & Cai, W. NanoLuc: a small luciferase is brightening up the field of bioluminescence. Bioconj. Chem. 27, 1175–1187 (2016).
18. Maguire, C. A., van der Mijn, J. C., Degeling, M. H., Morse, D. & Tannous, B. A. Codon-optimized Lucilia italica luciferase variants for mammalian gene expression in culture and in vivo. Mol. Imaging 11, 13–21 (2012).
19. Mavrikis, K. J. et al. Disordered methionine metabolism in MTAP/CDKN2A-deleted cancers leads to dependence on PRMT5. Science 351, 1208–1213 (2016).
20. Wei, G. et al. Chemical genomics identifies small-molecule MCL1 repressors and BCL-xL as a predictor of MCL1 dependency. Cancer Cell 21, 547–562 (2012).
21. Sulahian, R. et al. Synthetic lethal interaction of SHOC2 depletion with MEX inhibition in RAS-driven cancers. Cell Rep. 29, 118–134.e8 (2019).
22. Kwon, J. W. & Hahn, W. C. A leucine-rich repeat protein provides a scaffold for VHL-recruiting dTAG molecules. Nat. Commun. 11, 574–587 (2016).
23. Negrers, J. E. et al. Synthetic lethal interaction between the ESCRT paralog enzymes VPS4A and VPS4B in cancers harboring loss of chromosome 18q or 16q. Cell Rep. 33, 108493 (2020).
24. Meyer, C. et al. The TIA1 RNA-binding protein family regulates Eif2AK2-mediated stress response and cell cycle progression. Mol. Cell 69, 622–635.e6 (2018).
25. Arruda, N. L. et al. Distinct and overlapping roles of STAG1 and STAG2 in cohesin localization and gene expression in embryonic stem cells. Epigenetics Chromatin 13, 32 (2020).
26. Cancer Genome Atlas Research Network, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N. Engl. J. Med. 368, 2059–2074 (2013).
28. Battini, J. L., Rasko, J. E. & Miller, A. D. A human cell-surface receptor for xenotropic and polytropic murine leukemia viruses: possible role in G protein-coupled signal transduction. Proc. Natl Acad. Sci. USA 96, 1385–1390 (1999).

29. Giovannini, D., Touhami, J., Charnet, P., Sitbon, M. & Battini, J.-L. Inorganic phosphate export by the retrovirus receptor XPR1 in metazoans. J. Cell Biol. Rep. 3, 1866–1873 (2021).

30. Bondeson, D. P., Paolella, B. R., Asfaw, A. & Rothberg, M. Phosphate dysregulation via the XPR1: KIDINS220 protein complex is a therapeutic vulnerability in ovarian cancer. Nature Cancer. 3, 681–695 (2022).

31. Sánchez-Ruïlola, L. et al. Protein kinase D intracellular localization and activity control kinase D-interacting substrate of 220-kDa traffic through a postsynaptic density-95/discs large/zonula occludens-1-binding motif. J. Biol. Chem. 281, 18888–18900 (2006).

32. Li, S., Prasanna, X., Salo, V. T., Vattulainen, I. & Ikonen, E. An efficient auxin-inducible degron system with low basal degradation in human cells. Nat. Methods 16, 866–869 (2019).

33. Sathyan, K. M. et al. An improved auxin-inducible degron system preserves native protein levels and enables rapid and specific protein depletion. Genes Dev. 33, 1441–1455 (2019).

34. Mathieson, T. et al. Systematic analysis of protein turnover in primary cells. Nat. Commun. 9, 689 (2018).

35. Seong, B. K. A. et al. TRIM8 modulates the EWS/FLI oncoprotein to promote survival in Ewing sarcoma. Cancer Cell 39, 1262–1278.e7 (2021).

36. Malone, C. F. et al. Selective modulation of a pan-essential protein as a therapeutic strategy in cancer. Cancer Discov. 11, 2282–2299 (2021).

37. Erb, M. A. et al. Transcription control by the ENL YEATS domain in acute leukaemia. Nature 543, 270–274 (2017).

38. Rago, F. et al. Degron mediated BRM/SMARCA2 depletion uncovers novel combination partners for treatment of BRG1/SMARCA4-mutant cancers. Biochem. Biophys. Res. Commun. 508, 109–116 (2019).

39. Legati, A. et al. Mutations in XPR1 cause primary familial brain calcification associated with altered phosphate export. Nat. Genet. 47, 579–581 (2015).

40. Levers, J. D. et al. Potent and selective small-molecule MCL-1 inhibitors demonstrate on-target cancer cell killing activity as single agents and in combination with ABT-263 (navitoclax). Cell Death Dis. 6, e1590 (2015).

41. Shimizu, M. et al. Metabolic profiles of pemoliodamine in human plasma simulated with pharmacokinetic data in control and humanized-liver mice. Xenobiotica 47, 844–848 (2017).

42. Mosure, K. W. et al. Preclinical pharmacokinetics and in vitro metabolism of asunaprevir (BMS-650032), a potent hepatitis C virus NS3 protease inhibitor. J. Pharm. Sci. 104, 2813–2823 (2015).

43. BasuRay, S., Wang, Y., Smagris, E., Cohen, J. C. & Hobbs, H. H. Accumulation of PNPLA3 on lipid droplets is the basis of associated hepatic steatosis. Proc. Natl Acad. Sci. USA 116, 9521–9526 (2019).

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Author contributions

D.P.B. and A.I. conceived of the “degron panel” approach and designed the lentiviral vectors. Z.M.-B. cloned the initial NanoLuc constructs and validated the approach. D.P.B., Z.M.-B., S.O., T.A.S., N.B., M.C., A.A.G., J.K., C.L., D.M., K.T., S.J.W., and A.I. evaluated CDT efficiency across targets and conducted additional experiments. T.C.A. synthesized dTAGV−1. D.P.B., B.R.P., F.V., Z.T., T.R.G., W.R.S., and A.I. supervised the work. D.P.B. and A.I. wrote the manuscript. D.P.B., T.R.G., W.R.S., and A.I. edited the manuscript.

Competing interests

F.V. receives research funding from Novo Ventures. T.R.G. has an equity interest in or receives consulting income from Sherlock Biosciences and Anji Pharmaceuticals and receives research funding from Calico Life Sciences, Bayer HealthCare, and Novo Holdings. A.I. receives cash compensation for consulting with Ridgeline Discovery. W.R.S is a board or SAB member and holds equity in Ideaya Biosciences, Civetta Therapeutics, Red Ridge Bio, and 2Seventy Bio and has consulted for Array, Astex, Epidarex Capital, Ipsen, PearlRiver Therapeutics, Merck Pharmaceuticals, Sanofi, Servier, and Syndax Pharmaceuticals and receives research funding from Pfizer Pharmaceuticals, Merck Pharmaceuticals, Ideaya Biosciences, Calico, Boehringer-Ingelheim, Bristol Myers Squibb, and Ridgeline Discovery. W.R.S. is a co-patient holder on EGFR mutation diagnostic patents. The remaining authors declare no competing interests.

Additional information

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