Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS

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Lenalidomide is a highly effective treatment for myelodysplastic syndrome (MDS) with deletion of chromosome 5q (del(5q)). Here, we demonstrate that lenalidomide induces the ubiquitination of casein kinase 1A1 (CK1α) by the E3 ubiquitin ligase CUL4–RBX1–DDB1–CRBN (known as CRL4CRBN), resulting in CK1α degradation. CK1α is encoded by a gene within the common deleted region for del(5q) MDS and haploinsufficient expression sensitizes cells to lenalidomide therapy, providing a mechanistic basis for the therapeutic window of lenalidomide in del(5q) MDS. We found that mouse cells are resistant to lenalidomide but that changing a single amino acid in mouse Crbn to the corresponding human residue enables lenalidomide-dependent degradation of CK1α. We further demonstrate that minor side chain modifications in thalidomide and a novel analogue, CC-122, can modulate the spectrum of substrates targeted by CRL4CRBN. These findings have implications for the clinical activity of lenalidomide and related compounds, and demonstrate the therapeutic potential of novel modulators of E3 ubiquitin ligases.

The immunomodulatory (IMiD) agents lenalidomide, thalidomide, and pomalidomide are the first drugs identified that promote the ubiquitination and degradation of specific substrates by an E3 ubiquitin ligase. These compounds bind CRBN, the substrate adaptor for the CRL4CRBN E3 ubiquitin ligase, and modulate the substrate specificity of the enzyme. Each of these drugs induces degradation of two lymphoid transcription factors, IKZF1 and IKZF3, leading to clinical efficacy in multiple myeloma and increased interleukin-2 release from T cells2–4. However, it has not yet been determined whether degradation of distinct substrates may mediate additional activities and whether all IMiD compounds have the same substrate specificity.

Lenalidomide is also a highly effective treatment for myelodysplastic syndrome (MDS) with deletion of chromosome 5q (del(5q)), inducing cytogenetic remission in more than 50% of patients5–7. In vitro, lenalidomide selectively induces apoptosis of del(5q) MDS cells8,9. No biallelic deletions or loss of function mutations on the remaining allele have been detected in any of the genes in the del(5q) common deleted region, implying that MDS with del(5q) is a disease of haploinsufficiency10,11. We hypothesized that ubiquitination of a distinct CRBN substrate explains the efficacy of lenalidomide in del(5q) MDS.

Lenalidomide induces degradation of CK1α

In order to identify lenalidomide-regulated CRL4CRBN substrates in myeloid cells, we applied stable isotope labelling of amino acids in cell culture (SILAC)-based quantitative mass spectrometry12 to assess global changes in ubiquitination13 and protein levels in the del(5q) myeloid cell line KG-1 (Fig. 1a, b, Extended Data Fig. 1, Extended Data Table 1a, b and Supplementary Table 1). Treatment with 1 μM lenalidomide significantly altered only seven K-ε-GG sites from five proteins specific out of 13,061 reproducible sites, demonstrating the highly specific effects of this drug. Moreover, lenalidomide significantly altered the protein abundance of 3 out of 5 differentially ubiquitinated proteins. Consistent with previous studies, lenalidomide treatment decreased ubiquitination of CRBN (P = 0.026) and increased ubiquitination of IKZF1 (P = 7.23 × 10−6 and P = 4.97 × 10−4 for two distinct sites), with a reciprocal decrease in IKZF1 protein abundance (P = 0.006)2,3.

In addition to IKZF1, we detected increased ubiquitination (P = 0.04) and decreased protein abundance (P = 0.006) of casein kinase 1A1 (CK1α) following treatment with 1 μM lenalidomide (Fig. 1a, b, Extended Data Fig. 1, Extended Data Table 1a, b and Supplementary Table 1). CK1α is encoded by the CSNK1A1 gene, which is located in the del(5q) common deleted region, and is expressed at haploinsufficient levels in del(5q) MDS10,11. CK1α has been implicated in the biology of del(5q) MDS12,13 and has been shown to be a therapeutic target in myeloid malignancies14, and is therefore an attractive candidate for mediating the effects of lenalidomide in del(5q) MDS.

CK1α is a substrate of CRL4CRBN

We sought to determine whether CK1α is a lenalidomide-dependent substrate of the CRL4CRBN E3 ubiquitin ligase. We confirmed that lenalidomide treatment decreases CK1α protein levels in multiple human cell lines and in the bone marrow and peripheral blood of acute myeloid leukaemia (AML) patients treated in vivo (Fig. 1c, Extended Data Fig. 2 and Extended Data Table 2). Lenalidomide treatment resulted in decreased protein levels of both wild-type isoforms of CK1α as well as two somatic CK1α mutations recently identified in del(5q) MDS patients15 (Extended Data Fig. 3). Lenalidomide decreased CK1α protein levels without altering CSNK1A1 mRNA expression (Fig. 1d and Extended Data Fig. 2c), consistent with a post-translational mechanism of regulation. The lenalidomide-dependent decrease in CK1α protein level was abrogated by treatment with the proteasome inhibitor MG132 and the NEDD8-activating enzyme inhibitor
MLN4924, which interferes with the activity of cullin-RING E3 ubiquitin ligases, implicating proteasome- and cullin-dependent degradation of CK1α (Fig. 2a). Homozygous genetic inactivation of CRBN by CRISPR-Cas9 genome editing eliminated lenalidomide-dependent degradation of CK1α, demonstrating CRBN-dependent degradation of CK1α (Fig. 2b and Extended Data Fig. 2d).

We next examined whether CK1α binds CRBN and is ubiquitinated by the CRBN E3 ubiquitin ligase. We observed co-immunoprecipitation of CK1α with endogenous and Flag-tagged CRBN only in the presence of lenalidomide (Fig. 2c and Extended Data Fig. 2e). Lenalidomide treatment increased the ubiquitination of endogenous CK1α in KG-1 cells (Fig. 2d) and in the presence of CRBN in vitro (Fig. 2e), confirming that CK1α is a direct target of CRBN. Using a chimaeric protein of CK1α and CK1c, which shares significant homology with CK1α but is not responsive to lenalidomide, we found that the amino-terminal half (amino acids 1–177) of CK1α is essential for lenalidomide-induced degradation (Extended Data Fig. 3d, e). Sequence alignment with the previously delineated lenalidomide-responsive degron in IKZF1/IKZF3 did not reveal any evident homology, suggesting that CK1α and IKZF1/IKZF3 may interact with the CRBN–lenalidomide complex in distinct manners.

**Effect of CSNK1A1 expression level**

We next explored the biological effects of CK1α depletion. CK1α is a serine/threonine kinase with multiple cellular activities. Most notably, CK1α inhibits p53 through MDM2 and MDMX and negatively regulates Wnt signalling as a component of the β-catenin destruction complex. In a haematopoietic-specific conditional knockout mouse model, homozygous inactivation of CSNK1A1 induces apoptosis via p53 activation, while heterozygous loss of CSNK1A1 causes β-catenin accumulation and stem cell expansion. Similarly, cells haploinsufficient for CSNK1A1 preferentially undergo apoptosis in response to the casein kinase 1 inhibitor D4476. Since del(5q) cells express about 50% of normal levels of CSNK1A1, these results led us to hypothesize that del(5q) cells would be more sensitive to the effects of lenalidomide-induced degradation of CK1α compared to normal cells with two copies of the gene.

To evaluate whether decreased CSNK1A1 expression sensitizes cells to lenalidomide, we transduced primary human CD34+ haematopoietic stem and progenitor cells with green fluorescent protein (GFP)-tagged lentiviral vectors expressing CSNK1A1 or control short hairpin RNAs. Cells expressing CSNK1A1 shRNAs were depleted in the absence of treatment, demonstrating that knockdown of
CSNK1A1 inhibits the growth or survival of haematopoietic cells (Extended Data Fig. 4). Treatment with lenalidomide enhanced the depletion of cells expressing CSNK1A1 shRNAs but had no effect on cells expressing control shRNAs, demonstrating that reduced CSNK1A1 levels sensitize haematopoietic cells to lenalidomide. We next evaluated whether overexpression of CSNK1A1 could reduce the lenalidomide sensitivity of del(5q) MDS cells. We obtained bone marrow samples from MDS patients with normal karyotype MDS or normal donors. Although lenalidomide also induced the degradation of IKZF1 in myeloid cells (Fig. 1a, b), overexpression of IKZF1 had a similar effect on del(5q) MDS, normal karyotype MDS, and normal donor CD34+ cells, suggesting that degradation of IKZF1 does not explain the therapeutic window of lenalidomide in del(5q) MDS (Extended Data Fig. 5). These findings demonstrate that increased expression of CSNK1A1 specifically rescues del(5q) cells from lenalidomide treatment.

**Species–specific effects of lenalidomide**

We next sought to use a conditional knockout mouse model to determine whether haploinsufficiency for CSNK1A1 sensitizes cells to lenalidomide treatment15. In initial experiments, we found that lenalidomide did not decrease CK1α protein levels in mouse Ba/F3 cells, primary murine leukaemia cells, or mice treated in vivo (Fig. 4a, b and Extended Data Fig. 6a–d), suggesting that mouse cells are intrinsically resistant to IMiD compounds. Consistent with these findings, mice do not develop the limb malformations observed in human embryos exposed to thalidomide22 and murine multiple myeloma cells do not respond to lenalidomide23. Since CRBN is the direct protein target of lenalidomide, we examined whether expression of human CRBN could confer lenalidomide sensitivity to mouse cells. Overexpression of human, but not mouse, CRBN in mouse Ba/F3 cells resulted in a lenalidomide-dependent decrease of CK1α protein levels, implying that amino acid differences between mouse Crbn and human CRBN are responsible for the species-specific response to lenalidomide (Fig. 4a, b and Extended Data Fig. 6c, d).

To identify the amino acids responsible for this difference, we tested human/mouse CRBN chimaeric proteins for their ability to confer lenalidomide-induced CK1α degradation in mouse Ba/F3 cells (Fig. 4a). Lenalidomide sensitivity was determined by the ratio of the percentage of GFP-positive cells in the lenalidomide condition to the percentage of GFP-positive cells in the DMSO condition after 5 days of treatment. A ratio greater than 1 for the CRBN or CSNK1A1 vector but not for the empty vector indicates that CSNK1A1 expression reduces lenalidomide sensitivity. Further information about the patients is given in Extended Data Fig. 5d.

**Figure 3** | Ectopic CSNK1A1 overexpression reduces lenalidomide sensitivity in primary MDS del(5q) cells. CD34+ cells derived from patient or control bone marrow were transduced with a lentiviral vector overexpressing CSNK1A1 and GFP or an empty control vector and treated with DMSO or 1 μM lenalidomide. Results are reported as a ratio of the percentage of GFP+ cells in the lenalidomide condition to the percentage of GFP+ cells in the DMSO condition after 5 days of treatment. A ratio greater than 1 for the CSNK1A1 vector but not for the empty vector indicates that CSNK1A1 expression reduces lenalidomide sensitivity. Further information about the patients is given in Extended Data Fig. 5d.

**Figure 4** | Amino acid changes in CRBN explain species-specific lenalidomide effects. a. Effect of the expression of human CRBN, mouse Crbn, chimaeras of human and mouse CRBN (mouse–human and human–mouse, breakpoint at residue 221 (human)/225 (mouse)) and variants of the mouse–human chimaera where single amino-acids in the C terminus were mutated to their corresponding mouse residue on lenalidomide-dependent CK1α degradation in mouse Ba/F3 cells. b. Expression of mouse Crbn(W399F) restores lenalidomide-dependent CK1α degradation in mouse Ba/F3 cells. See also Extended Data Fig. 6d. c. Effect of CRBN, Crbn and Crbn(I391V) on lenalidomide sensitivity of an IKZF1-luciferase fusion protein expressed in human 293T cells. Data are mean ± s.e.m. (n = 3 biological replicates). d. Alignment of human and mouse CRBN IMiD binding region. Non-conserved amino acids are red. Amino acids involved in IMiD binding14,15 are indicated by blue bars. Mouse W403 is indicated with a green bar. e. Superposition of the IMiD binding domains of human CRBN (blue, PDβ accession 4TZ4) and mouse Crbn (yellow, PDβ accession 4TZC). Residues are labelled according to human isoform 2 (blue numbers) and mouse isoform 2 (yellow numbers). f. The V387 residue is indicated on the surface of human CRBN with a black arrow. g. The corresponding mouse residue, I391, is indicated on the surface of mouse Crbn with a black arrow. Mouse W403 is indicated by a red arrow. Results are representative of 3 (a, b, c) independent experiments. Uncropped blots are shown in Supplementary Fig. 1.
human CRBN (Fig. 4a). Substitution of the isoleucine at this position in mouse Crbn for the human valine (Crbn(I391V)) was sufficient to confer lenalidomide-induced CK1α degradation in mouse cells (Fig. 4b and Extended Data Fig. 6c, d). Similar effects of these two point mutants were observed on lenalidomide-induced degradation of IKZF1 and IKZF3 in human 293T cells (Fig. 4c and Extended Data Fig. 6e, f), suggesting that a single amino acid change in CRBN determines lenalidomide-responsiveness for multiple substrates.

We modelled the effects of this mouse-human amino acid substitution on CRBN–IMiD drug complex4,25. V387 of human CRBN (equivalent to I391 of mouse Crbn) is located in the IMiD drug binding region of CRBN, but does not directly interact with lenalidomide (Fig. 4d). To investigate how the substitution of isoleucine for valine in mouse Crbn confers lenalidomide-responsiveness, we superimposed the structures for the mouse and human IMiD-binding regions bound to lenalidomide as solved in Chamberlain et al. (2014)25. No backbone changes are present at the site of the valine-isoleucine species differences (Fig. 4e), but the isoleucine residue is well-defined in the electron density with the long arm of the side chain oriented towards the indole NH moiety of W403 in the mouse structure (Extended Data Fig. 7). The increase in steric bulk of the isoleucine side chain, relative to valine, results in a bulge in the solvent accessible surface of the mouse protein adjacent to both W403 and lenalidomide (Fig. 4f, g). It has been proposed that IMiD binding produces a hotspot for substrate interactions by placement of the hydrophobic phthalimide or isoindolinone ring in an environment of potential hydrogen bond donors and acceptors from the surface of CRBN25. In this case, the larger side chain of the isoleucine residue found in rodents may sterically clash with substrate proteins such as IKZF1 and CK1α, blocking access to key hydrogen bonds from CRBN, such as from the indole NH from tryptophan 403 (mouse numbering). Steric clashes and occlusion of key bonds with substrate proteins thereby provides a potential explanation for why IMiD compounds bind mouse Crbn1 but do not promote degradation of IKZF1 and CK1α.

Having determined the mechanism of lenalidomide resistance in mouse cells, we expressed the Crbn(I391V) cDNA in haematopoietic cells from Csnk1a1 conditional knockout mice to determine the effects of Csnk1a1 haploinsufficiency on lenalidomide sensitivity. We isolated

**Figure 5 | Effects of lenalidomide treatment on Csnk1a1+/− mouse haematopoietic cells.** a, Csnk1a1+/− Mx1Cre+ or Mx1Cre− c-Kit+ haematopoietic stem and progenitor cells (CD45.2) and competitor cells (CD45.1) were transduced with Crbn(I391V), mixed in equal ratios, and treated with lenalidomide or DMSO. The relative percentage of CD45.1+/− and CD45.2+/− cells was followed by flow cytometry over 5 days. b, Effects of 0.1 μM lenalidomide on the chimaerism of Csnk1a1+/− Mx1Cre+, Csnk1a1+/− Mx1Cre−, or Mx1Cre− cells (CD45.2) transduced with Crbn(I391V) in comparison to CD45.1 competitor cells. Data are shown as mean ± s.e.m., n = 3 biological replicates. c, Quantitative RT–PCR analysis of p21 expression in Csnk1a1+/− or control cells transduced with Crbn(I391V) and treated with DMSO or lenalidomide. Data are normalized to DMSO and shown as mean ± s.d., n = 3 biological replicates. d, Ratio of CD45.2+ cells and CD45.1+ cells in late apoptosis (Annexin V+/−/PI−) after transduction with Crbn(I391V) and four day treatment with 0.1 μM lenalidomide. CD45.2+ cells are either Csnk1a1+/− Mx1Cre+ or Mx1Cre−. Data are normalized to DMSO treatment. Data are mean ± s.e.m., n = 4 biological replicates. Results for b, c, and d are representative of three independent experiments with hCRBN or Crbn(I391V). P values are from an unpaired two-sided t-test.
CD45.2+ c-Kit+ haematopoietic stem and progenitor cells from Csk1a1+/-Mx1Cre+ and Mx1Cre+ control littermates treated with poly (LC) to induce gene excision in haematopoietic cells. We transduced these cells with a retroviral vector expressing Crbn138IV, and cultured them in competition with similarly transduced isogenic CD45.1 c-Kit+ cells in the presence or absence of lenalidomide (Fig. 5a). Lenalidomide had no effect on control cells, but Csk1a1+/-Mx1Cre+ cells were significantly depleted in the presence of lenalidomide (Fig. 5b). The enhanced sensitivity of Csk1a1+/-Mx1Cre+ cells to lenalidomide was associated with induction of the p53 target gene p21 (Fig. 5c) and increased levels of apoptosis (Fig. 5d), and was rescued by heterozygous deletion of Trp53 (Fig. 5b), demonstrating a critical down-stream role for the p53 pathway. These results are consistent with the clinical observation that TP53 mutations confer lenalidomide resistance in MDS with del(5q)26.

Differential substrate specificity

Thalidomide, lenalidomide, and pomalidomide target IKZF1 and IKZF3 for ubiquitination and degradation and are active in multiple myeloma, but only lenalidomide has been shown to be clinically effective in del(5q) MDS2,5–7. We therefore asked whether different thalidomide analogues induce degradation of the same substrates. We used tandem mass tag (TMT) quantitative proteomics27 in the MDS-L cell line to compare the activities of lenalidomide and CC-122, a novel CRBN-binding agent that shares the glutarimide ring and has recently entered clinical trials (Fig. 6a). As expected, treatment with lenalidomide significantly decreased protein levels of both IKZF1 and CK1z. In striking contrast, treatment with 1 μM CC-122 caused an even greater decrease in IKZF1 (P = 2.77 × 10^-10, 72 h) than 10 μM lenalidomide (P = 2.10 × 10^-8, 72 h), but had no effect on CK1z protein levels (P > 0.05) (Fig. 6b and Extended Data Fig. 8a, b).

We confirmed the TMT mass spectrometry findings by western blot for IKZF1 and CK1z in MDS-L and KG-1 cells (Fig. 6c and Extended Data Fig. 8c, d). While all compounds induced degradation of IKZF1, thalidomide and CC-122 did not affect CK1z protein levels, even at high concentrations, and pomalidomide had only weak effects on CK1z protein levels. Although CC-122 has a greater potency than lenalidomide for degradation of IKZF1 and IKZF3, it was ineffective in decreasing CK1z protein levels compared to lenalidomide, suggesting that subtle chemical modifications can affect substrate preference (Fig. 6a–c). Furthermore, treatment with excess CC-122 abrogated the thalidomide-induced degradation of CK1z, demonstrating that lenalidomide and CC-122 compete for the same glutarimide binding site on CRBN (Fig. 6d). Consistent with the role of CK1z as a negative regulator of Wnt signalling, we observed increased levels of β-catenin after treatment with lenalidomide but not CC-122 or thalidomide (Extended Data Fig. 8e–g). These experiments demonstrate that despite structural similarity, the substrate specificities of thalidomide analogues differ. Notably, only lenalidomide has a strong effect on CK1z, suggesting that it may indeed be the most proper modulator of CRL4<sub>CRBN</sub> for the treatment of del(5q) MDS (Fig. 6e).

Intriguingly, lenalidomide, but not thalidomide or pomalidomide, has been reported to induce the formation of two β-strands composed of CRBN residues 346–36325. Although conformational differences are difficult to interpret in the absence of a substrate-bound structure, the formation of these β-strands is expected to make significant changes in the surface of CRBN near the IMiD binding site27 and thus it may contribute to the differential recruitment of IKZF1 and CK1z. The interaction of specific thalidomide analogues with particular substrates may therefore be governed by unique structural determinants, revealing the biological and clinical potential for members of this class of drugs to induce degradation of distinct sets of proteins.

Discussion

We demonstrate that lenalidomide targets CK1z for degradation, and that heterozygous deletion of CSNK1A1 in del(5q) MDS provides a therapeutic window for selective targeting of the malignant cells by lenalidomide. The concept that genes within heterozygous deletions could cause vulnerabilities in cancer cells was first proposed 20 years ago28 and has been more recently demonstrated as CYCLOPS genes (cancer vulnerabilities unveiled by genomic loss) in cell lines29. Our data demonstrate that in del(5q) MDS, lenalidomide-induced degradation of CK1z below haploinsufficient levels induces p53 activity and growth inhibition, as CK1z is a negative regulator of p53. Deletion of contiguous genes on chromosome 5q, such as RPS14, may further sensitize del(5q) cells to p53 activation30,31. This mechanism of activity is consistent with the acquisition of TP53 mutations in del(5q) MDS patients who develop resistance to lenalidomide. Degradation of CK1z may also contribute to other clinical effects of lenalidomide such as activity in the activated B-cell (ABC) subtype of diffuse large B-cell lymphoma32 and lenalidomide-induced myelosuppression. Further investigation is required to determine the complete biological effects from degradation of each substrate.

Lenalidomide, like thalidomide and pomalidomide, binds CRBN and induces degradation of specific substrates. We found that a single amino acid difference between mouse and human CRBN renders mouse cells insensitive to IMiD compounds. This discovery enabled us to demonstrate, using a genetically engineered mouse model, that Csk1a1 haploinsufficiency sensitizes cells to lenalidomide. Non-conserved amino acid changes in CRBN may also explain why thalidomide does not cause teratogenity in mice and was approved for use in pregnant women, leading to the birth of more than 10,000 newborns with limb malformations and other disabilities.

Thalidomide, lenalidomide, and pomalidomide all induce CRL4<sub>CRBN</sub>-mediated degradation of IKZF1 and IKZF3, but the subtle differences in chemical structure between these molecules cause dramatic changes in potency. We now find that thalidomide and a novel compound, CC-122, induce the degradation of IKZF1 but not CK1z. CC-122 may have a greater therapeutic window for the treatment of B cell malignancies and other diseases that depend on IKZF1 and IKZF3, but would not be predicted to have activity in del(5q) MDS.

CC-122, like thalidomide and its analogues, has a glutarimide ring that anchors the molecule in CRBN, and structural variation in the remainder of the molecule is thought to determine substrate specificity3,4,5. These findings provide evidence that thalidomide-related molecules have distinct biological activities, mediated by degradation of distinct sets of substrates, and that these compounds will be the first in a larger class of drugs with therapeutic utility through the targeting of specific proteins for degradation.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Information The original mass spectra may be downloaded from MassIVE (http://massive.ucsd.edu) using the identifier: MSV000079014. The data are accessible at ftp://massive.ucsd.edu/MSV000079014. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to B.L.E. (bebert@partners.org).
METHODS

The experiments were not randomized, and no statistical methods were used to predetermine sample size.

Reagents. Lenalidomide (Toronto Research Chemicals, Selleck Chemicals, and Celgene), Histone H2A (Millipore and Celgene), Ponalidomide (Selleck Chemicals and Celgene), MG-132 (Selleck Chemicals), CC-122 (Celgene), PR619 (Lifesciences), MLN4924 (Active Biochem), and Leptomycin B (Santa Cruz) were dissolved in DMSO at 10 to 100 mM and stored at −20 °C for up to 6 months. For cell culture experiments drugs were diluted at least by 1:1,000 so that the final DMSO concentration was 0.1% or lower.

Cell lines. KG-1, Ba/F3, K562, MMIS, Jurkat, HEL, and 293T cells were obtained from American Type Culture Collection (ATCC) and their identity was not further authenticated. MDS-L cells were provided by Kaoru Tohyama, Kawasaki Medical School (Japan). Cells were cultured in RPMI 1640 (Mediatech) or DMEM (Mediatech) supplemented with 10% heat-inactivated fetal bovine serum (FBS/Omega Scientific) and 1% penicillin, streptomycin, and 1-glutamine (Mediatech). Cells were grown at 37 °C in a humidified incubator under 5% CO2. Ba/F3 cells were cultured in the presence of 10 ng ml−1 mouse IL-3 (Milteny) and MDS-L cells were cultured with 10 ng ml−1 human GM-CSF. 293T cells were transfected using TransIT-LT1 (Mirus Bio) according to the manufacturer’s protocol. Cell lines were intermittently tested for mycoplasma.

Cell culture and treatment for K-cGG and proteome profiling. KG-1 cells were cultured for 2 weeks (−6 cell doublings) in RPMI depleted of t-arginine and t-lysine (Caisson Labs Inc.) and supplemented with 10% dialysed FBS (Sigma) and t-arginine (Arg6) and t-lysine (Lys0) (light). 15N4-L-arginine (Arg10) and 15N2-L-lysine (Lys8) (heavy) to generate light-, medium- and heavy-labelled fractions (final fraction 1 250 mm column (Agilent, 3.5 μm bead size)) were then subjected to a moderated t-test to assess statistical significance. This statistic is similar to the ordinary t-statistic, with the exception that the standard errors are calculated using an empirical Bayes method using information across all proteins, thereby making inference about each individual protein more robust. The nominal P values arising from the moderated t-statistics are corrected for multiple testing by controlling the false discovery rate (FDR), as proposed by Benjamini and Hochberg. Proteins with an FDR adjusted P value of less than 0.05 were deemed to be reproducibly regulated. Figures containing scatter plots of SILAC data show all points regardless of the reproducibility measure. Statistical significance was assessed using only reproducible data points.

The original mass spectra may be downloaded from MassIVE (http://massive.ucsd.edu) using the identifier: MSV000079014. The data are accessible at ftp://massive.ucsd.edu/MSV000079014. Plasmids and virus constructs. The following cDNAs were cloned in the pRSF91 retrovirus backbone (gift of C. Baum, Hanover Medical School) or pEFTa-RRES- XV (gift of C. Baum). CSNK1A1 Isol1 (gift of W. G. Kaelin), CSNK1E (ccsbBroadEN_00379), mouse Crbn Isolform 2 (Thermo Scientific), and human CRBN Isolform 2 (ccsbBroadEn_08244). Human IKZF1 isolform 1 was synthesized using gBlock(s) (IDT) with internal BstXI and BsrGI sites removed using synonymous substitutions. For certain
experiments was immunoprecipitated overnight using anti-Flag M2 Affinity Gel (Sigma-Aldrich) in the presence of 10 μM MG132 and DMSO or 1 μM lenalidomide. The beads were washed 3 times with IP lysis buffer (Pierce) and protein was eluted from the affinity gel with 250 μg ml⁻¹ Flag peptide (Sigma) after incubation for 30 min at 4 °C. Protein lysates were then analysed as described above.

For immunoprecipitation of endogenous CRBN 5 × 10⁶ 293T cells were treated with DMSO or 10 μM lenalidomide and 10 μM MG132 for 4 h. Protein lysates were incubated overnight at 4 °C with 1 μg of a polyclonal mouse anti-CRBN antibody (abcam) in the presence of lenalidomide or DMSO and MG132. Protein G Sepharose beads were added for one hour. The beads were washed once with IP lysis buffer (Pierce) and protein was eluted from the beads by incubation with LDS loading buffer (Life Technologies) at 70 °C for 10 min.

In vivo ubiquitination. For assessment of endogenous ubiquitination of CK1α 2 × 10⁷ KG-1 cells were treated with DMSO, 1 μM lenalidomide for 4 h and then lysed in IP lysis buffer containing 10 mM NEM and 10 μM MG132. Ubiquitinated proteins were pulled down by Ubiquitin 1 Tandem UBA (TUBE2) Agarose (Boston Biochem) for 4 h at 4 °C and washed 3× with IP lysis buffer. Protein was eluted by incubation with Laemmli buffer (Bio rad) at 95 °C for 5 min, separated by SDS–PAGE, transferred to PVDF membrane and probed with anti-CK1α.

In vitro ubiquitination. 293T cells were transfected with either HA-CK1α or Flag–CRBN-expressing vectors. After 48 h, cells were lysed in Pierce IP lysis buffer (Thermo Scientific) and immunoprecipitated overnight with Flag–Sepharose beads (Anti-Flag M2 Affinity Gel, Sigma) or HA-Sepharose beads (EZView Red anti-HA affinity gel, Sigma). The beads were washed 3× in IP lysis buffer and 2× in E3 Ligase Reaction buffer (Boston Biochem) and eluted with 250 μg ml⁻¹ Flag peptide (Sigma) or 100 μg ml⁻¹ HA peptide for 30 min at 4 °C. The eluates were mixed in a 1:1 ratio and added to a ubiquitination reaction mixture containing 200 nM E1 (UBE1), 2 μM UbH5a, 1 μM UbH5c, 1 μg l⁻¹ K9 ubiquitin, 1 μg ubiquitin aldehyde, 1× Mg-ATP, 1× E3 Ligase Reaction Buffer (all Boston Biochem), 10 μM MG132, 100 nM MG101 and 1 μM lenalidomide, 10 μM lenalidomide, or DMSO (1:1:00) as appropriate in a total volume of 25 μl.

Negative controls did not include E1 and E2 enzymes. After a 90 min incubation at 30 °C, the reaction was denatured by adding 5× SDS containing loading buffer (Boston Biochem), boiled at 95 °C for 5 min, separated by SDS–PAGE and transferred to a PVDF membrane in order to detect HA-CK1α and its ubiquiti- nated forms with a CK1α-specific antibody. The membrane was then stripped and re-probed with anti-Flag antibody. Immunofluorescence. 50,000 293T cells were grown on Lab-Tek 8 well chamber slides (Nunc) for 24 h and then treated with DMSO or 10μM lenalidomide for various durations. At the conclusion of treatment, the media was decanted and the wells were washed 1× with PBS. Cells were fixed in 4% formaldehyde in PBS for 15 min, washed 3× 5 min in PBS and blocked for 1 h at room temperature in PBS with 0.3% Tween-20 and 5% BSA. Primary antibody was anti-CRBN (C-19, Santa Cruz), which was diluted 1:100 in PBS with 0.3% Tween-20 and 1% BSA (antibody dilution buffer) and incubated for 2 h at room temperature. After 3× 5 min washes in PBS, Alexa Fluor 488 donkey anti-Goat (Life Technologies) was added at the dilution, incubated for 30 min at room tempera- ture. After 3× 5 min washes in PBS slides were coverslipped with Vectashield mounting media with DAPI (Vector Laboratories). Slides were analysed by fluor- escence microscopy at 100× using a Nikon Eclipse 90i and NIS Elements. Channels were merged using ImageJ.

Purification, culture, and lentiviral infection of human CD34⁺ cells for shRNA experiments. Research cord blood units were obtained from The New York Blood Center according to an Institutional Review Board-approved protocol. Cord blood CD34⁺ haematopoietic cells were isolated from Ficol purified PBMCs from an Indirect CD34 MicroBead kit (Miltenyi) and an Auto MACS Pro purification kit (Miltenyi) using the standard procedure. The cells were cultured in serum-free media (SEFM, StemSpan) containing 50 ng ml⁻¹ recombinant human TPO (Miltenyi), 40 ng ml⁻¹ human FLT3 ligand (Miltenyi), 25 ng ml⁻¹ recombinant human SCF (Miltenyi), and 10 ng ml⁻¹ IL-3 (Miltenyi). For shRNA experiments, CD34⁺ cells were transduced with a VSV-G pseudotyped TRC pLKO.0 lentiviral vector expressing GFP instead of the puromycin resistance gene. Infection was performed after 24 h in culture in a 96-well using spinnfection in the presence of 2 μg ml⁻¹ polybrene (hexadimethrine bromide, Sigma). 48 h after transduction the number of transduced cells was analysed by flow cytometry and was used as baseline. Then cells were cultured in 1 μM lenalidomide or DMSO and the relative number of infected cells was assessed by flow cytometry for 3 weeks.

Purification, culture, and lentiviral infection of patient samples. Viably frozen bone marrow mononuclear cells were obtained from healthy donors or patients with del(5q) MDS according to IRB approved protocols at the University of Pennsylvania and Roswell Park Cancer Institute. Informed consent was obtained
from all subjects. Samples were thawed and CD34⁺ haematopoietic cells were isolated 20–24 h later using an Indirect CD34 MicroBead kit (Miltenyi) and an Auto MACS Pro (Miltenyi). Cells were grown in serum free media (SFEM, StemSpan) supplemented with 25 ng ml⁻¹ SCF, 40 ng ml⁻¹ FLT3 ligand, 50 ng ml⁻¹ thrombopoietin, 40 μg ml⁻¹ lipids, 100 U ml⁻¹ Pen/Strep and 2 mM glutamine. 6–8 h after CD34⁺ isolation, cells were transduced with concentrated VSV-G pseudotyped pEFea-GFP-IREs-hcSNK1A1, pEFea-GFP-IREs-hIKZF1 or empty vector control virus via spinfection in the presence of 4 μg ml⁻¹ polybrene (Sigma, diluted to 2 μg ml⁻¹ after spinfection). After 3 days, the initial percentage of transduced cells was determined by flow cytometry and remaining cells were split to treatment with either DMSO or 1 μM lenalidomide. The relative abundance of transduced cells in each condition was assessed after 5 days by flow cytometry. Control cord-blood CD34⁺ cells were isolated as above. Adult bone marrow CD34⁺ cells were purchased as single-donor lots from AllCells (Alameda, CA). The number of replicates for each patient sample, vector, and treatment was limited by the number of cells available and was as follows: one for samples 1–5, 8, 9 and 12; two for samples 6, 7 and 10; three for sample 11; four for sample 13. Samples were combined from three experiments.

For qPCR validation of CSNK1A1 or IKZF1 mRNA expression, cord blood CD34⁺ cells were transduced with lentivirus expressing GFP and the cDNA of TP53 sequencing of patient samples. Genomic DNA was extracted from the CD34⁺ fraction of the patient bone marrow samples using a DNA Blood Mini Kit (Qiagen). PCR and sequencing was performed as described in the International Agency for Research on Cancer’s Direct Sequencing Protocol (http://p53.iarc.fr/download/t53_directsequencing_iarc.pdf). Mutations were identified using Mutation Surveyor (Softgenetics). Benign polymorphisms were identified using the International Agency for Research on Cancer’s Portal (http://p53.iarc.fr/TP53GeneVariations.aspx).

For in vivo treatment, female C57BL/6 mice were treated with once daily intraperitoneal injection of 10 mg per kg lenalidomide dissolved in DMSO and diluted in 100 μl PBS or with DMSO in 100 μl PBS. Bone marrow was harvested after 14 days and lysed with IP lysis buffer (Pierce). Due to experimental design, the treatment groups could not be blinded.

**Quantitative mass spectrometry in MDS-L cells.** MDS-L multiplexed quantitative mass spectrometry samples were processed and analysed by the Thermo Fisher Scientific Center for Multiplexed Proteomics at Harvard Medical School. Samples were prepared as previously described³ with the following modification. All solutions were reported at final concentrations. Lysis buffer (8 μl urea, 1% SDS, 50 mM Tris pH 8.5, protease and phosphatase inhibitors from Roche) was added to the cell pellets to achieve a protein concentration between 2–8 mg ml⁻¹. A micro-BCA assay (Pierce) was used to determine the final protein concentration in the cell lysate. Proteins were reduced and alkylated as previously described. Proteins were precipitated using methanol/chloroform. In brief, four volumes of methanol was added to the cell lysate, followed by one volume of chloroform, and finally three volumes of water. The mixture was vortexed and centrifuged to separate the chloroform phase from the aqueous phase. The precipitated protein was washed with one volume of ice cold methanol. The washed precipitated protein was allowed to air dry. Precipitated protein was resuspended in 4 M urea, 50 mM Tris pH 8.5. Proteins were first digested with LysC (1:50; Promega) for 12 h at 37 °C. The LysC digestion is diluted down to 1 M urea, 50 mM Tris pH 8.5 and then digested with trypsin (1:100; enzyme-protein) for another 8 h at 25 °C. Peptides were desalted using a C₁₈ solid phase extraction cartridges as previously described. Dried peptides were resuspended in 200 mM EPPS, pH 8.0. Peptide quantitation was performed using the micro-BCA assay (Pierce). The same amount of peptide from each condition was labelled with tandem mass tag (TMT) reagent (1:3; peptide:TMT label) (Pierce). The 6-plex and 10-plex labelling reactions were performed for 2 h at 25 °C. Modification of tyrosine residue with TMT was reversed by the addition of 5% hydroxyl amine for 15 min at 25 °C. The reaction was quenched with 0.5% TFA and samples were combined at a 1:1:1:1:1:1 ratio for 6-plex experiments or 1:1:1:1:1:1:1:1:1:1:1:1 for 10-plex experiments. Combined samples were desalted and offline fractionated into 24 fractions as previously described.

**Liquid chromatography-MS3 spectrometry (LC-MS/MS) in MDS-L cells.** 12 of the 24 peptide fractions from the basic reverse phase step (every other fraction) were analysed with an LC-MS3 data collection strategy on an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific) equipped with a Proxeon Easy nLC 1000 for online sample handling and peptide separations. Approximately 5 μg of peptide resuspended in 5% formic acid + 5% acetonitrile was loaded onto a 100-μm inner diameter fused-silica micro capillary with a needle tip pulled to an internal diameter less than 5 μm. The column was packed in-house to a length of 35 cm with a C₁₈ reverse phase resin (GP118 resin 1.8 μm, 120 Å, Sepax Technologies). The peptides were separated using a 120 min linear gradient from 1% methanol to 90% methanol at 0 (1000 ng peptide) and 120 ng (200 000 ng peptide) after CN digestion was diluted down to 100 nM with buffer A (3% ACN + 0.125% formic acid) at a flow rate of 600 nl min⁻¹ across the column. The scan sequence for the Fusion Orbitrap began with an MS1 spectrum (Orbitrap analysis, resolution 120,000, 400–1400 m/z scan range, AGC target 2 × 10⁶, maximum injection time 100 ms, dynamic exclusion of 75 s). Top speed (1 s) was selected for MS2 analysis, which consisted of CID (quadrupole isolation set at 0.5 Da and ion trap analysis, AGC 4 × 10⁶, NCE 35, maximum injection time 150 ms). The top ten precursors from each MS2 scan were selected for MS3 analysis (synchronous precursor selection), in which precursors were fragmented by HCD before Orbitrap analysis (NCE 55, max AGC 5 × 10⁶, maximum injection time 150 ms, isolation window 2.5 Da, resolution 15000, product ions selected for MS3). Combined samples were desalted and offline fractionated into 24 fractions as previously described.

**LC-MS3 data analysis for MDS-L cells.** A suite of in-house software tools were used to for. RAW file processing and controlling peptide and protein level false discovery rates, assembling proteins from peptides, and protein quantification from peptides as previously described.³ MS/MS spectra were searched against a Uniprot human database (February 2014) with both the forward and reverse sequences. Database search criteria are as follows: tryptic with two missed cleavages, a precursor mass tolerance of 50 ppm, fragment ion mass tolerance of 1.0 Da, static alkylation of cysteine (57.02146 Da), static TMT labelling of lysine residues and N termini of peptides (229.16932 Da), and variable oxidation of methionine (15.99491 Da). TMT reporter ion intensities were measured using a 0.03 Da window (6-plex) or 0.003 Da window (10-plex) around the theoretical m/z for each reporter ion in the MS3 scan. Peptide spectral matches with poor quality MS3 spectra were excluded from quantitation (<100 summed signal-to-noise across 6 channels and <0.5 precursor isolation specificity for 6-plexes or <200 summed signal-to-noise across 10 channels and <0.5 precursor isolation specificity for 10-plexes).
A moderated t-test was applied across all proteins to assess statistical significance. This statistic is similar to the ordinary t-statistic, with the exception that the standard errors are calculated using an empirical Bayes method using information across all proteins, thereby making inference about each individual protein more robust. This test assumes normality and uses an estimated standard deviation intended to handle relatively few replicates per condition. Posterior residual standard deviations are used in place of ordinary standard deviation in the moderated t-test applied. This shrinkage of protein-wise sample variances to a pooled estimate provides more stable inference when sample numbers are reduced. The nominal \( P \) values arising from the moderated t-statistic are corrected for multiple testing by controlling the false discovery rate (FDR), as proposed by Benjamini and Hochberg. Proteins with an FDR adjusted \( P \) value of less than 0.05 were deemed to be reproducibly regulated.

**IMiD compound substrate selectivity in KG-1 and MDS-L cells.** Cells (2–4 \( \times 10^6 \)) were plated in 10-cm dishes and incubated overnight (18–24 h). Cells were treated with DMSO, lenalidomide (1–10 \( \mu \)M), CC-122 (1–10 \( \mu \)M), pomalidomide (1–10 \( \mu \)M), or thalidomide (10–100 \( \mu \)M) for 6 h. Drug-treated cells were collected, washed with PBS and cell pellets were lysed in RIPA buffer containing protease and phosphatase inhibitors for 30–45 min followed by sonication and centrifugation. Protein lysates were quantified using BCA protein assay kit and 10–15 \( \mu \)g of protein was used for western analysis. CC-122 and lenalidomide competition experiment was conducted as above except cells were pre-treated with DMSO or 10 \( \mu \)M CC-122 for 90 min followed by treatment with lenalidomide (0.3–10 \( \mu \)M) or DMSO for 6 h.

The synthesis and characterization of CC-122 is described in the Supplementary Methods section.

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38. Weekes, M. P. et al. Quantitative temporal viromics: an approach to investigate host-pathogen interaction. *Cell* **157**, 1460–1472 (2014).
Extended Data Figure 1 | Effect of lenalidomide on specific ubiquitination sites. Median log_2 ratios for different lysine residues in CK1α isoform 2, IKZF1 isoform 1, and CRBN isoform 2 for 1 or 10 μM lenalidomide-treated KG-1 cells versus DMSO-treated cells. SILAC experiments were performed in two biological replicates with flipped SILAC labelling. Only lysine residues detected in both replicates are shown. Error bars show range.
**Extended Data Figure 2 | Effect of lenalidomide in human cells.**

**a**, Time course of effect of lenalidomide treatment on CK1α protein levels in KG-1 cells. **b**, Immunoblot of CK1α protein levels in the bone marrow (1, 2) and peripheral blood (3, 4) mononuclear cells of AML patients treated with lenalidomide as part of a clinical trial. Pre-treatment samples are taken at the screen or before the first treatment (C1D1). Subsequent time points are cycle 1 day 15 (C1D15), cycle 2 day 1 (C2D1) or cycle 1 day 8 (C1D8) of lenalidomide treatment. Further details about these patients (n = 4) can be found in Extended Data Table 2. **c**, MM1S, K562, and Jurkat cells were treated with different concentrations of lenalidomide for 24 h. CK1α protein levels were detected by western blot and CSNK1A1 mRNA expression levels were measured by RQ-PCR. Data are mean ± s.d., n = 3 each with three technical replicates. **d**, Immunoblot confirming loss of CRBN expression in 293T cells with the CRBN gene disrupted by CRISPR-Cas9 genome editing. **e**, Immunoprecipitation with a CRBN-specific antibody in 293T cells treated with DMSO or 10 μM lenalidomide for 5 h in the presence of 10 μM MG132. Results in **a**, **c**, **d**, and **e** are each representative of two independent experiments. Uncropped blots are shown in Supplementary Fig. 1.
**Extended Data Figure 3** | Sequence determinants of CK1α degradation.

**a**, 293T cells were transfected with plasmids expressing Flag–CK1α isoform 1 or isoform 2 together with a human CRBN-expressing plasmid. Cells were treated with DMSO or 10 μM lenalidomide for 16 h. Cells expressing Flag–CK1α isoform 1, which contains a nuclear localization domain, were incubated in the absence or presence of the nuclear export inhibitor leptomycin B.

**b**, 293T cells expressing Flag–CK1α isoform 2 wild-type or two different point mutations identified in patient samples were treated with DMSO or 10 μM lenalidomide for 16 h.

**c**, Immunofluorescence for CK1α after treatment with DMSO or 10 μM lenalidomide. Enlarged area is indicated by a box in Merge. FITC channel represents staining for CK1α. No changes in CK1α localization are seen upon lenalidomide treatment. Experiment was performed twice in biological duplicate. In each condition, at least 25 cells were assessed.

**d**, Chimaeric proteins of casein kinase 1A1 (CK1α) and casein kinase 1E (CK1ε), which shares significant homology with CK1α but is not responsive to lenalidomide, that were used in e to determine the lenalidomide-responsive region in CK1α.

**e**, Flag-tagged (chimaeric) proteins from d were transfected in 293T cells together with a CRBN-expressing plasmid. Cells were treated with 1 μM lenalidomide for 24 h and protein was detected with a Flag-specific antibody. Data are representative of two (a, c), three (b) or four (e) independent experiments. Uncropped blots are shown in Supplementary Fig. 1.
Extended Data Figure 4 | CSNK1A1 knockdown increases lenalidomide sensitivity in haematopoietic cells. a, Knockdown validation by western blot. b–d, CD34⁺ cells were transduced with GFP-labelled lentivirus expressing either control shRNA targeting luciferase (b) or shRNA targeting CSNK1A1 (c, d) and treated with DMSO or 1 μM lenalidomide. The percentage of GFP⁺ cells was assessed by flow cytometry over time. Results are representative of 3 independent experiments each with n = 3 biological replicates.
Extended Data Figure 5 | Expression of CSNK1A1 and IKZF1 in patient samples. a, mRNA expression of CSNK1A1 in cord blood CD34+ cells infected with lentivirus expressing human CSNK1A1 or empty vector. CD34+ cells were infected with GFP-tagged lentivirus and GFP+ cells were sorted three days later. Values are mean ± s.d., n = 4 biological replicates, each with 3 technical replicates. b, mRNA expression of IKZF1 in cord blood CD34+ cells infected with lentivirus expressing IKZF1 or empty vector as in a. Values are mean ± s.d., n = 3 biological replicates, each with 3 technical replicates. c, CD34+ cells derived from patient or control bone marrow were transduced with a lentivirus expressing human IKZF1 (hIKZF1) and GFP or an empty control vector and treated with DMSO or 1 μM lenalidomide. The percentage of GFP+ cells was assessed by flow cytometry after five days for each vector-drug combination. Results are reported as a ratio of the percentage of GFP+ cells in the lenalidomide condition to the percentage of GFP+ cells in the DMSO condition. Results are combined from three experiments. d, Characteristics of patient samples used for CSNK1A1 and IKZF1 expression experiments. Results of TP53 sequencing, including exons with adequate coverage, is given in the rightmost column. All samples sequenced had wild-type TP53. ND, not done due to limited patient material. WT, TP53 exon sequence has only known benign polymorphisms.

| Sample No. | Karyotype | Category | TP53 Status (exons sequenced) |
|------------|-----------|----------|------------------------------|
| 1          | del(5q) MDS / 47,XX,del(5)[q15q33]+[1]/46,XX[3] | del(5q) MDS Pre-Lenalidomide | WT (2-11) |
| 2          | 46,XX,del(5)[q13q33][17] / 46,XX[3] | del(5q) MDS Pre-Lenalidomide | ND |
| 3          | 46,XY,del(5)[q15q35][23] / 46,XY[2] | del(5q) MDS Pre-Lenalidomide | ND |
| 4          | 46,XX,del(5)[q15q33],(12;13)(q15;q11)[16]/46,XX[3] | del(5q) MDS Pre-Lenalidomide | WT (2-11) |
| 5          | 46,XX,del(5)[q13q33][14] / 46,XX[6] | del(5q) MDS Pre-Lenalidomide | WT (2-11) |
| 6          | normal / 46,XY[6] | Normal Karyotype MDS | WT (4-7, 9-10) |
| 7          | normal / 46,XY[6] | Normal Karyotype MDS | WT (2-11) |
| 8          | normal / 46,XY[6] | Normal Karyotype MDS | WT (2-11) |
| 9          | normal / 46,XY[6] | Normal Karyotype MDS | WT (3-4, 6-11) |
| 10         | normal / 46,XY[6] | Normal Karyotype MDS | ND |
| 11         | normal / 46,XY[6] | Normal Karyotype MDS | ND |
| 12         | normal / 46,XY[6] | Normal Karyotype MDS | ND |
| 13         | normal / 46,XY[6] | Normal Karyotype MDS | ND |
| ND         | TP53 sequencing not done due to limited sample size or normal donor sample | | |
| WT         | TP53 sequence is wild-type (known benign polymorphisms only) in the exons sequenced | | |
**Extended Data Figure 6** | Effect of lenalidomide on mouse cells.  

**a**, CK1α protein levels are unaffected in mouse Ba/F3 cells and primary mouse AML cells (MA9) treated with a range of lenalidomide doses. Data are representative of two independent experiments (n = 2).  

**b**, CK1α expression in bone marrow cells of mice treated with DMSO (n = 5) or lenalidomide (n = 5).  

**c**, CK1α protein levels in Ba/F3 cells transduced with empty vector, mouse Crbn, human CRBN or Crbn(I391V) and treated with lenalidomide.  

**d**, Quantification of CK1α protein levels in Ba/F3 cells using ImageJ. Graphs show the fraction of normalized CK1α protein levels as compared to control (DMSO) treated cells of the respective line. Bars represent mean ± s.e.m. from three independent experiments as in **c**.  

**e**, Effect of lenalidomide on an IKZF3–luciferase (e) and IKZF1–luciferase fusion protein (f) in 293T cells expressing human, mouse or different chimaeras or mutations of CRBN. Data are shown as mean ± s.e.m. (n = 3, biological replicates) and are representative of three (e) or five (f) independent experiments. Uncropped blots are shown in Supplementary Fig. 1.
Extended Data Figure 7 | Difference electron density map of mouse residue I391 calculated in the absence of a side chain showing the favoured orientation of the residue. The density is contoured at 3.8σ following a single round of Refmac5 refinement.
Extended Data Figure 8 | Comparison of the effects of thalidomide derivatives. **a**, Comparison of log₂ ratios for CK1α and IKZF1 in MDS-L cells after treatment with lenalidomide or CC-122 for 24 or 72 h assessed by tandem mass tag (TMT) quantitative proteomics. Analysis was performed with n = 4 for DMSO control and n = 3 for each drug treatment time point. **b**, Adjusted P values for CK1α and IKZF1 proteomic data in MDS-L cells. **c**, Western blot validation of IKZF1 and CK1α levels in DMSO (n = 4), lenalidomide (n = 3) and CC-122 (n = 3) treated samples used for MDS-L proteomic analysis. **d**, Western blot validation of the effects of the different agents on CK1α and IKZF1 protein levels in KG-1 cells. **e**, Effect of lenalidomide, pomalidomide (Pom), and thalidomide (Thal) on protein levels of CK1α, β-catenin, and IKZF1 in KG-1 cells treated for 24 h with the indicated drug concentrations. **f**, Effect of CC-122 and lenalidomide on β-catenin protein levels in KG-1 cells after 72 h. **g**, Effect of lenalidomide on CK1α and β-catenin protein levels in HEL cells. Data are representative of two (e, g) or three (c, d) independent experiments. Uncropped blots are shown in Supplementary Fig. 1.
## Extended Data Table 1 | Statistically significant SILAC results with 1 μM lenalidomide

### (a)

| K-c-GG site | Replicate 1 | Replicate 2 | Average Log₂ fold change | adj.P.Val | Direction of change |
|-------------|-------------|-------------|--------------------------|-----------|---------------------|
| IKZF1       | 2.78        | 3.18        | 2.98                     | 7.23E-06  | up                  |
| IKZF1       | 1.73        | 2.17        | 1.95                     | 0.000497  | up                  |
| MARCB8      | 1.58        | 0.92        | 1.25                     | 0.036986  | up                  |
| CK1α        | 1.06        | 1.08        | 1.07                     | 0.035843  | up                  |
| CRBN        | -1.07       | -1.21       | -1.14                    | 0.026006  | down                |
| RNF166      | -1.49       | -1.37       | -1.43                    | 0.003102  | down                |
| RNF166      | -1.58       | -1.39       | -1.48                    | 0.003102  | down                |

### (b)

| Protein    | Replicate 1 | Replicate 2 | Average Log₂ fold change | adj.P.Val | Direction of change |
|------------|-------------|-------------|--------------------------|-----------|---------------------|
| ZNF692     | -1.89       | -2.20       | -2.05                    | 0.013806  | down                |
| IKZF1      | -1.62       | -1.54       | -1.58                    | 0.005638  | down                |
| CK1α       | -1.59       | -1.53       | -1.56                    | 0.005638  | down                |
| RNF166     | -1.41       | -1.64       | -1.52                    | 0.015257  | down                |
| ZFP91      | -0.69       | -0.69       | -0.69                    | 0.047677  | down                |
| LEMD3      | -0.66       | -0.68       | -0.67                    | 0.047677  | down                |
| NRM         | -0.64       | -0.68       | -0.66                    | 0.047677  | down                |
| LBR         | -0.67       | -0.65       | -0.66                    | 0.047677  | down                |
| UNC84A, SUN1 | -0.68     | -0.64       | -0.66                    | 0.047677  | down                |
| C12orf57    | 0.66        | 0.70        | 0.68                     | 0.047677  | up                  |

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a, List of significantly regulated K-c-GG sites with 1 μM lenalidomide vs DMSO. P-value is adjusted as described in the methods section. b, List of significantly regulated proteins with 1 μM lenalidomide vs DMSO. P-value is adjusted as described in the methods section. Average log₂ fold change of two biological replicates.
### Extended Data Table 2 | Characteristics of the patient samples from the AML-001 trial used in Extended Data Fig. 2b

| Coded Patient Number (source of cells) | Sex | Primary Race | AML Classification | Peripheral Blood Blast Count | Age at Randomization | Prior MDS History? | MDS Primary Or Secondary | Study Arm Randomized To | Cycle 1 Dosing |
|---------------------------------------|-----|--------------|--------------------|-------------------------------|----------------------|-------------------|-------------------------|--------------------------|-----------------|
| 1 (BMMC)                              | Male | White        | AML not otherwise specified | $\geq 1 \times 10^9/L$ | 71 | Yes | Primary | Lenalidomide | 50 mg daily, except drug withheld days 4-12 |
| 2 (BMMC)                              | Male | White        | AML with myelodysplasia-related changes | $\geq 1 \times 10^9/L$ | 80 | Yes | Primary | Lenalidomide | 50 mg daily, except drug withheld days 3-6 and 24-28 |
| 3 (PBMC)                              | Male | Asian        | AML with myelodysplasia-related changes | $<1 \times 10^9/L$ | 75 | No | | Lenalidomide | 50 mg daily |
| 4 (PBMC)                              | Male | White        | AML with myelodysplasia-related changes | $\geq 1 \times 10^9/L$ | 81 | Yes | Primary | Lenalidomide | 50 mg daily |

BMMC: Bone marrow mononuclear cells
PBMC: Peripheral blood mononuclear cells