Systematic identification of cancer cell vulnerabilities to natural killer cell-mediated immune surveillance

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Abstract

Only a subset of patients respond to therapies that stimulate T-cell responses to cancer, highlighting the need for alternative strategies to promote anti-cancer immunity. Here, we performed genome-scale CRISPR-Cas9 screens in a human MHC-deficient leukemic cancer cell line to systematically identify perturbations that enhance natural killer (NK) effector functions. Our screens defined critical components of the tumor-immune synapse and highlighted the importance of cancer cell interferon-γ (IFNγ) signaling in promoting NK activity. Surprisingly, we found that loss of the cullin4-RING E3 ubiquitin ligase (CRL4) substrate adaptor DCAF15 strongly sensitized cancer cells to NK-mediated clearance. DCAF15 disruption led to an inflamed state in leukemic cancer cells associated with increased expression of the lymphocyte costimulatory molecule CD80, which triggered NK activation. Biochemical analysis revealed that DCAF15 interacts directly with cohesin complex members SMC1 and SMC3. Treatment with indisulam, an anticancer drug reported to function through DCAF15 engagement, was able to phenocopy genetic disruption of DCAF15. In AML patients, reduced DCAF15 expression was associated with improved survival. These findings suggest that inhibition of DCAF15 may have useful immunomodulatory properties in the treatment of myeloid neoplasms.
Introduction

Major advances in tumor control have recently been achieved by targeting immune inhibitory signaling pathways. Treatment with “checkpoint inhibitors,” antibodies targeting PD1, PD-L1, or CTLA4, lead to durable responses across a wide range of indications, but only in a subset of patients. Treatment response is positively correlated with tumor mutational burden and infiltration of CD8+ effector T cells, which recognize tumor cells via peptides bound to major histocompatibility complex class I (MHC-I) molecules, suggesting that checkpoint inhibitors work best at clearing highly immunogenic cancers with repressed T cell responses. Substantial efforts are being made to extend the benefits of immunotherapy to additional patients. This includes approaches such as combining checkpoint inhibitors with other therapies, drugging additional lymphocyte-suppressive pathways, and boosting the activity of other arms of the immune system.

Resistance to therapy has long been a major problem in cancer treatment. Drugs targeting tumor growth processes can profoundly reduce tumor burden, but resistance invariably arises, driven by the substantial genetic and phenotypic heterogeneity present within human tumors. Recent clinical and experimental data have similarly highlighted the ability of cancer cells to escape checkpoint inhibitor-induced immune control. B2M and JAK1/2 mutations have been identified in melanoma patients with acquired resistance to checkpoint inhibitors. These mutations impair recognition of the tumor by the adaptive immune system, either by directly disrupting antigen presentation or by rendering the cells insensitive to IFNγ, an important inducer of MHC-I expression. Functional genetic screens using T cell-cancer cell cocultures have highlighted similar mechanisms of resistance in vitro. Even treatment-naïve tumors can be highly immuno-edited, presenting with IFNγ pathway mutations, reduced MHC-I expression and loss of the peptide sequences that can serve as antigens. Together, these findings highlight a critical need for therapies that can either increase MHC expression or work in a MHC-independent fashion.

Anti-tumor immunity is not solely mediated by the adaptive immune compartment. Innate immune cells, most notably natural killer (NK) cells, can have both direct tumoricidal activity and also help to fully elaborate long-lasting anti-tumor responses. NK cells are cytotoxic lymphocytes...
capable of mounting rapid responses to damaged, infected, or stressed cells, including cancer cells. T and NK cells share effector functions, releasing cytokines and exocytosing lytic granules upon activation to kill target cells. However, NK activation status is controlled by the integrated signals from germline-encoded NK-activating and -inhibiting receptors (aNKRs/iNKRs). Generally speaking, iNK ligands are expressed by normal and healthy cells, whereas aNK ligands are upregulated after DNA damage or viral insult. MHC-I molecules provide a potent inhibitory signal sensed by NK cells, enabling the innate immune system to respond productively to MHC-deficient cells. As a result, there is considerable interest in amplifying NK responses to cancers, as well as developing NK-based cell therapies.

Here, we performed genetic screens in an MHC-deficient leukemic cell line to systematically identify modulators of NK-mediated anti-cancer immunity. These screens, unexpectedly, pointed to the potential therapeutic utility of targeting the CRL4 substrate adaptor DCAF15 in myeloid malignancies. Disruption of DCAF15 strongly sensitized cancer cells to NK-mediated killing, resulting from increased cancer cell expression of lymphocyte costimulatory molecules. Proteomic experiments revealed that DCAF15 interacted with the cohesin complex components SMC1 and SMC3. Treatment with indisulam, an anticancer drug that modulates DCAF15 function, reduced interaction with cohesin members and mimicked DCAF15 loss-of-function immunophenotypes.

Results

A genome-scale CRISPR screen identifies modulators of NK effector functions

K562 human chronic myelogenous leukemia cells are a NK-sensitive cancer cell line that weakly expresses MHC-I. To enable a genome-scale CRISPR screen to identify genes that affect the susceptibility of K562 cells to NK-mediated killing in a co-culture assay, we employed NK-92 cells, a human lymphoma-derived cell line phenotypically similar to activated NK cells. These cells exhibit interleukin-2 (IL2)-dependent growth, express a large number of aNKRs and few iNKRs, and display potent cytolytic activity against K562 cells. For screening purposes, a clonal isolate of K562 cells expressing high levels of spCas9 was generated and validated.
In pilot experiments, labelled K562 cells were co-cultured with NK-92 cells to determine an effector-to-target (E:T) ratio that applied sufficient selective pressure in the context of a screen (Fig. S1D-F). IL2 was removed during the co-culture to promote the eventual death of NK-92 cells, allowing the collection of genomic DNA preparations undiluted by effector cell DNA. A multi-day timeframe between NK challenge and screen readout was used to capture tumor cell fitness changes related both to the direct cytolytic activities of NK cells as well as the longer-term effects from NK-released cytokines.

For the co-culture screen, cas9-expressing K562 cells were infected with a genome-scale single guide RNA (sgRNA) library targeting all unique coding genes and miRNAs, as well as one thousand non-targeting controls. Seven days post-infection, cells were either grown normally or challenged with NK-92 cells at a 1:1 or 2.5:1 E:T ratio, reducing K562 cell counts 19-fold or 43-fold, respectively, by the end of the screen (Fig. 1A-B). Deep sequencing was used to compare changes in sgRNA abundance between the challenged and unchallenged state after 8 days of co-culture, and genes were ranked using the MAGeCK software. There was good agreement between the results from screens performed at the different E:T ratios (Fig. S2A).

Inspection of the screening results revealed two broad classes of “hits”— sgRNAs targeting components of the tumor-immune synapse or components of the IFNγ signaling pathway (Fig. 1C-D). Disruption of Intercellular Adhesion Molecule 1 (ICAM1) was the top-ranked NK evasion mechanism, scoring many orders of magnitude stronger than any other gene—an observation consistent with the critical role of ICAM1-LFA1 interactions in establishing initial target-lymphocyte adhesion and polarizing cytotoxic granules towards the synapse. Single guide RNAs targeting multiple other tumor-immune synapse components were also enriched after NK challenge, including NCR3LG1 (#26-ranked gene by MaGeCK score), the activating ligand for NKP30 on NK cells; CD58 (#37), an adhesion molecule that binds CD2; and CD84 (#80), a SLAM-related receptor that binds homotypically to promote activation and cytokine secretion in lymphocytes. Other than NECTIN2/PVRL2, sgRNAs targeting NK-inhibitory surface proteins did not score prominently as NK-sensitization mechanisms, consistent with the weak MHC-I expression on K562 cells and the limited repertoire of NK inhibitory receptors expressed.
on NK-92 cells. NECTIN2 transmits both stimulatory or inhibitory signals to NK cells, depending on whether it is bound to DNAM1 (CD226) or TIGIT, respectively.

Besides ICAM1, the top 10 highest scoring NK evasion mechanisms were dominated by sgRNAs targeting the proximal components of the IFNγ signaling pathway, including STAT1, JAK1, IFNGR2, JAK2 and INFGR1 (Fig. 1D). Significantly, IFNγ is not directly cytotoxic or cytostatic to K562 cells (see and Fig. 5), suggesting that the sensitizing effect of the cytokine was downstream of its immunomodulatory effects. Consistent with the important role of IFNγ pathway engagement, sgRNAs targeting negative regulators of the interferon response were strongly depleted after NK challenge. Disruption of the protein tyrosine phosphatases PTPN2 and PTPN1 were the #2 and #5 ranked NK-sensitizing mechanisms, respectively. Presumably, these proteins suppress IFNγ-induced immunomodulation by dephosphorylating STAT and JAK proteins, as has been reported in CRISPR screens using T-cell coculture systems or syngeneic tumor models. Taken together, these findings indicate that our in vitro functional genomics screens effectively revealed known components of physiologically-relevant immune synapse and cytokine pathways.

Prospective identification of novel genes affecting sensitization to NK cells

Mechanisms of NK sensitization identified in the screen were diverse, revealing many strongly-scoring genes not previously linked to either interferon signaling or NK cell biology (Fig. 1E). Most surprisingly, disruption of DCAF15, an uncharacterized substrate adaptor for CRL4 ubiquitin E3 ligases, was the top-ranked sensitization mechanism. DCAF15 is a member of the large family of DDB1 and Cul4-associated factors (DCAFs). CRL4 complexes enable cells to mark proteins for destruction by the proteasome system, thereby contributing to the regulation of intracellular protein homeostasis. As substrate adaptors for CRL4, DCAF proteins provide specificity to the complex, determining which client proteins are ubiquitinated. As with most substrate adaptors, the normal client repertoire of DCAF15 is undefined, and relatively little is known about DCAF15’s biological function.

We also noted that disruption of of two cohesin-related genes, STAG2 and HDAC8, scored as NK sensitization factors (ranked #26 and #19, respectively). Cohesin is a ring-shaped complex
involved in chromatin replication, organization and repair, with STAG2 acting as a core complex member and HDAC8 controlling chromatin accessibility. Cohesin dysregulation has cell context-specific consequences, including DNA damage and aneuploidy; in leukemic cells, cohesin mutations are thought to enforce stem cell programs by altering chromatin organization.

A phenotypic screen based on MHC-I upregulation to identify modulators of the IFN\(\gamma\) response

The prominent role for IFN\(\gamma\) signaling in the immune response to cancer cells, both clinically and in our screens, prompted us to define more specifically which NK-sensitizing genes are involved in modulating the IFN\(\gamma\) response. MHC-I levels are highly upregulated in K562 cells after IFN\(\gamma\) exposure, increasing 5.9+/−0.98 fold after 24hrs of exposure to IFN\(\gamma\). This induction was increased to 10.01+/−1.63-fold when PTPN2 was disrupted, whereas no upregulation was detected when STAT1 was disrupted (Fig. 2A-B and S3A-B). We therefore used IFN\(\gamma\)-induced cell surface MHC-I expression as a proxy for the strength of the interferon response. K562 cells transduced with a genome-scale CRISPR library were treated with IFN\(\gamma\) for 24hrs and MHC-I expression was measured by flow cytometry. The brightest 20% and dimmest 20% of cells were sorted, and deep sequencing was used to compare sgRNA abundance between the populations (Fig. 2C, Table S3).

As expected, cells with impaired MHC-I upregulation were highly enriched for sgRNAs targeting the IFN\(\gamma\)-JAK-STAT pathway (IFNGR1/2, JAK1/2, STAT1, IRF1/2), as well as the antigen processing/presentation machinery (B2M, TAP1/2, TAPBP, PDIA3, HLA-C/B) (Fig. 2D). Conversely, disruption of PTPN2 or STAG2 scored well at inducing an exuberant MHC-I response. Surprisingly, sgRNAs targeting epigenetic factors were highly enriched within the brightest MHC-I expressing cells—most prominently, members of the BCOR complex PCGF1 and KDM2B, members of the PRC2 complex EZH2 and SUZ12, as well as factors affecting histone methylation/acetylation status.

Rank-rank comparisons between the NK and MHC screens were informative in defining a core group of genes that appear to play a role in the IFN\(\gamma\) response in K562 cells (Fig. 2E). Comparing sgRNAs enriched after NK challenge with those causing impaired MHC-I upregulation clearly
delineated the known proximal components of the IFNγ signaling pathway (IFNGR1/2, JAK1/2, STAT1), and highlighted several poorly characterized genes such as GSE1, SPPL3 and NR2F2 (Fig. 2E).

Surprisingly, comparing sgRNAs depleted after NK challenge with those causing an exaggerated MHC-I response highlighted the CRL4 substrate adaptor DCAF15 most prominently, alongside the cohesin members STAG2 and HDAC8 (Fig. 2E). As expected, negative feedback regulators of the IFNγ pathway (PTPN1 and PTPN2) were also recovered by this analysis. We focused on understanding the function of DCAF15, given its prominence in both the NK sensitization (#1 ranked hit at 2.5 E:T ratio; #12 ranked hit at 1:1 E:T ratio) and MHC upregulation (#13 ranked hit) screens.

**Disruption of DCAF15 enhances NK effector functions**

We validated and benchmarked novel NK cell sensitizers using a FACS-based competitive coculture system that mimicked the conditions of the screen (Fig. 3A). Fluorescently-labelled K562 cells expressing control or test sgRNAs were co-cultured in the presence of NK cells, and changes in the relative ratios of cell types were measured by flow cytometry after 48 hours (Fig. 3B-C and S4A-D). Differences in the basal growth rates between genotypes were controlled for by co-culturing the cancer cells in the absence of NK cells. As expected, deletion of the adhesion molecule ICAM1 conferred very strong resistance to NK cells (19.7-fold, N=12). Disabling signaling downstream of IFNγ by deleting STAT1 provided a more modest level of protection (2.45-fold, N=8).

K562s are very sensitive to NK-mediated killing, thereby yielding a large dynamic range for detection of resistance-promoting factors, while limiting the ability of the assay to detect similarly large increases in sensitization. Nevertheless, we were able to validate that multiple independent sgRNAs targeting DCAF15 promoted sensitization to NK cells (1.6-fold change, N=48). As positive controls, disruption of NECTIN2 or PTPN2 induced a similar degree of sensitization (NECTIN2: 1.95-fold change, N=6; PTPN2: 1.4-fold change, N=12). These findings implicate DCAF15 as a novel modulator of NK-mediated cancer cell immunity.
Loss of the cullin-RING E3 ligase substrate adaptor DCAF15 leads to an inflamed state distinct from dysregulated IFNγ signaling

DCAF15 was a strong hit in both the NK sensitization and MHC upregulation screens, suggesting that DCAF15 disruption sensitizes K562 cells to NK-mediated killing by dysregulating the IFNγ response. Consistent with the screening results, polyclonal cells expressing DCAF15 sgRNAs ("DCAF15 KO cells") displayed 2.45-fold higher levels of MHC-I than control knockout cells after 24hrs of IFNγ exposure, an effect comparable in magnitude to PTPN2 disruption (Fig. 4A). We then tested whether DCAF15 KO cells exhibited hallmarks of dysregulated JAK-STAT signaling, using disruption of PTPN2 as a positive control. We were unable to see any difference in induction of STAT1 phosphorylation after IFNγ exposure in DCAF15 KO cells, or differences in steady state levels of STAT1/2, JAK1/2 or IFNGR1 (Fig. 4B and data not shown). DCAF15 KO cells appeared healthy and proliferated at a normal rate (Fig. S5A). Upon long-term exposure to IFNγ, DCAF15 disruption did not induce a synthetic fitness defect with IFNγ (Fig. 4C). In contrast, PTPN2 KO cells showed higher levels of STAT1Py701 induction after IFNγ exposure, and their proliferative rate was temporarily reduced after transient exposure to IFNγ, or more substantially slowed down by continuous treatment with the cytokine (Fig. 4B-C).

To explore the mechanism by which DCAF15 was contributing to the NK response, RNA-seq was performed on DCAF15 or PTPN2 KO cells basally and after 24hrs of IFNγ exposure. Clustering analysis clearly showed that PTPN2 KO cells were transcriptionally distinct from control or DCAF15 KO cells (Fig. 4D). PTPN2 KO cells clustered differentially along the IFNγ principal component and were enriched for interferon-associated Gene Ontology (GO) terms both before and after cytokine exposure (Fig. S5B-C). In contrast, DCAF15 KO cells did not show substantial differences in their response to IFNγ (Fig. 4D). However, DCAF15 KO cells were enriched for GO terms associated with NK-mediated cytotoxicity, antigen presentation and cell adhesion, consistent with our phenotypic characterization of these cells (Fig. 4E). Together, these findings indicate that while DCAF15 KO K562 cells exhibit a relatively normal response to IFNγ...
stimulation, they are nonetheless in an inflamed state primed to interact with cytotoxic lymphocytes.

**Increased NK cell triggering by DCAF15 knockout cells mediated by CD80 expression**

Intriguingly, differential RNA analysis showed that one of the most highly upregulated genes in DCAF15 KO cells was CD80 (Fig. 4F; Q value 3.7e-23, Beta value 0.98). CD80 is an important co-stimulatory molecule for lymphocytes. CD80 and other B7 family members regulate T cell activation and tolerance by ligation to CD28, CTLA4 or PDL1. CD80 expression is typically upregulated after the activation of antigen-presenting cells (APCs), providing critical secondary signals to T cells. While mostly studied in the context of T cell biology, B7 family molecules are known to be activating receptors for NK cells, with both CD28-dependent and -independent ways that NK cells receive this signal.

K562 cells are an undifferentiated and multipotential CML cell line, well-studied for their ability to differentiate towards many different lineages, including APC-like states. We hypothesized that upregulation of CD80 in DCAF15 KO cells may reflect a broader acquisition of APC-like properties. Immunophenotyping of unstimulated DCAF15 KO cells revealed higher levels of the APC markers CD80, CD40 as well as class I and II MHC molecules (Fig. 5A; 2.23-fold CD80 increase; 2.01-fold CD40 increase; 1.44-fold MHC-I increase; 1.22-fold MHC-II increase). DCAF15 KO cells did not display higher levels of the APC maturation marker CD83 (Fig. 5A) or altered expression of various receptors identified in the screen to be important for NK adhesion, activation, or inhibition (Fig. S6A). Importantly, the changes to the DCAF15 KO cell immunophenotype could be rescued by constitutive expression of a sgRNA-resistant DCAF15 open-reading frame (Fig. S6B-D).

Transducing tumors with B7 family members can enhance anti-tumor immunity by enabling the tumor cells to directly deliver antigenic and costimulatory signals to T and NK cells. In our model system, we confirmed that NK-92 cells were competent to receive CD80 stimuli--expressing CD28 but not CTLA4 (Fig. S6E). K562 cells stably over-expressing wild-type CD80 were generated (Fig. 5B; 49-fold higher CD80 levels than endogenous). Over-expression was
sufficient to increase K562 sensitivity to NK-92 mediated killing (Fig. 5C), whereas overexpression of a mutant form of CD80 carrying point mutations that abrogate CD28 binding \(^{46}\) (CD80\(^{Q65A,M72A}\)) had no effect.

We next determined whether the increased CD80 expression in DCAF15 KO cells was important for their altered NK sensitivity. K562 cells were pretreated with either control or CD80 blocking antibodies prior to exposure to NK-92 cells (Fig. 5D). NK sensitivity was measured by the ability of K562 cells to trigger NK cell degranulation, as measured by NK cell CD107a expression. As expected, ICAM1 KO cells triggered less NK degranulation than control cells (by 52±12%) and were not significantly affected by CD80 antagonism (Fig. 5E). DCAF15 KO cells showed a 17±8% increased ability to trigger NK cells and were approximately twice as sensitive to CD80 antagonism compared to control cells (Fig. 5E-F). Following CD80 antagonism, degranulation triggered by DCAF15 KO was not significantly different from untreated control cells. Taken together, these results indicate that DCAF15 disruption in K562 cells induces an APC-like immunophenotype conducive to promoting lymphocyte responses, with higher CD80 expression especially important for increased NK cell triggering.

**The anti-leukemia drug indisulam inhibits DCAF15 function**

Aryl sulfonamide drugs have demonstrated promising anti-cancer properties in hematological malignancies \(^{47}\). Recently, it was discovered that these agents work via interaction with the CRL4-DCAF15 E3 ubiquitin ligase, promoting its action on the essential splicing factor RBM39 as a neosubstrate \(^{48,49}\). This mechanism of action is conceptually similar to that of the “IMiD” thalidomide analogs, which interact with the CRL4-cereblon E3 ubiquitin ligase to promote neosubstrate interactions with various lymphocyte transcription factors \(^{50-52}\). Presumably, sulfonamides and IMiDs also impair the degradation of the normal client proteins when they induce neomorphic activity of the substrate adaptor. This has not been proven, however, as it is difficult to systematically determine the normal substrate repertoire of adaptor proteins.

We hypothesized that treating K562 cells with low concentrations of the aryl sulfonamide indisulam would phenocopy DCAF15 depletion (Fig. 6A). Three-day dose-response experiments
revealed that K562 cells were sensitive to indisulam, and that DCAF15 disruption reduced this sensitivity, consistent with previous reports \(^\text{(49)}\) and Fig. 6B. Dose-response experiments across a panel of 16 hematological cancer cell lines confirmed the reported \(^\text{(48)}\) positive relationship between DCAF15 mRNA expression levels and indisulam sensitivity (Fig. S7A; \(R^2=0.33, P=0.02\)).

We empirically determined that 100nM indisulam treatment lowered RBM39 levels while minimally affecting cell viability and proliferation over a four-day period (Fig. 6C-D and \(^\text{49}\)). Remarkably, this treatment regime was able to recapitulate the increased CD80 expression seen in DCAF15 KO cells (Fig. 6E; 2.14-fold increase), and more modestly, the effects on MHC-I and CD40 expression (1.36-fold and 1.27-fold increase, respectively). CD80 upregulation was first detected 24hrs after treatment initiation and plateaued after 48hrs (Fig. S8B). Importantly, indisulam treatment did not further upregulate CD80 in DCAF15 KO cells, suggesting that the drug’s effect on CD80 levels was entirely mediated through DCAF15 (Fig. 6F).

To extend these observations to other cell lines, a panel of hematological cancer cell lines was screened to identify those with detectable CD80 expression (Fig. S7C). The CML cell line KU812 expressed similar levels of CD80 as K562, whereas the Daudi lymphoma cell line expressed significantly higher basal CD80 levels. These cells lines were subjected to similar 4-day low-dose regimes of indisulam, which only modestly affected the growth and viability of the cells (Fig. S7D). Remarkably, both Daudi and KU812 cells up-regulated CD80 levels after indisulam treatment, to an extent comparable to that which was observed in K562 cells (Fig. 6G; 2.45-fold for Daudi, 1.71-fold for KU812). Indisulam was not able to induce de novo CD80 expression in CD80-negative cell lines (Fig. S7E). Thus, in certain cellular contexts, aryl sulfonamides are immuno-modulatory agents that alter co-stimulatory protein levels by disrupting the normal functions of DCAF15.

**Reduced DCAF15 expression is associated with improved survival in AML patients**

Given the \textit{in vitro} findings, we hypothesized that lower DCAF15 expression in myeloid malignancies could be associated with better clinical outcomes. We tested this hypothesis using publicly available acute myeloid leukemia (AML) datasets \(^\text{53,54}\). In both adult and pediatric AML,
lower expression of DCAF15 mRNA was associated with increased median overall survival time (Fig. 6H-I). The improved survival of DCAF15-low patients was not driven by a correlation between DCAF15 expression and more aggressive AML subtypes (Fig. S7F-G). Taken together, these findings indicate that lower DCAF15 function, achieved pharmacologically or by genetic means, is associated with favorable immunophenotypes in vitro and improved outcomes in AML patients.

CRL4-DCAF15 directly interacts with cohesin complex members

The normal substrate repertoire of DCAF15 is unknown. To systemically identify direct DCAF15 client proteins, we undertook proximity-based proteomic analysis of DCAF15 interaction partners (Fig. 7A). DCAF15 was fused to a promiscuous bacterial biotin ligase 55 (“DCAF15-BioID”) and stably expressed in K562 cells, enabling recovery of interaction partners by streptavidin pull-down. In pilot experiments, we confirmed that exogenous C-terminally tagged DCAF15 was able to rescue DCAF15 KO phenotypes and associate with CRL4 complex members DDB1 and CUL4A (Fig. S6B-D, Fig. S8A). During stable DCAF15 overexpression, we observed that the basal concentration of biotin in the media (~3µM) was sufficient to induce BioID activity in the absence of exogenous (50µM) biotin supplementation (Fig. 7B). However, proteasome inhibition by MG132 increased accumulation of DCAF15-BioID and biotinylated species. As a control, results were compared to a GFP-BioID fusion, expected to generically biotinylate proteins. GFP-BioID accumulated much more readily than DCAF15-BioID, and its biotinylation activity was not affected by MG132 treatment.

After 24hrs of biotin and MG132 treatment, biotinylated protein species were recovered under stringent denaturing conditions. Isobaric labelling and mass spectrometry were used to quantitatively compare the DCAF15 interactome to the GFP interactome (Fig. 7C and Table S5). This approach clearly recovered DCAF15 and the core CRL4 complex, including DDA1, DDB1 and CUL4A (DCAF15: 155.6-fold change, P=2.5e-152; DDA1: 24.5-fold change, P=6.5e-139; DDB1: 4.86-fold change, P=8.1e-21; CUL4A: 3.8-fold change, P=8.5e-18).
Surprisingly, two of the most differentially biotinylated proteins were the cohesin complex members SMC1A and SMC3 (SMC1: 2.39-fold change, P=0.00098; SMC3: 2.76-fold change, P=5e-5). We confirmed the interaction between DCAF15-BioID and endogenous SMC1 and 3 by streptavidin-pulldown followed by western blotting (Fig. 7D). To determine whether this association with cohesin was a generic feature of CRL4 complexes or specific to the CRL4 loaded with DCAF15, we examined the interaction partners of a different substrate adaptor. DCAF16 is a nuclear-localized CUL4 substrate adaptor, which, like DCAF15, interacts with DDB1 despite lacking a canonical WD40 docking domain. When stably expressed in K562 cells, DCAF16-BioID fusions accumulated similarly to DCAF15-BioID, interacted with DDB1 but did not biotinylate SMC proteins (Fig. 7E). As low concentrations of indisulam phenocopy certain aspects of DCAF15 depletion (Fig. 6), we wondered whether indisulam treatment would alter the interaction of DCAF15 with cohesin. DCAF15-BioID cells were treated with indisulam for 72 hours, with biotin and MG132 added for the final 18 hours. This treatment regime lead to substantial indisulam-dependent biotinylation of RBM39 and reduced recovery of biotinylated SMC proteins (Fig. 7E). These findings indicate that cohesin proteins may be bona fide ubiquitination substrates for DCAF15, as DCAF15 interacts with SMC proteins in a way that is increased by proteasome inhibition, reduced by indisulam treatment and specific to CRL4-DCAF15.

Discussion

T cell immunity necessitates antigen recognition in the context of MHC, a requirement unchanged by checkpoint inhibitor therapy. Clinical and experimental data have revealed that disrupting optimal antigen presentation levels is a common mechanism by which cancer cells escape recognition by the adaptive immune system. These findings have highlighted the need for therapeutic strategies that can promote antigen presentation, or that work in a MHC-independent fashion. One such approach involves modulating the NK cell compartment to promote anti-cancer immunity. NK cells integrate the signals from multiple activating and inhibitory receptors to strike the right balance between auto-immunity and sanitization. Here, we discovered that the combinatorial logical of NK activation proved particularly amenable to CRISPR screening, leading to the discovery and validation of a novel factor that enhances NK effector functions when
disrupted. The biology was explored within the context of co-cultured NK-92 and K562 cells, a relatively simple model of the interaction between cancer cells and unlicensed NK cells. Despite this limitation, the screens clearly identified known lymphocyte adhesion factors and aNK ligands, such as ICAM1, NCR3LG1, CD58 and CD84. Other strongly-scoring genes encoding cell surface proteins, such as CEACAM7, IL23R and PTGFRN, may represent novel activating stimuli for NK cells. Extending our genetic screening approach to other types of tumor cells and natural killer subtypes will likely enable a more complete understanding of the relative importance of various aNKRs and iNKRs, and could facilitate the identification of candidate ligands for orphan NK receptors.

In this screening context, the dominant cytokine signature was clearly associated with IFN\(\gamma\). There was no evidence of a contribution from TNF\(\alpha\), the other prominent cytokine secreted by NK cells. Unlike other published tumor:cytotoxic lymphocyte co-culture screens \(^8,10\), neither TNF\(\alpha\) nor IFN\(\gamma\) was directly cytostatic or cytotoxic to our cancer cell model, likely explaining why components of the immune synapse scored so much more prominently. The importance of IFN\(\gamma\) in our screen reflected the immunomodulatory nature of the cytokine, helping to prime the cancer cells to undergo a productive interaction with lymphocytes. A notable exception was seen with disruption of PTPN2, a negative regulator of IFN\(\gamma\) signaling. Loss of PTPN2 both altered tumor cell immunophenotypes and reduced tumor cell growth after IFN\(\gamma\) treatment. PTPN2 disruption has proven to enhance activated T-cell mediated killing, as well as potentiate the effect of immunotherapy in syngeneic tumor models \(^9,10,56\). These data suggest that targeting PTPN2 may be a generalizable strategy to sensitize tumor cells to multiple arms of the immune system.

The importance of the cancer cell IFN\(\gamma\) response in promoting optimal lymphocyte responses prompted us to systemically identify perturbations that augment cytokine-induced MHC-I upregulation. As expected, this screen clearly delineated the proximal components of the IFN\(\gamma\)-JAK-STAT pathway, as well as the antigen processing/presentation machinery. Surprisingly, disruption of a poorly characterized substrate adaptor for the CRL4 E3 ligase was a top scoring hit in both the MHC-I and NK screens. We therefore focused our efforts on understanding the role of DCAF15 function. We determined that DCAF15 KO cells did not have a grossly dysregulated response to IFN\(\gamma\). Instead, the higher levels of MHC-I following DCAF15 disruption reflected a
broader phenotypic change reminiscent of APC activation, with the upregulation of a variety of co-stimulatory and antigen-presenting molecules (Fig. 7F). The upregulation of CD80 in DCAF15 KO cells was especially important for increasing NK-92 cell triggering. Given the array of changes elicited by DCAF15 disruption, it will be important to determine how the triggering of primary NK cell populations is altered by DCAF15 KO cells. It is conceivable that unlicensed NK cells, lacking expression of inhibitory self-MHC receptors, may be most responsive to the DCAF15 KO immunophenotype.

Our data suggest that the surface factors upregulated by DCAF15 disruption are not direct client proteins of the substrate receptor, but rather represent events secondary to altered turnover of the normal DCAF15 client protein(s). We pioneered a novel approach to systemically purify substrates of DCAF family members, as conventional biochemical methods are poorly suited to recover the transient, low-affinity interactions between substrate adaptors and client proteins. The use of DCAF-BioID fusion proteins and proteasome inhibition protects labile substrates from degradation and robustly recovers biotinylated proteins under stringent, denaturing conditions. We anticipate this will be a generalizable strategy for discovering client proteins for the whole family of CUL4 CRL substrate receptors.

Proteomic analysis and subsequent validation experiments showed that DCAF15-BioID interacted with core CRL4 complex members, and surprisingly, cohesin members SMC1 and SMC3. We were intrigued by this interaction given the similar CRISPR screening scoring pattern of DCAF15 to the cohesin factors STAG2 and HDAC8 (Fig. 2E); the shared roles of cohesin and CRL4 E3 ligases in DNA metabolism, organization, replication and repair \(^{34,57,58}\); and the ability of cohesin mutations to dysregulate hematopoietic differentiation in myeloid malignancies \(^{35}\). While not proven here, we hypothesize that SMC1 and SMC3 are direct ubiquitination substrates for DCAF15, given that their interaction was increased on proteasome inhibition, reduced by indisulam treatment, and not seen with unrelated DCAF substrate adaptors. Rather than globally controlling SMC protein levels, we speculate that CRL4-DCAF15 complexes ubiquitinate cohesin at specific genomic sites to regulate chromatin topology or repair (Fig. 7F). In this model, disruption of either STAG2, HDAC8 or DCAF15 impair cohesin function with overlapping phenotypic consequences.
Recently, it was discovered that aryl sulfonamide drugs including indisulam are capable of binding to DCAF15 and altering CRL-DCAF15 substrate specificity towards the splicing factor RBM39\textsuperscript{48,49}. The cytotoxic effects of indisulam were driven by splicing defects resulting from RBM39 degradation. Our studies confirmed indisulam-induced RBM39 degradation and the indisulam-dependent interaction between DCAF15 and RBM39. We also discovered indisulam-induced phenotypes attributable to inhibition of DCAF15’s normal functions. Concentrations of indisulam with limited RBM39 degradation or cytotoxicity had immunomodulatory properties that phenocopied genetic DCAF15 disruption, and were sufficient to reduce the interaction of DCAF15 with SMC1/SMC3, putative endogenous client proteins. We also determined that AML patients with naturally occurring lower levels of DCAF15 had improved overall survival. While these clinical data are preliminary in nature, they provide a rationale for drugging DCAF15 in myeloid neoplasms, achieved through judicious dosing of existing anti-cancer sulfonamides or the development of pure DCAF15 inhibitors.
FIGURES

Figure 1. A genome-scale CRISPR screen identifies modulators of NK effector functions
A) Overview of the genome-scale NK CRISPR screening system.
B) K562 population doublings (PDs) during the CRISPR screen, as measured by total number of live cells. Note that early timepoints from the co-culture reflect the presence of both K562 and NK-92 cells.
C) Analysis of the NK CRISPR screen results. Changes in sgRNA abundance were compared between the 2.5:1 E:T co-culture condition and day 15 dropout cells using the MAGeCK algorithm. The top 10 enriched or depleted genes are shown, as rank-ordered by Mageck score; other manually selected genes are highlighted with their rank indicated. FDR, false discovery rate.
D) Overview of high-scoring components of the tumor-immune synapse and IFNγ signaling pathway recovered by the screen. sgRNAs against genes enriched after exposure to NK cells are marked in red, while depleted sgRNAs are marked in green.

Supplemental Figure 1.
A) Western blot showing spCas9 expression in clonal isolates of K562 cells infected with a Cas9-expressing lentivirus. Asterisk highlights the clone used for screening purposes.
B) Measurement of Cas9 activity using an EGFP disruption assay. Cas9-expressing K562 cells were infected at a low MOI with a lentivirus expressing EGFP and a sgRNA targeting EGFP. The percent of cells that lost EGFP expression was measured over time by flow cytometry.
C) Measurement of Cas9 activity using sgRNAs targeting mismatch-repair (MMR) complex members. A Cas9-expressing K562 clonal isolate was infected at a low MOI with a lentivirus expressing sgRNAs against core members of the MMR complex. Protein disruption was measured by western blot 10 days after sgRNA infection. Note that certain MMR complex members depend on other binding partners for stability.
D) Experimental design for determining selective pressure of different effector:target cell ratios. K562 cells were labelled with carboxyfluorescein succinimidyl ester (CFSE) prior to co-culture with NK-92 cells.
E) Effect of increasing effector:target cell ratio on total number of viable target cells over time. During early timepoints, CFSE fluorescence was used to generate target cell counts. At later timepoints, total cell counts accurately reflected target cell counts due to NK-92 cell death.

F) Representative image of K562:NK-92 co-cultures at 5:1 E:T ratio. NK-92 cells (unlabeled) form aggregate structures enveloping the target cells (green). Scale bar, 250µM.

Supplemental Figure 2.
A) Analysis of the NK CRISPR screen results performed at a lower E:T ratio. sgRNA abundance was compared between the 1:1 effector:target ratio co-culture condition and day 15 dropout cells using the MAGeCK algorithm. The computed false discovery rate (FDR) is plotted against the -log10 transformation of the MAGeCK score. The top 5 enriched or depleted genes are shown, as rank-ordered by MAGeCK score; other manually selected genes are highlighted with their rank indicated in parentheses.

Supplemental Table 1. Design of genome-scale CRISPR library. sgRNA sequences and coordinates of the intended target locus are provided.

Supplemental Table 2. NK CRISPR screen data. Normalized sgRNA counts and MAGeCK analysis output are provided.

Figure 2. A phenotypic screen based on MHC-I upregulation to identify modulators of the IFNγ response
A) Flow cytometry measurement of MHC-I expression in K562 cells transduced with the indicated sgRNAs after 24hrs of 10ng/ml IFNγ treatment.
B) Fold upregulation of MHC-I expression after IFNγ treatment in K562 cells transduced with the indicated sgRNAs. Mean and standard deviation are shown. *** P value= 0.0002, ** P value=0.03, Mann-Whitney test.
C) Design of CRISPR screen for IFNγ-induced upregulation of MHC-I expression. SSC, side-scatter.
D) Analysis of the MHC-I upregulation CRISPR screen results. The MAGeCK algorithm was used to compare sgRNA abundance between cells in the bottom two and top two deciles of MHC-I expression. The false discovery rate (FDR) is plotted against the -log10 transformation of the MAGeCK score. The top 5 to 10 enriched or depleted genes are shown, as rank-ordered by MAGeCK score; other manually selected genes are highlighted with their rank indicated in parentheses.

E-F) Comparison of the NK and MHC screening results. Results of each screen were rank-ordered based on their MAGeCK score. Select genes are highlighted.

Supplemental Figure 3.
A) Western blot of total STAT1 levels in K562 cells infected with control or STAT1 sgRNAs.
B) Western blot of PTPN2 levels in K562 cells infected with control or PTPN2 sgRNAs.

Supplemental Table 3. Raw MHC-I screen data. Normalized protospacer counts and MAGeCK analysis output are included.

Figure 3. Disruption of DCAF15 enhances NK effector functions
A) Experimental design of competitive co-culture experiments, with FACS data illustrating a sgRNA that enhances NK-mediated killing.
B) Representative results from competitive co-culture experiments performed at a 2.5:1 E:T ratio.
C) Competitive co-culture results performed at a 2.5:1 E:T ratio and measured 48-96hrs after challenge with NK-92 cells. Mean and standard deviation are shown. *** P-value <0.0001, Mann-Whitney test.

Supplemental Figure 4.
A) Measurement of cell surface NECTIN2 expression by flow cytometry in K562 cells expressing control or NECTIN2 sgRNAs. Gates show background levels of fluorescence.
B) Measurement of cell surface ICAM1 expression by flow cytometry in K562 cells expressing control or ICAM1 sgRNAs. Gates show background levels of fluorescence.
C) Measurement of DCAF15 mRNA expression by RNAseq in K562 cells transduced with control or DCAF15 sgRNAs.

Figure 4. DCAF15 disruption leads to an inflamed state distinct from dysregulated IFNγ-JAK-STAT signaling

A) Flow cytometry measurement of cell surface MHC-I expression in K562 cells transduced with the indicated sgRNAs after 24hrs of 10ng/ml IFNγ treatment. *** P <0.0001; ** P = 0.0044, Mann-Whitney test.

B) Western blots against total and phosphorylated STAT1 after 30 minutes of 1ng/ml IFNγ treatment in K562 cells infected with the indicated sgRNAs.

C) Growth rate of K562 cells expressing the indicated sgRNAs, cultured normally (“untreated”), treated 24hrs with 10ng/ml IFNγ (“IFNγ pulse”), or retreated with 10ng/ml IFNγ every day (“IFNγ constant”).

D) Principle component analysis (for principle components (PC) 1 and 3) of transcriptomes from K562 cells expressing the indicated sgRNAs and treated +/- 10ng/ml IFNγ for 24hrs.

E) Selected GO terms, identified by RNA-seq, enriched in DCAF15 KO cells. Negative log10 transformation of the Benjamini-Hochberg corrected P value.

F) Volcano plot of genes differentially expressed between DCAF15 KO cells compared to control KO cells. Selected genes are highlighted. The FDR-corrected P-value generated from a likelihood ratio test (Q-value) is plotted against an approximate measure of the fold change in expression (Beta value).

Supplemental Figure 5.

A) Growth rate of K562 cells expressing sgRNAs against DCAF15 or a control gene. Results from 2-3 independent experiments and 2-3 different sgRNAs per gene. Mean and standard deviation are shown. DCAF15 sgRNAs, 1.1+/−0.01 population doublings per day. Control sgRNAs, 1.13+/−0.03 population doublings per day.

B) Gene set enrichment analysis (GSEA) of genes differentially upregulated in PTPN2 KO cells. nES, normalized enrichment score. Categories with a FDR Q-value of <0.1 are shaded in blue.

C) GSEA of genes differentially upregulated in PTPN2 KO cells after IFNγ treatment.
**Supplemental Table 4.** List of differentially expressed genes determined by RNA-seq of control, DCAF15 or PTPN2 KO K562 cells.

**Figure 5. Increased NK triggering by DCAF15 knockout cells mediated by CD80 expression**

A) Flow cytometry measurements of the indicated cell surface markers in K562 cells expressing the indicated sgRNAs. N=9-24 samples per condition. *** P<0.0001, ** P=0.001, ns P>0.1, Mann-Whitney Test.

B) Flow cytometry measurement of CD80 surface expression in control K562 cells or those lentivirally over-expressing CD80. Gate shows level of background fluorescence in unstained cells.

C) Competitive co-culture results between indicated K562 cell types and NK-92 cells, performed at 1:1 E:T ratio. * P=0.005, ** P=0.0007, NS P>0.1, unpaired T test. CD80\textsuperscript{mut} refers to CD80\textsuperscript{Q65A,M72A} mutant.

D) Experimental design of CD80 blockade experiment.

E) Effect of blocking antibodies to CD80 on NK-92 activation, measured by CD107 flow cytometry on NK-92 cells after 4hrs of co-culture. Data points from experiments performed on the same day are joined by lines of the same color. ** P=0.03, * P=0.06, ns P>0.1, Wilcoxon matched-pairs signed rank test. Experiment performed 4 times, 2x sgRNAs per condition.

F) Percent decrease in NK-92 degranulation after CD80 antibody treatment of indicated target cells. Data points from experiments performed same day are joined by lines. Mean is indicated. ** P=0.03, Wilcoxon matched-pairs signed rank test.

**Supplemental Figure 6.**

A) Flow cytometry measurements of the indicated cell surface markers in K562 cells expressing the indicated sgRNAs. N=2-14 samples per condition. Ns P>0.1, * P=0.04, ** P=0.01, Mann-Whitney Test.

B) Experimental design for rescuing DCAF15 disruption in K562 by lentiviral expression of a sgRNA-resistant DCAF15 open reading frame.

C) Western blot of DCAF15 rescue construct expression.
D) Flow cytometry measurements of the indicated cell surface markers in K562 cells expressing the indicated sgRNAs and rescue constructs. N=6-24 samples per condition. *** P<0.0001, ** P=0.004, Mann-Whitney test.

E) Flow cytometry measurement of CTLA4 and CD28 expression on NK-92 cells. Gates show background fluorescence levels in unstained cells.

**Figure 6. The anti-leukemia drug indisulam inhibits DCAF15 function**

A) Proposed model for DCAF15 gain- and loss-of-function phenotypes triggered by indisulam treatment.

B) Dose response of K562 cells expressing the indicated sgRNAs to indisulam. Relative viability measured by ATP content. N=2 experiments. Mean and standard deviation are shown.

C) Growth of K562 cells treated with 0.1μM indisulam over 4 days. N=12 per timepoint. Mean and standard deviation are shown.

D) Western blot for total RBM39 levels in K562 cells after 0.1μM indisulam treatment for the indicated number of days.

E) Flow cytometry measurements of the indicated cell surface markers in K562 cells after indisulam treatment. Mean and standard deviation are shown. *** P<0.0001, ** P=0.0085, * P=0.013, mean significantly different from 1, one sample T test.

F) CD80 expression measured by flow cytometry in indisulam-treated K562 cells expressing the indicated sgRNAs. Mean and standard deviation are shown. N=4-6 samples. ** P=0.0095, * P=0.0286, ns P>0.1, Mann-Whitney test.

G) Effect of indisulam treatment on CD80 expression in the indicated cell lines. N=3-12 samples. Mean and standard deviation are shown. *** P<0.0001, ** P= 0.0066, mean significantly different from 1, one sample T test.

H) Kaplan-Meier analysis of overall survival in adult AML patients from TCGA LAML project stratified by DCAF15 expression. “DCAF15 high” and “DCAF15 low” represents patients in top or bottom 50% of DCAF15 expression, respectively. N=142 patients. 95% confidence interval shown. Median survival, 16.17 vs 12.18 months. P-value from log-rank test.
Kaplan-Meier analysis of overall survival in pediatric AML patients from TARGET project stratified by DCAF15 expression. “DCAF15 high” and “DCAF15 low” represents patients in top or bottom 20% of DCAF15 expression, respectively. N=76 patients. 95% confidence interval shown. Median survival, 21.17 vs NA. P-value from log-rank test.

Supplemental Figure 7.

A) Relationship between DCAF15 mRNA expression and indisulam sensitivity across a panel of cell lines.

B) Time-course of CD80 expression after indisulam treatment in K562 cells measured by flow cytometry. Blue shading indicates period of CD80 upregulation. N=2-4 samples per timepoint. Mean and standard deviation are shown.

C) CD80 expression measured by flow cytometry across a panel of cancer cell lines. Dashed line indicates background level of antibody staining, with cell lines in red have expressing above background levels of the protein. CML, chronic myelogenous leukemia. BL, Burkitt’s lymphoma. AML, acute myeloid leukemia. AMoL, acute monocytic leukemia. T-ALL, T-acute lymphoblastic leukemia. MM, multiple myeloma. ALCL, anaplastic large cell lymphoma.

D) Growth of Daudi and KU812 cells treated with 0.1µM indisulam over 4 days. N=3 per timepoint. Mean and standard deviation are shown.

E) Fold change in CD80 expression in the indicated cell lines after 4 days treatment with 0.1µM indisulam.

F) Box and whisker plot of DCAF15 expression in TCGA LAML samples, stratified by subtype using the French American British (FAB) AML classification scheme. Analysis from UALCAN portal 59. Subtypes with significantly different median expression values are indicated.

G) FAB classification of adult AML patients in the TCGA LAML project, stratified by top 50% (“DCAF15 high”) or bottom 50% (“DCAF15 low”) of DCAF15 expression.

Figure 7. CRL4-DCAF15 directly interacts with cohesin complex members

A) Experimental system for discovering DCAF15 interaction partners by proximity ligation.
B) Indicated constructs were stably expressed in K562 cells, and biotinylated proteins detected by HRP-conjugated streptavidin ("α-biotin"). Asterisks denote major endogenous biotin-containing proteins. “MG132” and “Biotin” refer to 18hrs treatment with 5μM MG132 or 50μM biotin.

C) Proteins identified by quantitative mass spectrometry as differentially biotinylated by DCAF15-BioID as compared to GFP-BioID. Log2-fold changes are plotted against precision of the measurement (1/ coefficient of variation). Colors denote the posterior probability that a protein fold change was small (referred to as “P-null” in the legend), as explained in the methods.

D-E) Affinity capture of biotinylated proteins by streptavidin beads (“biotin IP”) and detection by western blot. Indicates constructs were stably expressed in K562 cells. “Indisulam” refers to 48hrs 0.1μM indisulam treatment prior to MG132 and biotin addition.

F) Model of proposed DCAF15 function.

Supplemental figure 8.

A) Exogenous DCAF15 interacts with CRL4 core complex members. Indicated constructs were stably expressed in K562 cells. Anti-Flag immunoprecipitations were performed and analyzed by western blot.

Supplemental Table 5. Comparison of biotinylated proteins recovered from K562 cells expressing DCAF15-BioID or GFP-BioID using isobaric labelling and mass spectrometry.

Supplemental Table 6. List of sgRNA sequences used.

Supplemental Table 7. List of antibodies used.

Supplemental Table 8. Primer design for sequencing sgRNA libraries.
Materials and methods

Cell lines

All cell lines were purchased from ATCC and were tested monthly for mycoplasma contamination. Cell lines other than NK-92 were maintained in RPMI supplemented with 10% FBS, 1mM GlutaMAX and 1% antibiotic, antimycotic. NK-92 cells were grown in Myelocult H5100 (Stem cell Technologies) supplemented with 100U/ml human IL-2 (Peprotech cat#200-02). IL-2 stock solution was made by reconstituting lyophilized cytokine to 10e6 U/ml in 50mM acetic acid, 0.1% BSA in PBS.

Construction of the CRISPR Library

CRISPR screening was performed using bespoke genome-scale libraries, to be described in detail elsewhere. In brief, the sgRNA library was designed with 120,021 sgRNAs present, representing 6 guides each against 21,598 genes and 4 guides each against 1,918 miRNAs, as well as 1000 non-targeting negative control guides (Table S1). sgRNAs targeting protein-coding genes were based on the Avana libraries. sgRNAs targeting miRNAs were based on the design of the Gecko v2.0 libraries. The protospacer library was synthesized by Twist Biosciences. The synthesized oligo library was amplified using emulsion PCR followed by purification. The vaccinia virus DNA polymerase was used to clone the protospacers into a lentiviral construct (In-fusion, Takara). The lentiviral construct was linearized by BfuA1 digestion (New England Biolabs). To ensure complete digestion, BfuA1 activity was stimulated by addition of 500nM of a double-stranded oligo containing a BfuA1 site (5’ atagcacctgtata 3’) (based on ).

The guide RNAs were expressed from a human U6 promoter, using a modified sgRNA design (A-U flip, longer stem-loop) previously described. An EF1a-Puro-T2A-cerulean-wpre ORF was used for selection purposes and for measuring infection rates. The library was electroporated into MegaX cells and plated across 37x 500cm² LB-carbenicillin plates. The library was recovered and pooled by scraping and column-based plasmid purification (Zymopure GigaPrep).

Preparation of virions
16M 293T cells were plated onto 177 cm\(^2\) dishes (9x plates total). 24hrs later, cell media was replaced with 32mls of DMEM+10% FBS (D-10). Cells were transfected with the library by lipofection (per plate: 158ul lipofectamine 2000, 8mls of Optimem, 3.95µg of VSVG, 11.8µg of Pax2, 15.78µg of library). The transfection mixture was left on the cells overnight, then changed to 25mls of D-10. Viral supernatant was collected 48hrs later. Debris was removed by centrifugation at 200g. Aliquots were flash-frozen and stored at -80°C.

Validation of the Cas9-expressing cell line used for screening

K562 cells were lentivirally infected with a EF1a-spCas9-T2A-blastR construct. Following 10µg/ml blasticidin selection, cells were dilution cloned. Clonal isolates with high levels of cas9 expression (as determined by western blot) were selected for further characterization. To determine the kinetics and efficacy of gene cutting under screening conditions (e.g., low MOI of the sgRNA construct), cells were infected with a lentiviral construct expressing both EGFP and a sgRNA targeting GFP. Following puromycin selection, the loss of EGFP expression was monitored by flow cytometry. In other experiments, the ability to effectively deplete endogenous proteins was determined by using a series of sgRNAs targeting the mismatch repair complex and measuring protein depletion by western blot.

The NK cell screen

Five hundred million spCas9-expressing K562 cells were mixed with CRISPR sgRNA library virions and 1L of media, then distributed across 34 6-well plates. Cells were spin-fected at 1900 rpm for 30 min at room-temperature with CRISPR library virus, conditions calculated to achieve a MOI of ~0.3 and 1000 cells per sgRNA library representation. MOI was measured by tracking the percent of the population expressing the cerulean marker found in the sgRNA library. Cells were incubated overnight in viral supernatant prior to being pooled, spun down to remove virions, and returned to spinner-flask culture (Bell-Flo Flask, Belloco Glass Inc). 24 to 48hrs post-infection, 2µg/ml puromycin selection was started for four days. Cells were maintained in log-growth phase with a minimal representation of 500M cells. Seven days post guide-infection, NK cell challenge was initiated. For the 2.5:1 E:T challenge, 100M K562 cells were mixed with 250M NK-92 cells in 1L of Myelocult, and split across 25 177 cm\(^2\) dishes. For the 1:1 E:T challenge, 100M K562 cells were mixed with 100M NK-92 cells in 400mls of Myelocult, and split across 10 177 cm\(^2\) dishes.
As a control, K562 cells were continuously propagated in RPMI media. 2 days after initiating the NK-92 challenge, the co-culture was switched to RPMI media. NK-92 cells, when grown in RPMI without IL-2, are rapidly lost from the culture. Six days after the media switch, 100M cell pellets were generated for library construction. All cell pellets were stored cryopreserved in cell-freezing media (Gibco #12648).

The MHC-based screen

Generation of K562 cells expressing the sgRNA library was performed as described above. On day 8 post-guide infection, 150M cells were stimulated with 10ng/ml IFNγ (R&D Systems 285-IF). 24hrs later, cells were spun down, washed, and stained in 3mls of 1:200 anti-HLA ABC-APC (W6/32 clone, BioLegend) in PBS, 2% FBS for 30min at 4°C. Approximately 10M of the brightest 20% and dimmest 20% of cells were sorted (BD FACSARia Fusion). Cell purity was determined to be >95% by re-analysis post-sorting. A replicate of this experiment was performed on day 14 post-guide infection.

CRISPR library preparation and sequencing

Genomic DNA was prepared by thawing cryopreserved cell pellets and proceeding with DNA extraction using column-based purification methods (NucleoSpin Blood XL, Machery-Nagel). Protospacer libraries were generated by a two-step PCR strategy, modified from 64. In the first round of PCR, 150μg of gDNA (equivalent to 15M K562 cells and 125-fold coverage of the library) was used in a 7.5ml PCR reaction to amplify the protospacers. This reaction was performed using a 500nM mixture of primers containing 0-9bp staggerers, to ensure base-pair diversity during Illumina sequencing (see supplemental table S7). The reaction was performed with Phusion master mix (New England Biolabs) and 3% DMSO with the following cycling conditions: 1 cycle X 30 s at 98°C, 21 cycles X 15 s at 98°C, 20 s at 63°C, 15 s at 72°C, 1 cycle X 2 min at 72°C. In the second round of PCR, 4ul of the initial PCR product was used as the template in a 200ul PCR reaction to make the sample compatible with Illumina chemistry and to add unique I5 and I7 barcodes to the sample. The reaction was performed with Phusion master mix (New England Biolabs), 500nM primers and 3% DMSO with the following cycling conditions: 1 cycle X 30 sec at 98°C, 12 cycles X 15 s 98°C, 20 s at 60°C, 15 s at 72°C, 1 cycle X 2 min at 72°C. The library was size-selected first by a 1:1 SPRI bead selection (AMPure XP beads, Beckman Coulter),
quantified by high-sensitivity dsDNA Qubit (ThermoFisher Scientific), and pooled. An agarose size selection step (PippinHT, Sage Science) was performed prior to sequencing on an Illumina Hiseq4000.

**Screen analysis**

Libraries were sequenced to a depth of ~20 million reads per condition. Reads were aligned to the sgRNA library using bowtie2. Protospacer count tables were generated from these alignments with python scripts and processed with MAGeCK. MAGeCK analysis was used to score and prioritize sgRNAs, using default settings in the algorithm. A subset of genes, mostly from highly related gene families, have more than 6 sgRNAs targeting them. As the MAGeCK scoring method tends to prioritize consistency of effect over magnitude of effect, genes with more than 6 guides targeting them were excluded from the analysis. MAGeCK scores were -log10 normalized, and values were plotted against FDR values.

**Competitive co-culture experiments**

Cell were transduced at a high MOI with a lentiviral sgRNA construct expressing the guide of interest, puromycin resistance and CMV promoter-driven expression of a red or green fluorescent protein. Knockout cell populations were maintained in a polyclonal state. Gene disruption was confirmed at the protein level by flow cytometry or western blot, and by RNA-seq when antibody reagents were not available. Complete knockout in >90% of the population was routinely achieved (Fig. S4A-D). Cells were counted and 0.25M red-labelled test cells were mixed with 0.25M green-labelled control cells (expressing a sgRNA against an olfactory receptor gene). The mixture was either grown in RPMI or mixed with 1.25M NK-92 cells in 4mls Myelocult media. The ratio of red-to-green cells was measured 2 to 4 days post-challenge, with the fold-change normalized to the ratio in the non-challenged state (to control for differences in basal cell growth rate).

**Flow cytometry**

0.5 to 1M cells were spun down and stained with APC-conjugated antibodies for 30mins at 4°C in PBS with 2% FBS. Cells were analyzed on a BD LSRSortessa. The geometric mean fluorescence intensity of singlet, DAPI-excluding cells was measured, and normalized to the background fluorescence of that particular genotype of cells.
**DCAF15 rescue experiments**

The DCAF15 open reading frame was synthesized based off reference sequence NM_138353, but with silent mutations designed to confer resistance to all three sgRNAs. To ensure resistance to sgRNA-directed gene cutting, silent mutations were introduced to the PAM domain and the proximal region of the protospacer (guide #1: 5’ GCTGCACACCAAGTACCAGGTGG to GCTGCACACCAAaTAtCAaGTaG. Guide #2: 5’ TGACATCTACGTCAGCACCGTGG to TGACATCTACGTcACaGTaG; Guide #3: 3’ GCAGCTTCCGGAAGAGGCGAGGG to GCAGCTTCCGGAAtaaaCGtGGt).

The rescue construct was expressed lentivirally from an EF1a promoter, with a c-terminal 3x flag epitope tag. Rescue cells were selected with hygromycin. The rescue construct was introduced two weeks after the initial introduction of the DCAF15 sgRNAs, and stable cell lines generated by a week of selection in 375µg/ml hygromycin. Expression of the construct was confirmed by lysing cells in PBS+0.1% NP40 (as per 48) and western blotting for the Flag tag.

**CD80 blocking experiments**

RFP-labelled target cells (0, 0.75M, or 2.5M) were resuspended in 0.5mls Myelocult + 5µg/ml control (MOPC-21 clone) or blocking CD80 (2D10 clone) antibodies. Cells were incubated for 30 mins at room temperature. 0.5M NK-92 cells in 0.5mls Myelocult were added to the well, as well as 1:200 anti-CD107a-APC antibody. Cells were co-cultured for 4hrs at 37°C. Flow cytometry was used to measured CD107A-APC expression in the NK-92 (RFP-negative) population. NK-92 cells were found to display a basal level of CD107a expression in the absence of effector cells. Increases above basal staining levels were used to define NK-92 degranulation. The experiment was repeated 4 times to establish biological replicates.

**CD80 over-expression experiments**

A full-length CD80 open reading frame, based on reference sequence NM_005191.4, was synthesized with a c-terminal 3x Flag tag and expressed lentivirally from the EF1a promoter. Over-expressing cells were selected with hygromycin. As a control construct, a mutant version of CD80 was synthesized with Q65A and M72A mutations in the “V-type” Ig-like domain. Each of these
residues makes contacts with CTLA4 in published CD80-CTLA4 co-crystal structures \(^6^5\) and alanine scanning experiments have shown that these residues are required for CD80 binding to CD28 or CTLA4 \(^4^6\).

**sgRNA sequences used**

See supplemental table 6.

**Antibodies used**

See supplemental table 8.

**Cell viability after in vitro cytokine stimulation**

Cells were plated at 0.25M/ml in media with or without 10ng/ml IFN\(\gamma\). Every day, an aliquot of cells was counted (Vi-CELL XR, Beckman Coulter), and cells were diluted in fresh media to maintain them in logarithmic growth phase. Continuously-treated cells were re-fed with fresh 10ng/ml IFN\(\gamma\) every day, whereas pulse-treated cells only received 24hrs of cytokine treatment.

**Western Blotting**

Cells were counted, spun down and washed in PBS prior to lysis in NP40 lysis buffer (25mM HEPES pH 7.5, 150mM NaCl, 1.5mM MgCl\(_2\), 0.5% NP40, 10% glycerol, 2mM DTT) with protease and phosphatase inhibitors. Lysates were normalized by cell count and/or protein concentration. 20-40\(\mu\)g of lysates were used for western blot analysis using standard procedures. Antibody binding was detected by enhanced chemiluminescence (SuperSignal Dura, Thermo Scientific).

**RNA-sequencing analysis**

Cells were seeded at 0.33M/ml density. Twenty-four hours later, 2M cells were collected. RNA was first purified by TRizol extraction and then further purified using column-based methods and polyA selection. RNaseq libraries were constructed using TruSeq Stranded mRNA Library Prep Kits (Illumina) and were sequenced on an Illumina Hiseq4000 machine using 150bp paired-end reads. Transcript abundances were quantified using Salmon\(^6^6\), with differential expression analysis performed by Sleuth \(^6^7\).
**Indisulam treatment**

Indisulam (Sigma SML1225) was reconstituted at 10mM in DMSO and stored in single use aliquots at -80°C. For indisulam dose-response experiments, 5000 cells were plated in 384-well plates (Greiner) and treated with indisulam over a 72hr period. A 12-point dose-response was performed between 10µM and 4.9µM of drug, as dispensed by a Tecan D300e. Cell viability was measured by ATP quantification (Cell Titer Glo, Promega). Dose-response measurements were fitted to a sigmoidal curve and an IC50 determined (Prism, GraphPad Software). DCAF15 expression data from different cell lines was downloaded from the Cancer Cell Line Encyclopedia (https://portals.broadinstitute.org/ccccle).

For low-dose indisulam experiments, cells were plated at 0.4M/ml in media with or without 100nM indisulam (1:100,000 dilution of stock). Cells were diluted every day in fresh media and drug to maintain them in logarithmic growth phase. Cells were analyzed for CD80 expression 96 hours after initiation of treatment.

**AML survival analysis**

FPKM RNAseq quantification for patient samples form the TARGET and TCGA LAML cohorts was obtained from the NIH NCI Genomic Data Commons DATA portal (https://portal.gdc.cancer.gov/). Clinical data for that TARGET AML and TCGA LAML cohorts were obtained from the Genomic Data Commons DATA portal and the Broad Institute TCGA Genome Data Analysis Center (http://gdac.broadinstitute.org/) respectively. Patients with matching clinical and transcript abundance data patients were stratified by DCAF15 expression as indicated. Survival time and vital status were defined as 'Overall Survival Time in Days' and 'Vital Status' respectively for the Target AML cohort. For the TCGA LAML study survival time for deceased patients ('patient.vital_status'=dead) was defined as 'patient.days_to_death' while for living patients ('patient.vital_status'=alive) 'patient.days_to_last_followup' was used for survival time. Survival analysis and Kaplan–Meier plots were generated using lifelines software for python (https://doi.org/10.5281/zenodo.2584900).

**DCAF15 proximity ligation**
K562 cells were infected with lentivirus expressing DCAF15-3flag-BioID-HA-T2A-BlastR, DCAF16-3flag-BioID-HA-T2A-BlastR, or 3flag-GFP-T2A-BioID-HA-T2A-BlastR. Stable cell lines were generated by 10µg/ml blasticidin selection. In triplicate, 20M cells were grown in media supplemented with 50µm biotin and 5µm MG132. 18hrs later, cell pellets were washed three times with ice cold PBS and lysed in mild lysis buffer (PBS 0.1% NP40 + PI/PPI), conditions determined to maximize the solubility of over-expressed DCAF15. 1mg of clarified lysate was used for enrichment of biotinylated species. Lysates were mixed with 60ul of streptavidin beads (Pierce #88817) for 4hrs at 4c in 500µl total volume. Beads were collected on a magnetic column, washed twice with 1ml RIPA buffer (25mM HEPES-KOH pH 7.4, 150mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1mM EDTA) and three times with 1ml urea buffer (2M urea, 10mM TRIS-HCl- pH 8.0).

For western blotting experiments, after urea washing, beads were equilibrated in mild lysis buffer. Elution was performed by incubating the beads 15min at 23°C, 15min at 95°C in 30µl 2.5X Laemlli buffer supplemented with 10mM Biotin and 20mM DTT. The eluate was brought down to 1X concentration with lysis buffer prior to western blotting. We note that detection of total biotinylated species by western blot was extremely sensitive to detection conditions. Membranes were blocked for 10min in PBS, 2.5%BSA, 0.4% Triton-X 100. 1ng/ml streptavidin-HRP in blocking buffer was added for 25min at room temperature. The membrane was washed for 15minutes in PBS 0.4% TritonX-100 prior to ECL exposure.

To measure BioID activity after indisulam treatment, cells were treated for 48hours with 0.1µm indisulam, followed by 24hrs in indisulam with 50µm biotin and 5µm MG132.

**Proteomic analysis of DCAF15 interaction partners**

For proteomics experiments, after the urea buffer washes, the samples were resuspended in denaturing buffer (8M urea, 50 mM ammonium bicarbonate pH 7.8). Proteins were reduced with dithiothreitol (5 mM, RT, 30min) and alkylated with iodoacetamide (15 mM RT, 45 min in the dark). Excess iodoacetamide was quenched with dithiothreitol (5 mM, room temperature, 20 min in the dark). The samples were diluted to 1 M urea using 50 mM ammonium bicarbonate and then digested with trypsin (37°C, 16h). After protein digestion, samples were acidified with
trifluoroacetic acid to a final concentration of 0.5% and desalted using C18 StageTips. Peptides were eluted with 40% acetonitrile/5% formic acid then 80% acetonitrile/5% formic acid and dried overnight under vacuum at 25°C (Labconco CentriVap Benchtop Vacuum Concentrator, Kansas City, Mo).

For tandem mass tag (TMT) labelling, dried peptides were resuspended in 50µL 200mM HEPES/30% anhydrous acetonitrile. TMT reagents (5 mg) were dissolved in anhydrous acetonitrile (250µL) of which 10µL was added to peptides to achieve a final acetonitrile concentration of approximately 30% (v/v). Following incubation at room temperature for 1 h, the reaction was quenched with 5% hydroxylamine/200mM HEPES to a final concentration of 0.3% (v/v). The TMT labelled peptides were acidified with 50µL 1% trifluoroacetic acid and pooled prior to desalting with SepPak (Waters) and dried under vacuum.

The pooled TMT-labeled peptides were fractionated using high pH RP-HPLC. The samples were resuspended in 5% formic acid/5% acetonitrile and fractionated over a ZORBAX extended C18 column (Agilent, 5µm particles, 4.6 mm ID and 250 mm in length). Peptides were separated on a 75-min linear gradient from 5% to 35% acetonitrile in 10mM ammonium bicarbonate at a flow rate of 0.5mL/min on an Agilent 1260 Infinity pump equipped with a degasser and a diode array detector (set at 214, 220, and 254nm wavelength) from Agilent Technologies (Waldbronn, Germany). The samples were fractionated into a total of 96 fractions and then consolidated into 12 as described previously. Samples were dried down under vacuum and reconstituted in 4% acetonitrile/5% formic acid for LC-MS/MS processing.

Peptides were analyzed on an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific) coupled to an Easy-nLC (Thermo Fisher Scientific). Peptides were separated on a microcapillary column (100 µm internal diameter, 25 cm long, filled using Maccel C18 AQ resin, 1.8µm, 120A; Sepax Technologies). The total LC-MS run length for each sample was 180 min comprising a 165 min gradient from 6 to 30% acetonitrile in 0.125% formic acid. The flow rate was 300 nL/min and the column was heated to 60°C.
Data-dependent acquisition (DDA) mode was used for mass spectrometry data collection. A high resolution MS1 scan in the Orbitrap (m/z range 500-1,200, 60k resolution, AGC $5 \times 10^5$, max injection time 100 ms, RF for S-lens 30) was collected from which the top 10 precursors were selected for MS2 analysis followed by MS3 analysis. For MS2 spectra, ions were isolated using a 0.5 m/z window using the mass filter. The MS2 scan was performed in the quadrupole ion trap (CID, AGC $1 \times 10^4$, normalized collision energy 30%, max injection time 35 ms) and the MS3 scan was analyzed in the Orbitrap (HCD, 60k resolution, max AGC $5 \times 10^4$, max injection time 250 ms, normalized collision energy 50). For TMT reporter ion quantification, up to six fragment ions from each MS2 spectrum were selected for MS3 analysis using synchronous precursor selection (SPS).

Mass spectrometry data were processed using an in-house software pipeline. Raw files were converted to mzXML files and searched against a composite human uniprot database (downloaded on 29th March 2017) containing sequences in forward and reverse orientations using the Sequest algorithm. Database searching matched MS/MS spectra with fully tryptic peptides from this composite dataset with a precursor ion tolerance of 20 ppm and a product ion tolerance of 0.6 Da. Carbamidomethylation of cysteine residues (+57.02146 Da) and TMT tags of peptide N-termini (+229.162932 Da) were set as static modifications. Oxidation of methionines (+15.99492 Da) was set as a variable modification. Linear discriminant analysis was used to filter peptide spectral matches to a 1 % FDR (false discovery rate) as described previously. Non-unique peptides that matched to multiple proteins were assigned to proteins that contained the largest number of matched redundant peptides sequences using the principle of Occam’s razor.

Quantification of TMT reporter ion intensities was performed by extracting the most intense ion within a 0.003 m/z window at the predicted m/z value for each reporter ion. TMT spectra were used for quantification when the sum of the signal-to-noise for all the reporter ions was greater than 200 and the isolation specificity was greater than 0.75.

Base 2 logarithm of protein fold-changes were estimated by fitting a previously described Bayesian model to the peptide level intensities. Protein estimates are reported as the mean of the posterior distribution for each parameter. Similarly, coefficients of variation are calculated by taking the
posterior variance divided by the posterior mean. The probability of a small change (“P_null”) was estimated as the frequency of posterior samples that fall within the interval (-1,1) on the log2 scale.

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**Competing Interests**

All authors are employees of Calico Life Sciences, LLC.

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NK cells

K562 Cas9+ sgRNA library

Perforins Granzymes Cytokines etc...

Sequence surviving cells

Day post sgRNA infection

Cumulative PDs

NK-92: K562 CRISPR screen

2.5:1 E:T

1:1 E:T

No NK cells

Stimulus type for NK

Target Cell

NK cell

ICAM1 NCR3LG1 CD58 PVRl2 (NECTIN2)

LFA1 NKp30 CD2 CD84 TIGIT

IFNγ response

IFNGR1 IFNGR2 JAK1 JAK2 STAT1 STAT1

IFNγ signalling pathway

CD58 (#37)

CD84 (#80)

NCR3LG1 (#26)

PTGFRN (#16)

CEACAM7 (#19)

STAG2 (#26)

HDAC8 (#19)

PTPN1/2 RPL28

ICAM1

Enriched after NK challenge
2.5:1 E:T

Depleted after NK challenge
2.5:1 E:T

STAT1 JAK1 IFNGR1 IFNGR2

GEMINI4 MGA COG7 RAD51D RPL28

PTPN1

GEMIN4 MGA COG7 RAD51D RPL28

SNF8 PTP2

DCAF15 PTPN2 PVRl2 (NECTIN2)

A

B

C

D

Fig. 1
**Fig. 2**

**A**

![Plot](image)

- Normalized Count
- MHC-I
- unstimulated
- 24hr IFNγ
- control
- sgRNA
- PTPN2
- FACS

**B**

![Plot](image)

- Fold MHC-I upregulation
- MHC-I

**C**

![Plot](image)

- IFNγ Response CRISPR screen
- MHC-I upregulation
- FACS
- sgRNA abundance, brightest vs. dimmest
- 20% of cells

**D**

![Plot](image)

- Impaired MHC-I response
- Screen rank, NK sensitization
- Antigen processing/presentation
- IFNγ signalling pathway

**E**

![Plot](image)

- Screen rank, NK resistance
- Screen rank, impaired MHC-I response

**Screen rank, exaggerated MHC-I response**

- IFNγ signalling pathway
- cohesin pathway

---

- **MHC-I**
- **SSC**
- **MHC-I upregulation** after 24hr IFNγ
- sgRNA abundance, brightest vs. dimmest
- 20% of cells
A

Competitive co-culture

- NK cells
  - control sgRNA
    - test sgRNA
    - 50%
  - control sgRNA
    - test sgRNA
    - 50%

+ NK cells
  - control sgRNA
    - test sgRNA
    - 10%
  - control sgRNA
    - test sgRNA
    - 90%

B

Control sgRNA vs. sgRNA against:

- control
- DCAF15
- PTPN2
- ICAM1
- STAT1

C

Log₂ (fold change)

Sensitization

Resistance

Control sgRNA vs sgRNA against:

Fig. 3
**Fig. 4**

(A) MHC signal after 24hrs

(B) sgRNAs against:

| Basal | IFNγ |
|-------|-------|
| Control | PTPN2 | ICAM1 | Control | DCAF15 | Control | PTPN2 | ICAM1 | Control | DCAF15 |
| α–STAT1| α–STAT1| α–tubulin |
| -75   | -75   | -50   |

(C) Cumulative PDs

(D) PC1 (7%) "IFNγ"

(E) DCAF15 sgRNA vs. Control sgRNA

(F) DCAF15 sgRNA vs. Control sgRNA
**Fig. 5**

**A**

| Antigen presentation | Dendritic Cell Markers |
|----------------------|------------------------|
| MHC-I                | CD80                   |
| MHC-II               | CD40                   |
|                      | Maturation             |

**B**

- **Log2 (Fold Depletion) After NK Co-culture**
- **SSC**
- **CD80**

**C**

- **% NK92 CD107a+**

**D**

- α–CD80 antibody + target cells
  - 30min
  - Add NK92
  - 5:1 T:E
  - Add α–CD107APC
  - 4hrs
  - Measure NK92 degranulation

**E**

- **% change in NK92 degranulation after CD80 antagonism**

**F**

- sgRNAs against: DCAF15, Control

---

**Fig. 5**
A) Neosubstrate degradation + Normal target accumulation? 

B) Relative Viability, 72hrs 

C) Cumulative PDs 

D) 0.1 μM indisulam, days 

E) Signal change over untreated cells 

F) CD80, signal over background 

G) CD80 MFI, Fold change over untreated 

H) TCGA_LAML 

I) TARGET_AML 

Fig. 6
**Fig. 7**

A) BioID vs. GFP BioID

B) Quantitative MS - DCAF15

C) DCAF15 BioID vs. GFP BioID

D) Biotin enrichment

E) Biotin enrichment

F) Multipotent leukemic cell

DCAF15 knockout or indisulam

cohesin disregulation?

APC-like Differentiation

Increased immune surveillance
Fig. S1

**A**

| K562 cas9 clone # |
|-------------------|
| 16    | 17 | 18 | 19 | 20 | 21 | 22 | 23 |

| α-ν5 (spCAS9) |
|---------------|
| 150           |

| α-tubulin |
|-----------|
| 50        |

**B**

EGFP Reporter assay of Cas9 Activity/Kinetics

![Graph showing % Cells GFP-negative over Day post guide infection](image)

**C**

| MMR Complex | sgRNA against: | controls |
|-------------|----------------|----------|
| α-MLH1      | MLH1           | None     |
| α-PMS2      | MSH2           | None     |
| α-MSH2      | MSH6           | None     |
| α-MSH6      | PMS2           | None     |
| α-tubulin   | GFP            | None     |

**D**

NK92 Effector

- CFSE-labelled target K562
- 48hrs co-culture myelocult
- Count CFSE+ cells
- Co-culture RPMI (NK-92 die)
- Count all cells on hemocytometer

**E**

CFSE+ cell number normalized to d0

![Graph showing CFSE+ cell number over Day](image)

**F**

5:1 E:T

![Images of Merge, Phase contrast, and K562 (CFSE)](image)
Fig. S2
Fig. S3

A

|        | controls #1 | STAT1 sgRNA #1 | STAT1 sgRNA #2 | α-STAT1 | α-tubulin |
|--------|-------------|----------------|----------------|---------|-----------|

B

|        | control PTPN2 #1 | PTPN2 sgRNA #1 | PTPN2 sgRNA #2 | α-PTPN2 | α-GAPDH |
|--------|------------------|----------------|----------------|---------|---------|

37

50
A

B

C

DCAF15 RNAseq Read Counts

Estimated Counts

| Replicate 1 | Replicate 2 | Replicate 1 | Replicate 2 |
|-------------|-------------|-------------|-------------|
| #1          | #2          | #1          | #2          |
| #3          | #3          | #1          | #2          |
| #3          | #3          | #1          | #2          |

Controls

DCAF15 sgRNAs
Fig. S5

A

Control sgRNAs
DCAF15 sgRNAs

Days
Cumulative PDs
0 1 2 3 4 5 6 7 8

B

GSEA
PTPN2KO Upregulated genes

(1-Q_value)

INTERFERON_ALPHA_RESPONSE
INTERFERON_GAMMA_RESPONSE
ALLOGRAFT_REJECTION
TNFA_SIGNALING_VIA_NFKB
MYC_TARGETS_V2
INFLAMMATORY_RESPONSE

nES
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

C

GSEA
PTPN2KO +IFNγ Upregulated genes

(1-Q_value)

INFLAMMATORY_RESPONSE
IL2_STAT5_SIGNALING
ALLOGRAFT_REJECTION
COAGULATION
CHOLESTEROL_HOMEOSTASIS
KRAS_SIGNALING_UP

0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

nES
0 1 2 3
**Fig. S6**

**Panel A**

- **Diagram** showing fold signal intensity over background for various cytokine receptors.
- **Legend** indicates the fold change in signal intensity with controls.

| Cytokine Receptor | Fold Signal Intensity over Background |
|-------------------|---------------------------------------|
| ICAM1             | +                                     |
| IFNGR1            | +                                     |
| CD58              | +                                     |
| Nectin2           | NS                                    |
| ULBP              | NS                                    |

**Panel B**

- **Diagram** illustrating the experimental setup:
  - K562 cas9+ (blastR) → DCAF15 sgRNA (puroR) → +/- EF1a-DCAF15-3fl (HygR) → silent mutations to all 3 sgRNAs.

**Panel C**

- **Table** showing sgRNAs against DCAF15:
  - **Controls**
    - #1
    - #2
  - **DCAF15 Rescue**
    - #1
    - #2
    - #3

**Panel D**

- **Diagram** showing fold change MFI vs. control sgRNAs for Dendritic Cell Markers.
- **Legend** indicates the fold change in MFI with DCAF15 sgRNA.

| DCAF15 sgRNA | Fold change MFI vs. control sgRNAs |
|--------------|------------------------------------|
| +            | +                                  |
| +            | +                                  |
| +            | +                                  |
| +            | +                                  |
| +            | +                                  |
| +            | +                                  |

**Panel E**

- **Diagram** showing NK-92 cell analysis.
- **Legend** indicates the scatter plot for CD28 and CTLA4.
**Fig. S7**

A. 

\[
\log_2(\text{Indisulam IC50, } \mu\text{M}) \quad \log_2(\text{DCAF15 RPKM})
\]

- SET-2
- MOLM-16
- L82
- KELLY
- NOMO1
- K562
- THP1
- DEL
- KASUMI1
- HEL 92.1.7
- RAJI

R² = 0.33
P = 0.02

B. 

CD80 signal over background

Duration of 0.1μM Indisulam Treatment, Days

C.

- CML
- AML
- AMO
- T-ALL
- MM
- ALCL

- K562
- KU812
- RAJI
- DAUDI
- MOLM16
- KG1A
- NOMO1
- OCI-AML1
- THP1
- JURKAT
- MOLP8
- SKMM2
- SUPM2
- DEL
- L82
- SUDHL1

D.

- Untreated
- 0.1μM indisulam

CD80 Fold change over untreated

Cumulative PDs

Days

E.

Low-dose indisulam effect

CD80 non-expressors

F.

- DCAF15 high
- DCAF15 low

DCAF15 Transcript per million

G.

- # of patients

French American British AML subtype

N=16 N=42 N=39 N=16 N=35 N=18 N=2 N=3

M0 M1 M2 M3 M4 M5 M6 M7

DCAF15 high DCAF15 low

Fig. S7
A

| 5μm MG132, 22hr | Input (20%) | Flag IP |
|-----------------|-------------|---------|
|                 | WT          | DCAF15 \(^{\text{III}}\) | GFP \(^{\text{III}}\) |
|                 | -           | +        | +        |
| ± 5μm MG132, 22hr | -           | +        | +        | ± - |

| α-DDB1 | α-CUL4A | α-GAPDH | α-FLAG |
|--------|---------|---------|--------|
| 100    | 75      | 37      | 75     |
|        |         |         | 50     |
|        |         |         | 37     |