The ubiquitin ligase Cullin-1 associates with chromatin and regulates transcription of specific c-MYC target genes

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Transcription is regulated through a dynamic interplay of DNA-associated proteins, and the composition of gene-regulatory complexes is subject to continuous adjustments. Protein alterations include post-translational modifications and elimination of individual polypeptides. Spatially and temporally controlled protein removal is, therefore, essential for gene regulation and accounts for the short half-life of many transcription factors. The ubiquitin–proteasome system is responsible for site- and target-specific ubiquitination and protein degradation. Specificity of ubiquitination is conferred by ubiquitin ligases. Cullin-RING complexes, the largest family of ligases, require multi-unit assembly around one of seven cullin proteins. To investigate the direct role of cullins in ubiquitination of DNA-bound proteins and in gene regulation, we analyzed their subcellular locations and DNA-affinities. We found CUL4A and CUL7 to be largely excluded from the nucleus, whereas CUL4B was primarily nuclear. CUL1, 2, 3, and 5 showed mixed cytosolic and nuclear expression. When analyzing chromatin affinity of individual cullins, we discovered that CUL1 preferentially associated with active promoter sequences and co-localized with 23% of all DNA-associated protein degradation sites. CUL1 co-distributed with c-MYC and specifically repressed nuclear-encoded mitochondrial and splicing-associated genes. These studies underscore the relevance of spatial control in chromatin-associated protein ubiquitination and define a novel role for CUL1 in gene repression.

Mammalian gene expression follows oscillatory patterns, caused by alternating binding of transcriptional activators and repressors to specific DNA elements. The exchange of regulators during such cycles is partially accomplished through protein removal and subsequent degradation by the ubiquitin–proteasome system. Therefore, it is not surprising that transcription factors and their co-regulators are among the most short-lived proteins. However, the specific factors that trigger the removal of chromatin-associated proteins, and the genomic locations of degradation remain ill-defined.

The ubiquitin–proteasome system is a multi-enzyme cascade that triggers the covalent attachment of ubiquitin polypeptides to target proteins. Ubiquitination can impact protein function and trafficking, or mark proteins for proteasomal digestion. The ubiquitin–proteasome system is responsible for the removal of most nuclear and cytosolic proteins. This pathway regulates transcription directly through epigenetic ubiquitination and through poly-ubiquitination that can lead to the removal of DNA-associated proteins. Furthermore, earlier work by our group and others indicates that the turnover of transcriptional regulators is site-selective and specific to some of the DNA regions to which these proteins are bound. Nuclear degradation by the ubiquitin–proteasome system is therefore not only target protein-selective, but also displays spatial specificity.

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The ubiquitin–proteasome system allows for specific ubiquitination of proteins through E3 ubiquitin ligases, of which there are around 600 subunits encoded in the human genome. However, these subunits often assemble into larger complexes with multiple variable subunits, increasing the actual number of functional E3 complexes multifold through combinatorial diversity. Cullin-RING ligase complexes represent about half of the encoded E3 genes, making it the largest family of ubiquitin ligases.

Cullin ligase complexes are comprised of ubiquitin-conjugating enzymes, adapter proteins, substrate recognition factors, and the eponymous cullin proteins. Cullins are rigid, rod-like proteins that act as structural scaffolds. Cullin ubiquitin ligases impact a variety of vital cellular functions, such as cell cycle progression, signaling, and DNA repair. Their specific role in transcriptional regulation is less well understood.

Cullins have recently garnered therapeutic interest for their role in PROTAC- and IMiD-based protein removal. Chemical linkers can be used to connect a cullin ligase complex with a specific substrate protein. In clinical practice, this approach is utilized to treat cancers. In particular, the complex consisting of CUL4 and the substrate receptor cereblon has shown promise by eliminating oncogenic transcription factors. The general advantage of cullin-mediated protein removal with PROTACs is that it enables inhibition through degradation of previously undruggable polypeptides. With this increased attention on cullins, we hypothesized that cullin complexes might be involved in ubiquitination and perhaps removal of transcriptional regulators in a site-specific manner and at defined chromatin locations.

In this study, we investigate the intracellular distribution of the cullins CUL1, 2, 3, 4A, 4B, 5, and 7. We further analyze their chromatin-association, their likely interactions with other transcription factor networks, and the downstream genes that they regulate. Our results show that the spatial distribution of CUL4A and CUL4B is mutually exclusive. Further, we show strong chromatin-association of CUL1, especially at genes under control by the transcription factor c-MYC, and at promoters with high levels of protein turnover. CUL1 represses a subset of these genes that control mitochondria and RNA splicing, and CUL1-deficient cells display signs of mitochondrial stress.

Results
Expression of cullins. To better assess the relative contribution of the ubiquitously expressed cullin backbone proteins to cellular function, we analyzed the individual cullin transcripts in 62 different human tissues from The Protein Atlas. CUL1 and CUL4B are the two most highly transcribed cullin genes in primary human tissues. When comparing 64 human cell lines, CUL1 and CUL3 are the two most highly expressed cullins.

Despite the presence of seven paralogs, cullins still have unique functions. For instance, CUL1 has been well studied as part of the SCF complex (SKP1, CDC53/Cullin, and F-box proteins) that regulates cell cycle progression and signaling. Notwithstanding the fact that CUL1 and its most divergent paralog CUL7 feature a combined 53.4% amino acid sequence similarity and identity, both are essential for viability and cannot be rescued by the presence of any other cullin.

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and visualization of tagged proteins using biochemical assays, microscopy, or chromatin-immunoprecipitation. We optimized DNA transfection with plasmids encoding the tagged constructs for similar expression levels of all seven cullins in human HeLa cells (Fig. 2A and Suppl. Figure 1) and identified their subcellular locations using immunofluorescence detection. CUL1, 2, 3, and 5 were expressed in both the nucleus and cytoplasm, while CUL4A and 7 were largely excluded from the nucleus. CUL4B is exclusively nuclear. Size bar indicates 10 μm. Shown are representative images.

**DNA association of cullins.** To identify ubiquitin ligases that catalyze the ubiquitination and possibly degradation of DNA-associated transcription factors, we first assessed how the seven cullins might directly affect gene regulation through chromatin association. We used Chromatin Immunoprecipitation (ChIP) to determine the DNA affinity of all seven cullins in HeLa cells. Unsurprisingly, we found that cytoplasmic CUL4A and CUL7 did not interact with DNA. When testing cullins with exclusive or partial nuclear expression, only CUL1 and CUL4B substantially associated with DNA (Fig. 3A). In particular, CUL1 displayed reproducible, genome-wide peaks by ChIP-sequencing (ChIP-seq) (Fig. 3B). These findings are confirmed by earlier work that showed nuclear expression of CUL1 in human cell lines and chromatin association in *S. cerevisiae*.

CUL1 peaks were significantly enriched at promoter regions upstream of transcription start sites (Fig. 3C,D). Cullins do not possess DNA binding domains and CUL1 is likely indirectly tethered to chromatin. Such indirect binding increases the functional space CUL1 controls. Further, CUL1 can bridge substrate proteins and ubiquitinating enzymes over distances of 100 Å. On the compacted DNA solenoid, this distance translates to a linear DNA length of approximately 3,000 bp. Thus, to identify potential DNA regions under control of CUL1, we extended the CUL1 peaks by 3 kb in either direction. These CUL1 regions strongly overlapped with the active chromatin mark H3K27ac and excluded the repressive mark H3K27me3 (Fig. 3E).

We previously defined DNA sites associated with high protein turnover by performing ChIP against ubiquitin. Our studies demonstrated that the addition of a proteasome inhibitor further increases the signal by enhancing degradation-prone ubiquitination in contrast to non-degradative ubiquitination. Overlaying such a nuclear degradation map in HeLa cells with DNA regions under CUL1 control revealed a 59.17% overlap, suggesting that the majority of CUL1-associated sites are also sites of detectable protein degradation (Fig. 3F). Conversely, the CUL1-associated sites represent 23.16% of all genomic protein degradation sites in HeLa cells.

To identify the potential degradation targets or protein networks controlled by CUL1 activity, we examined CUL1-associated areas for known DNA binding motifs. We found the E-Box, a hallmark motif of the c-MYC/MAX heterodimer, to be highly enriched within CUL1-associated sites (Fig. 4A). Further, 67.3% of CUL1 target genes showed c-MYC occupancy at their promoters, suggesting that genes controlled by c-MYC may also be regulated by CUL1 (Fig. 4B).

**Proximal gene regulation by CUL1.** To functionally assess how CUL1 affects the expression of potential target genes, we performed unbiased RNA-sequencing in control HeLa cells or cells in which CUL1 was stably knocked down. Probable CUL1 target genes can be divided into seven main gene ontologies based on CUL1 affinity close to the transcription start site (< 1 kb): cell cycle genes (146 genes, p < 5.7E−15 for gene ontology enrichment), genes involved in RNA splicing (74 genes, p < 2.5E−14), nuclear-encoded mitochondrial genes (210 genes, p < 2.2E−13), ribonucleoprotein complexes (75 genes, p < 3.4E−10), transcriptional regulators (379 genes, p < 1.3E−6), genes of the ubiquitin–proteasome pathway (123 genes, p < 1.6E−6), and genes involved in cilium biology (37 genes, p < 5.5E−4). Of these seven gene ontologies with CUL1 affinity, two subsets were
significantly altered in their expression upon knockdown of CUL1: nuclear-encoded mitochondrial genes and genes encoding splicing factors were upregulated in CUL1-deficient HeLa cells (Fig. 4C).

CUL1 has a prominent role in cell cycle regulation. However, little is known about its function in transcriptional or metabolic control. To validate whether CUL1 depletion alters expression of nuclear-encoded mitochondrial and splicing-associated genes, we used shRNA to generate two independent CUL1-deficient HeLa cell lines (Fig. 5A and Suppl. Figure 2). We then specifically analyzed genes for which we had established close affinity of CUL1 and c-MYC to the promoter regions by ChIP-seq. CUL1 knockdown cells displayed a significant

Figure 3. 3xFLAG-Cullin Chromatin Immunoprecipitation-sequencing (ChiP-seq). (A) ChiP-seq of HeLa cells expressing each of the 3xFLAG-Cullins show that only CUL1 and CUL4B substantially associate with DNA. Marks indicate peaks with chromosomes listed from 1 to 22 and X, Y (top to bottom). The graph was generated with MACS2 and CEAS. (B) A comparison of CUL1 DNA binding peaks of two independent biological replicates. (C) CUL1 DNA affinity is enriched for promoter regions compared to the overall genomic prevalence of promoters (50% vs. 2.4%, \( p = 2.3E−322 \)). Indicated are pie chart percentages comparing the entire genome (left) with CUL1-associated regions (right). Data was calculated with MACS2 and CEAS. (D) CUL1 peak distribution upstream of transcription start sites (TSS) shown as relative density plot. (E) CUL1-associated DNA regions are significantly enriched for H3K27ac, but are devoid of previously reported H3K27me3 marks (\( p < 2E−300 \), Chi-squared test with Yates correction). (F) CUL1 peaks are significantly enriched at sites of proteasome-dependent degradation. ChiP peaks from 3xFLAG-Ubiquitin-expressing HeLa cells treated with proteasome inhibitor represent degradation-prone ubiquitination \(^*\) “Deg. Ubiq.” (\( p < 2.33E−308 \), Chi-squared test with Yates's correction).

Figure 4. CUL1 target gene analysis. (A) CUL1 peaks are significantly enriched for the E-box DNA binding motif of the c-MYC/MAX heterodimer (\( p = 1.00714E−30 \)). (B) Confirming the in silico motif enrichment, we also found significant overlap of CUL1 target genes with previously reported c-MYC target genes in HeLa cells (\( p < 2.06E−19 \), Chi-squared test with Yates' correction). (C) Nuclear-encoded mitochondrial genes (\( p < 6.67E−7 \), Wilcoxon rank-sum test) and splicing-associated genes (\( p < 4.20E−6 \)) are significantly upregulated in CUL1-deficient HeLa cells, as shown in this box plot. RNP refers to ribonucleoprotein complex genes; UPS refers to ubiquitin–proteasome system genes. Asterisks denote statistical significance. Gene ontologies were defined with DAVID.
Figure 5. CUL1 represses splicing-associated genes. (A) HeLa cells stably transduced with shRNA against CUL1 show a stable reduction in CUL1 expression based on immunoblot against endogenous CUL1. Knockdown construct KD 1 represents TRCN0000010781; CUL1 protein levels are reduced to 11.82% relative to GAPDH control. Knockdown construct KD 2 is TRCN0000003391; CUL1 protein levels are reduced to 26.17% relative to GAPDH control. The left lane contains lysate from cells transduced with control vector TRCN0000241922. Protein lysates were normalized by Bradford assay. Densitometry was performed with ImageJ. Uncropped immunoblots are shown in Suppl. Figure 2. (B) Analysis of transcript expression changes upon CUL1 knockdown for genes that show bona fide peaks for c-MYC and both CUL1 replicates in their promoter regions. Splicing-associated genes show a significant upregulation upon CUL1 depletion ($p = 2.46E^{-2}$, Wilcoxon rank-sum test). (C) CUL1 knockdown cells show a significant increase in transcripts of genes encoding splicing factors by RT-qPCR compared to cells expressing the TRC control vector (data are expressed as mean ± standard deviation, all significant $p$ values < 1.21E$^{-2}$, two-sided homoscedastic t test). RPS14 was used as reference transcript for $\Delta\Delta$Ct quantification. (D) HeLa cells transiently overexpressing 3xFLAG-CUL1 show a significant reduction in splicing-associated gene transcripts compared to the cells expressing the 3xFLAG vector alone (data are expressed as mean ± standard deviation, all significant $p$ values < 2.42E$^{-8}$, two-sided homoscedastic t test). RPS14 was used as reference transcript for $\Delta\Delta$Ct quantification. (E) Genome browser tracks of CUL1, H3K27ac, degradative ubiquitin, and c-MYC at select splicing-associated CUL1 and c-MYC target genes. Tracks from 3xFLAG-Ubiquitin-expressing HeLa cells treated with proteasome inhibitor represent degradative ubiquitination sites, “Deg. Ubiq.” Red boxes indicate promoter regions. Asterisks denote statistical significance.
increase in mRNA transcripts of these splicing-associated target genes and nuclear-encoded mitochondrial genes compared to cells expressing the control shRNA vector (Figs. 5B, 6A). Given that c-MYC-addicted cancer cells depend upon the spliceosome and that c-MYC drives mitochondrial biogenesis43, these data suggest an antagonistic relationship between c-MYC and CUL1. We performed RT-qPCR on select splicing-associated target genes and nuclear-encoded mitochondrial genes in dependence of CUL1 expression. Our studies confirmed the increased transcription of most target genes we tested in CUL1-deficient cells (Figs. 5C, 6B). Overexpression of CUL1 had the opposite effect and reduced expression of these target genes, suggesting the ubiquitin ligase has a repressor-like function on transcription from these c-MYC-associated gene promoters (Figs. 5D, 6C). Genome browser tracks show the close proximity of CUL1 affinity, c-MYC binding, and protein degradation at active (H3K27ac-positive) target promoters (Figs. 5E, 6D).

To further investigate how CUL1-regulated transcription of metabolic genes affects cellular function, we analyzed the mitochondrial oxygen consumption in cells with normal or reduced CUL1 expression. Basal respiration was increased by an average of 60% in cells in which CUL1 was knocked down (Fig. 7A). In addition to increased respiration, we found evidence for elevated mitochondrial stress in the absence of CUL1. The morphology of mitochondrial networks showed significantly enhanced levels of fusion, which is consistent with damaged mitochondria that are attempting to repair and restore metabolic function44,45 (Fig. 7B, C). Overall, our results indicate that CUL1 is associated with the promoters of approximately 210 nuclear-encoded mitochondrial genes and a significant number of these genes are repressed by CUL1. De-repression increases mitochondrial activity, but also leads to morphological changes in mitochondria that are consistent with stress.

Discussion
We here identify a novel role of the ubiquitin ligase CUL1 as a transcriptional repressor. A substantial number of genes controlled by c-MYC also show promoter association with CUL1. The promoters of these genes feature distinct ubiquitin peaks upon proteasome inhibition, indicating high levels of protein turnover. Our data suggest that CUL1 directly represses a subset of these genes involved in mitochondrial biology and splicing. CUL1 and c-MYC both show synergistic function in cancers and can act as oncogenes46,47. While this seemingly contradicts the antagonistic function between CUL1 and c-MYC we describe here, a key role of CUL1 is, notably, to promote cell cycle progression. CUL1 contributes to this progression through bulk degradation of
This clinically relevant degradation through cereblon occurs in the nucleus. Our results argue that this activity of ubiquitination of the transcription factors IKZF1 and IKZF3 upon treatment with thalidomide or its derivatives is mediated by CUL4B, not CUL4A. In support of our findings, an earlier report identified a nuclear localization sequence in CUL4B29. Except for CUL4A and CUL7, which are mostly excluded from the nucleus, all other cullins show some or specific expression in the nucleus. It is, therefore, possible that CUL2, 3, 4B, and 5 participate in the bulk ubiquitination of nuclear proteins and that CUL1 further engages in the site-selective ubiquitination of proteins at specific genomic regions.

Cullins represent the largest family of ubiquitin ligases. Here, we show a surprising variability in intracellular distribution of the seven cullins. Our data suggests that both CUL1 and CUL4B have the capacity to ubiquitinate DNA-bound proteins. In particular, CUL1 demonstrated the strongest association with chromatin and regulated protein turnover was examined by quantifying the levels of DNA-associated ubiquitination upon proteasome inhibition8. Such treatment leads to a massive redistribution of ubiquitin, shuttling the limiting amounts of this protein from non-degradative to degradative use. Further evidence that CUL1 is specifically engaged in protein degradation can be found in numerous publications10,46,48. Interestingly, CUL1 has been described as a ubiquitin ligase that targets c-MYC for degradation through the substrate receptor FBXW749. We have not found evidence that CUL1 knockdown or after the introduction of dominant-negative CUL1 (not shown). However, it is possible that c-MYC degradation by CUL1 occurs in a site-selective manner at specific promoters, in which case there may only be a negligible change to total c-MYC levels. We have previously observed such spatially selective degradation for other transcriptional regulators8. In summary, the identities of target proteins of chromatin-associated ubiquitination by CUL1 and their fates remain unsolved and are subjects of ongoing studies by our laboratory.

Previous reports on the subcellular locations of cullins were inconsistent, especially concerning the clinically relevant proteins CUL4A and CUL4B. Both cullins bind to cereblon30, a substrate-binding protein that triggers ubiquitination of the transcription factors IKZF1 and IKZF3 upon treatment with thalidomide or its derivatives. Degradation of IKZF1 and IKZF3 is therapeutically exploited in the treatment of hematological malignancies. This clinically relevant degradation through cereblon occurs in the nucleus51. Our results argue that this activity of ubiquitination of the transcription factors IKZF1 and IKZF3 upon treatment with thalidomide or its derivatives is mediated by CUL4B29. Except for CUL4A and CUL7, which are mostly excluded from the nucleus, all other cullins show some or specific expression in the nucleus. It is, therefore, possible that CUL2, 3, 4B, and 5 participate in the bulk ubiquitination of nuclear proteins and that CUL1 further engages in the site-selective ubiquitination of proteins at specific genomic regions.

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Methods
Phylogram. Phylogram was created with Jalview52 based on Clustal-Omega protein sequence alignments53.
Plasmids. Cullins were cloned into the 3xFLAG/pCMV7.1 vector (Sigma Aldrich, #E7533). CUL1 (GenInfo identifier #32307160), CUL3 (#380714661), CUL4B (#121114297), and CUL7 (#270265834) were cloned via NotI and KpnI sites. CUL2 (#311771638), CUL4A (#311772959), and CUL5 (#67514034) were cloned via Sall and Xbal. Plasmids are available through Addgene (#155019-155025).

Cell culture. HeLa cells (ATCC, #CCL2) were cultured in DMEM, 1X (Dulbecco’s Modification of Eagle’s Medium with 4.5 g/L glucose and t-glutamine) (Corning, #10-017-CV) supplemented with 10% FBS Opti-Gold, performance enhanced (GenDEPOT, #F0900-050) and 1% penicillin streptomycin (Gibco, #15140-122).

Western Blots. HeLa cells were transfected with 2 µg of 3xFLAG-CUL using Lipofectamine reagent 2000 (Invitrogen, #1168-019). Media was changed at 24 h, and at 48 h cells were washed with cold 1xPBS and frozen at − 80 °C. Cells were then thawed and lysed in RIPA cell lysis buffer (1X) with EDTA (GenDepot, #R4100-010) and protease inhibitor cocktail (1%, GenDepot, #P3100-010) for 1 h on ice with vortexing every 15 min. Lysates were pelleted at 4 °C at 1,400 × g for 30 min and supernatant was collected in a separate tube. Protein concentration was determined using protein assay dye reagent concentrate (Bio-Rad, #500-0006) and normalized against bovine serum albumin (BSA) (Sigma-Aldrich, #A97906-50G). Protein was then mixed with 4 × Laemmli sample buffer (Bio-Rad, #161-0747) and β-mercaptoethanol (Sigma-Aldrich, #M6250-100ML) according to manufacturer specifications and loaded onto Mini-PROTEAN TGX stain-free gels (Bio-Rad, #4568123). Proteins were separated using the Bio-Rad PowerPac and 1X Tris/glycine/SDS buffer (Bio-Rad, #1610732) and subsequently transferred to Trans-Blot Turbo transfer pack membranes (Bio-Rad, #1704156) using Trans-Blot Turbo transfer system (Bio-Rad). Western blot analysis against 3xFLAG was carried out using monoclonal anti-FLAG M2 antibody produced in mouse (GenDepot, #F0900-050) and β-mercaptoethanol (Sigma-Aldrich, #M6250-100ML) according to manufacturer specifications and loaded onto Mini-PROTEAN TGX stain-free gels (Bio-Rad, #4568123). Proteins were separated using the Bio-Rad PowerPac and 1X Tris/glycine/SDS buffer (Bio-Rad, #1610732) and subsequently transferred to Trans-Blot Turbo transfer pack membranes (Bio-Rad, #1704156) using Trans-Blot Turbo transfer system (Bio-Rad). Western blot analysis against 3xFLAG was carried out using monoclonal anti-FLAG M2 antibody produced in mouse (Sigma-Aldrich, #IF1804-1IMG) with β-actin rabbit monoclonal antibody (Cell Signaling Technology, clone D6A8) as a loading control. Western blot against CUL1 was performed with recombinant anti-Cullin1/CUL-1 antibody (Abcam, ab75817). The membrane was stripped in 62.5 mM Tris/10%SDS/0.5% β-mercaptoethanol at 37 °C for 30 min, re-equilibrated in 5% milk/TBST and re-probed as above with anti-GAPDH antibody—loading control (HRP) (Abcam, ab204481). SuperSignal West Pico PLUS chemiluminescent substrate (Thermo Scientific, #34577) was used to detect horseradish peroxidase (HRP)-conjugated proteins, and bands were visualized using the Bio-Rad ChemiDoc imaging system.

Lentiviral production and CUL1 knockdown. TRC Lentiviral shRNA vectors were acquired from Horizon Discovery (Cat# RHS4533-EG8454; TRCN0000003391 and TRCN0000010781), using TRCN0000024192 as negative control vector. Other tested vectors include TRCN000003392, TRCN000003393, and TRCN000003394. COS-1 cells (ATCC, #CRL-1650) were transfected with Lipofectamine reagent 2000 (Invitrogen, Cat #1168-019), 2 µg TRC shRNA vector and packaging plasmids. Virus was concentrated with Lenti-X Concentrator (Takara Bio, #631231) and treated with Polybrene (2 µg/mL, Millipore, #TR-1003-G). HeLa cells were infected with virus and subjected to puromycin selection (2 µg/mL, Gibco, #A11138-03).

Immunofluorescence microscopy. Coverslips were autoclaved then treated with 0.1 µg/mL poly-d-lysine (Millipore, #A-003-E). HeLa cells were plated onto the coverslips, then transfected as described. Cells were fixed with 4% para-formaldehyde in PBS (Thermo Scientific, #28906) for 15 min, permeabilized with 0.5% Triton X-100 (Fisher Scientific, #9002-93-1) for 15 min, and blocked with 10% BSA (Sigma-Aldrich, #A97906-50G) for 1 h. Cells were then incubated with monoclonal anti-FLAG M2 antibody produced in mouse (see above) and protein G beads (Thermo Fisher, #10003D) as negative control vector. Other tested vectors include TRCN0000003391 and TRCN0000010781, using TRCN0000241922 as negative control vector. Other tested vectors include TRCN000003392, TRCN000003393, and TRCN000003394. COS-1 cells (ATCC, #CRL-1650) were transfected with Lipofectamine reagent 2000 (Invitrogen, Cat #1168-019), 2 µg TRC shRNA vector and packaging plasmids. Virus was concentrated with Lenti-X Concentrator (Takara Bio, #631231) and treated with Polybrene (2 µg/mL, Millipore, #TR-1003-G). HeLa cells were infected with virus and subjected to puromycin selection (2 µg/mL, Gibco, #A11138-03).

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Chromatin immunoprecipitation (ChIP). ChIP experiments with the 3xFLAG tag were performed as previously published6. In short, HeLa cells were grown in T175 flasks and harvested at 90% confluency. Each flask contained approximately 5 million cells and at least 10 million cells were harvested for each experimental condition. 3F-Ubiquitin ChIP was performed with stably transduced HeLa cells6. 3F-Cullin ChIP was performed with HeLa cells that were transfected with Lipofectamine 2000 (Thermo Fisher, #11668019) 48 h prior to harvest with 25 µg of 3F-Cullin and 5 µg of a GFP spike-in control vector to validate consistent transfection efficiency across different cullin constructs at > 80%. For 3F-Ubiquitin ChIP, proteasome inhibition was performed for 3 h prior to ChIP with 25 µM lactacystin or 0.1% v/v DMSO control (Cayman Chemical, #70980). Cells were washed and fixed in 1% para-formaldehyde in PBS (Thermo Scientific, #28906) at room temperature for 10 min, followed by quenching with glycine. Cells were manually detached by scraping and washed prior to lysis. 5 million cells were lysed per 5 mL dilution buffer (150 mM NaCl, 20 mM Tris pH 7.4, 2 mM EDTA) with the addition of Triton X-100 (1%, VWR, #IB07100), protease inhibitor cocktail (1%, Genesee, #P13010-010), and RNase cocktail (1%, Thermo Fisher, #AM2288) for 10 min at 4 °C with constant mixing. Nuclei were isolated through centrifugation (350 × g, 5 min, 4 °C) and immediately sonicated in dilution buffer containing 0.04% SDS, RNase, and protease inhibitor cocktail using a Bioruptor Pico water bath sonicator (Diagenode) at 4 °C. Shearing was optimized to yield DNA fragments of 200–500 bp. After removal of insoluble material through centrifugation, the nuclear lysate was aliquoted for input material or diluted to 0.01% SDS and immunoprecipitated over night with monoclonal anti-FLAG M2 antibody produced in mouse (see above) and protein G beads (Thermo Fisher, #10003D) that were blocked with DNA-free BSA (Thermo Fisher, #15561020). The following day, beads were washed twice
Next generation sequencing. The Genomic and RNA Profiling Core at Baylor College of Medicine performed next generation sequencing as previously described. Libraries for ChIP-seq were synthesized and prepared for multiplexing according to New England BioLabs’ protocol for Illumina sequencing (Ultra Next DNA library prep kit I and II, #E7370S and #E7645S). As indexing primers, we used NEBNext Multiplex oligos (#E7335S and #E7500S). Libraries for RNA-seq were synthesized and prepared for sequencing with the KAPA stranded RNA-seq kit with RiboErase (HMR) (Roche, #KK8483) with ERCC ExFold RNA spike-in mix (Thermo Fisher, #4456739). Indexing primers for RNA-seq were custom-synthesized by IDT.

ChIP-Seq: The Genomic and RNA Profiling Core first conducted sample quality checks using the NanoDrop spectrophotometer and Agilent Bioanalyzer 2100 (High Sensitivity DNA Chip, #5067-4626). To quantitate the adapter ligated library and confirm successful P5 and P7 adapter incorporations, we used the Applied Biosystems Viia7 real-time PCR system and a KAPA Illumina/universal library quantification kit (#KK4824). We then sequenced the libraries on the Nextseq500 system using the high output v2.5 flowcell.

Library quantification by qPCR and Bioanalyzer: A qPCR assay was performed on the libraries to determine the concentration of adapter ligated fragments using the Applied Biosystems Viia7 quantitative PCR instrument and a KAPA library quant kit (#KK4824). All samples were pooled equimolarly and re-quantitated by qPCR, and also re-assessed on the Bioanalyzer.

Cluster Generation by Bridge Amplification: Using the concentration from the Viia7 qPCR machine above, 1.8 pM of equimolarly pooled library is loaded onto a NextSeq 500 high output v2.5 flowcell (Illumina #20024906) and amplified by bridge amplification using the Illumina NextSeq 500 sequencing instrument. PhiX Control v3 adapter-ligated library (Illumina, #PC-1103001) is spiked-in at 1% by weight to ensure balanced diversity and to monitor clustering and sequencing performance. A single-end 75 cycle run was used to sequence the flowcell on a Nextseq 500 sequencing system to achieve a minimum of 25 million reads per sample. Fastq file generation and data delivery was achieved using Illumina’s Basespace sequence hub.

RNA-seq: The Genomic and RNA Profiling Core first conducted sample quality checks using the NanoDrop spectrophotometer and Agilent Bioanalyzer 2100 (High sensitivity DNA Chip, #5067-4626). To quantitate the adapter ligated library and confirm successful P5 and P7 adapter incorporations, we used the Applied Biosystems Viia7 real-time PCR system and a KAPA Illumina/universal library quantification kit (#KK4824). We then sequenced the libraries on the Nextseq500 system using the high output v2.5 flowcell.

Library quantification by qPCR and Bioanalyzer: A qPCR assay was performed on the libraries to determine the concentration of adapter ligated fragments using the Applied Biosystems Viia7 quantitative PCR instrument and a KAPA library quant kit (#KK4824). All samples were pooled equimolarly and re-quantitated by qPCR, and also re-assessed on the Bioanalyzer.

Cluster Generation by Bridge Amplification: Using the concentration from the Viia7 qPCR machine above, 1.8 pM of equimolarly pooled library is loaded onto a NextSeq 500 high output v2.5 flowcell (Illumina, #20024907) and amplified by bridge amplification using the Illumina NextSeq 500 sequencing instrument. PhiX Control v3 adapter-ligated library (Illumina, #PC-1103001) is spiked-in at 1% by weight to ensure balanced diversity and to monitor clustering and sequencing performance. A paired-end 75 cycle run was used to sequence the flowcell on a NextSeq 500 sequencing system to achieve a minimum of 50 million reads per sample. Fastq file generation and data delivery was achieved using Illumina’s Basespace sequence hub.

Data processing. ChIP-seq fastq files were processed with Cutadapt ver. 1.12 and mapped to the HG19 genome with Bowtie ver. 1.0.9. Peak calling was performed with MACS2 ver. 2.1.0.20140616 with a false discovery rate < 0.05. Peaks were compared to input DNA as well as ChIP DNA from cells transfected with the 3xFLAG/pCMV7.1 control vector (Sigma Aldrich, #E7533). Mapping to functional genomic sites and target genes was performed with CEAS ver. 1.0.2. Gene ontologies were defined with DAVID (https://david.ncifcrf.gov) ver. 6.8. Target site and peak overlaps were analyzed with Bedtools ver. 2.23.0 and fold enrichment was calculated based on randomized peaks of equal number and size and intra-chromosomal permutation. Wig files were created from MACS2 output with Sambtools ver. 0.1.19-96b5294a53. Wig and bigwig files were visualized using the Integrative Genomics Viewer (IGV) version 2.3 from the Broad Institute64-66. The following ENCODE data was utilized: c-MYC (ENCF045UZK, ENCF224GZD), H3K27ac (ENCF388WMD), and H3K27me3 (ENCF252BLX)67,68. The c-MYC reference file used in this study is based on common peaks between both entries. Similarly, Venn diagram comparisons for CUL1 binding are based on common peaks between two biological ChIP replicates. As outlined in the manuscript, domains under CUL1 control were estimated by extending peak regions 3,000 bp in both directions. The analysis of gene expression changes by RNA-seq in Figs. 5B and 6A was performed using the bona fide peaks (not extended regions) of both CUL1 replicates and c-MYC. ChIP bed files were subjected to motif analysis using the SeqPos module in Cistrome69. Parameters were defined as sequencing positions p < 0.05, peak size 600 bp, using fold enrichment.

RNA-seq fastq files were processed with Cutadapt ver. 1.12 and mapped to the HG19 genome with TopHat2/Bowtie2 ver. 2.1.071. Gene expression changes were quantified with Cufflinks and Cuffdiff ver. 2.1.1.71

RNA extraction. RNA was extracted from confluent HeLa cells with RNeasy kit with RNase-free DNasel treatment (Qiagen, #74134 and #79254).
**RT-qPCR.** RNA was subjected to RT-qPCR using Invitrogen SuperScript III Platinum SYBR Green one-step RT-qPCR kit with ROX (Thermo Fisher, #11746-500) according to the manufacturer’s instruction.

Primer sequences:

- **ATP5F1_F:** GGT GTA ACA GGA CCC TAT GTACT
- **ATP5F1_R:** GAA GGT CTC TGC GCT AAT CAC
- **OXCT1_F:** TGG AGA TGA CGT AAG GGA ACG
- **OXCT1_R:** GGA GAG GGA TTC CTA TGC CCA
- **SLC25A25_F:** TGA CCA TCG ACT GGA ACG AGT
- **SLC25A25_R:** ACA TCA AAG ATC GTG GAA TGCTT
- **PHB_F:** TGT CAT CTT TGA CCG ATT CCG
- **PHB_R:** GCG TCA TTG TTG AAT GCA GACA
- **PHB2_F:** GTG CGC GAA TCT GTG TTC AC
- **PHB2_R:** GAT AAT GGG GTA CTG GAA CCAAG
- **SNRPA1_F:** GGT GCT ACG TTA GAC CAG TTTG
- **SNRPA1_R:** GTC CCT CAC CTA TAC GGC ATATT
- **RBM28_F:** ATG TCC GCA TTG TCT TGC ATC
- **RBM28_R:** GGC CAT CCA GTT TAA GCC CA
- **SNRPE_F:** TGC AGC CCA TCA ACC TCA TC
- **SNRPE_R:** GCC TTC TAT CCG CAT ATT CACTT
- **ZRANB2_F:** GTG GTC GGG AGA AAA CAA CTG
- **ZRANB2_R:** CCC AAT TCA CAT TGC TGC AAGT
- **CUL1_F:** AGC CAT TGA AAA GTG TGG AGAA
- **CUL1_R:** GCG TCA TTG TTG AAT GCA GACA
- **RPS14_F:** CCA TGT CAC TGA TCT TTC TGGC
- **RPS14_R:** TCA TCT CGG TCT GCC TTT ACC

**Oxygen consumption assays.** Seahorse XFp cell culture miniplates (Agilent, #103025-100) were treated with Cell-Tak cell and tissue adhesive (0.024 mg/mL Corning, #354240) according to manufacturer specifications and 30,000 cells/well were plated. Agilent Seahorse XF base medium (#103193-100) was supplemented with 25 mM glucose, 2 mM sodium pyruvate, and 2 mM l-glutamine. Basal respiration was normalized by cell count.

**Mitotracker and MiNA analysis.** HeLa cells were incubated with 500 nM Mitotracker Red CMXRos (Invitrogen, #M7512) for 30 min, then placed on coverslips, permeabilized, and mounted as described. Images were taken as z-stacks at 100 × with the Zeiss CellDiscoverer7 and processed with Zeiss ZEN 3.1 (blue edition) Deconvolution (Defaults—Excellent). Using ImageJ, images were converted to RGB, auto-thresholding was applied (yen algorithm), and pictures were subjected to MiNA analysis.

**Cell cycle analysis.** HeLa Control or CUL1 knockdown cells were transiently transfected for 48 h with Lipofectamine 2000 (Thermo Fisher, #11668027) and the FastFUCCI construct on 6-well plates. Microscopy was performed on a live-cell imager (CD7, Zeiss) at 20 × magnification. Analysis of 6 × 6 fields of view per cell type was performed with Zeiss ZEN 3.1 software. Cells were grown in phenol red-free DMEM medium with supplementation. The plasmid pBOB-EF1-FastFUCCI-Puro was a gift from Kevin Brindle & Duncan Jodrell (Addgene plasmid # 86849; [https://n2t.net/addgene:86849](https://n2t.net/addgene:86849); RRID:Addgene_86849).

**Data availability**

Raw and processed ChiP-seq files are available at the Gene Expression Omnibus under GSE147426.

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**Author contributions**
M.A.S., P.I., F.Y.S., H.E.C., and A.C. prepared reagents and performed the experiments. Cloning was conducted by F.Y.S. and A.C. M.A.S., L.M., E.S., and A.C. designed and contributed to the experiments, and L.M. also provided technical guidance. M.A.S., L.M., E.S., and A.C. interpreted the results. A.C. supervised the project. M.A.S. and A.C. drafted the manuscript with input from the co-authors. The final version of the manuscript was approved by all authors.

**Competing interests**
The authors declare no competing interests.

**Additional information**

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