Silencing of retrotransposons by SETDB1 inhibits the interferon response in acute myeloid leukemia

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A propensity for rewiring genetic and epigenetic regulatory networks, thus enabling sustained cell proliferation, suppression of apoptosis, and the ability to evade the immune system, is vital to cancer cell propagation. An increased understanding of how this is achieved is critical for identifying or improving therapeutic interventions. In this study, using acute myeloid leukemia (AML) human cell lines and a custom CRISPR/Cas9 screening platform, we identify the H3K9 methyltransferase SETDB1 as a novel, negative regulator of innate immunity. SETDB1 is overexpressed in many cancers, and loss of this gene in AML cells triggers desilencing of retrotransposable elements that leads to the production of double-stranded RNAs (dsRNAs). This is coincident with induction of a type I interferon response and apoptosis through the dsRNA-sensing pathway. Collectively, our findings establish a unique gene regulatory axis that cancer cells can exploit to circumvent the immune system.

Introduction

Dysregulation of the epigenome can lead to global and local changes in gene expression (Dawson and Kouzarides, 2012), transforming normal cells and providing license to proliferate, metastasize, curb cell death, and evade the host immune system (Hanahan and Weinberg, 2011). Cancer genome sequencing efforts have focused on defining tumor-specific changes in unique coding regions and structural variants, as well as noncoding mutations that impact transcription factor–binding sites or the activity of functional RNAs (Mwenifumbo and Marra, 2013). However, evidence has begun to accumulate suggesting that de-regulation of transposable elements (TEs), which comprise at least 45% of the human genome, may allow for tumor evolution through integration into and disruption of tumor suppressor loci or introduction of active promoter and enhancer elements within oncogenes (Lander et al., 2001; Haubold and Wiehe, 2006).

In the human and murine germlines, TEs, which include long terminal repeat (LTR)–containing endogenous retroviruses (ERVs), non-LTR–containing long interspersed nuclear elements (LINEs), and short interspersed nuclear elements (SINEs), are thought to be marked for transcriptional silencing via H3K9 trimethylation (H3K9me3) and/or DNA methylation (Kassiotis and Stoye, 2016). Indeed, loss of these marks in embryonic stem cells by depletion of distinct lysine methyltransferases, DNA methyltransferases, or their cofactors leads to up-regulation of TEs and, either through direct or indirect effects, a host of developmental regulatory and TE-neighbor ing genes (Karimi et al., 2011). Suppression of TEs via specific histone modification is likely necessitated by the unique, DNA-hypomethylated state observed throughout distinct stages of early development (Ehrlich, 2002). H3K9me3 levels decrease in committed cells, such as mouse embryonic fibroblasts and neural precursor cells, compared with embryonic stem cells, and the primary mechanism of TE suppression in somatic cells is thought to be proximal DNA methylation driven by DNA methyltransferases (Mikkelsen et al., 2007; Kassiotis and Stoye, 2016).

Though genomic alterations induced by mobilized TEs may permit cellular transformation, their unrestrained expression can induce an immune response or, potentially, genomic instability, either of which could reduce cancer cell survival (Rodić et al., 2015; Zeller et al., 2016). Indeed, recent studies have found this phenomenon to be a potential vulnerability in cancer cells, as desilencing of endogenous retroelements with...
Results

Identification of SETDB1 as a critical regulator of AML cell survival through a CRISPR/Cas9 genetic screen

To explore epigenetic vulnerabilities in AML, we developed a loss-of-function (LOF) cell viability screen in the human p53-deficient, MLL-AF9 translocation–driven THP-1 AML cell line, which we engineered to stably express Streptococcus pyogenes Cas9 (Tsuchiya et al., 1980; Cong et al., 2013; Jinek et al., 2013; Mali et al., 2013). In parallel, we generated a custom pooled, lentivirus-based single-guide RNA (sgRNA) expression library that targets ∼350 known human epigenetic and transcriptional modifiers. THP-1–Cas9 cells were infected with the sgRNA pool at 1,000-fold representation (Fig. S1 A), and comparative analysis of sgRNA abundance at both an early (day 7, reference sample) and late (day 21) time point was performed via high throughput sequencing. Confirming the utility of our approach, we observed the depletion of sgRNAs specific to known regulators of AML cell growth or viability, including KDM1A (LSD1), DOT1L, MLL, and BRD4 (Fig. 1 A), as well as positive control sgRNAs targeting known essential genes (MCL1 and PLK1). Interestingly, the top hit in our screen, as determined by the fraction of depleted sgRNAs per gene and overall fold change related to sgRNA depletion, was SETDB1 (Fig. 1 A). An analysis of The Cancer Genome Atlas (TCGA) and other publicly available datasets suggests that SETDB1 expression is elevated in AML relative to normal blood cells and that SETDB1 is overexpressed or amplified across a broad range of malignancies (Fig. 1 B; Rodriguez-Paredes et al., 2014; Sun et al., 2014, 2015; Fei et al., 2015; Liu et al., 2015). However, a molecular mechanism related to SETDB1–dependent maintenance of AML cell growth and viability has yet to be fully established.

Acute myeloid leukemia (AML) represents the most common form of myeloid leukemia in adults, and alterations in epigenetic modifiers are known to contribute to myeloid malignancies (Plass et al., 2008; Shih et al., 2012; Sasca and Huntly, 2016). For instance, the H3K4 methyltransferase gene MLL exhibits translocation in 4–7% of AMLs. In addition, a high frequency of mutations are seen in genes that impact DNA methylation, including TET2 (7–23%), which converts 5-methylcytosine to 5-hydroxymethylcytosine (the first product in the TET2-2OG pathway) and regulates gene expression. Set1/SETDB1, as well as other SET domain proteins, can form a complex with DNA damage–triggered cell death caused by Cas9, we performed an inducible shRNA-mediated depletion of SET DB1 mRNA (Wang et al., 2015; Aguirre et al., 2016; Munoz et al., 2016). To determine whether there is a general requirement for SETDB1 in AML cell survival, we engineered seven additional human AML cell lines to express Cas9 protein and treated these with the two distinct SETDB1 sgRNAs, an NTC sgRNA, or a positive control sgRNA targeting the essential gene PLK1. Like THP-1 cells, markedly reduced cell growth was observed in MOLM-13, ML-2, HL-60, OCI-AML3, and MV-4-11 cells upon treatment with SETDB1-specific sgRNAs, whereas the UKE-1 and EOL-1 lines were insensitive (Fig. 1 F). Our results are consistent with recent CRISPR/Cas9 screens that identified a cell line–dependent, but perhaps AML-specific, requirement for SETDB1 (Hart et al., 2015; Tzelepis et al., 2016; Wang et al., 2017). Although reduced viability of SETDB1-depleted liver cancer cell lines has been shown to be driven by p53 mutation status, we could not identify an overt genetic signature that could explain sensitivity to SETDB1 loss in the evaluated AML lines (Fei et al., 2015). Therefore, we sought a deeper understanding of the mechanisms by which SETDB1 contributes to AML cell survival and death.

To validate and further examine the SETDB1 LOF phenotype in THP-1–Cas9 cells, we performed viability and apoptosis assays on cells exposed to three distinct SETDB1-specific sgRNAs or a nontargeting control (NTR) sgRNA (Fig. 1 C and D). SETDB1-targeting sgRNAs depleted SETDB1 protein, induced apoptosis in standard 2D tissue culture conditions, and reduced colony formation in 3D culture (Fig. 1 C and D; and Fig. S1, B–D). Analysis of transduced cell populations show that the SETDB1–specific sgRNAs introduced frame-shifting alleles (indels) with high frequency, and the proportion of deleterious mutations was consistent with penetration of the apoptotic phenotype associated with each sgRNA (Fig. 1 E). To rule out the possibility that SETDB1 disruption synergizes with DNA damage–triggered cell death caused by Cas9, we performed an inducible shRNA-mediated depletion of SET DB1 mRNA (Wang et al., 2015; Aguirre et al., 2016; Munoz et al., 2016). Consistent with the knockout (KO) phenotypes, SETDB1 knockdown resulted in reduced protein levels, loss of viability, and induction of apoptosis markers (Fig. S2, A–D). To determine whether there is a general requirement for SETDB1 in AML cell survival, we engineered seven additional human AML cell lines to express Cas9 protein and treated these with the two distinct SETDB1 sgRNAs, an NTC sgRNA, or a positive control sgRNA targeting the essential gene PLK1. Like THP-1 cells, markedly reduced cell growth was observed in MOLM-13, ML-2, HL-60, OCI-AML3, and MV-4-11 cells upon treatment with SETDB1-specific sgRNAs, whereas the UKE-1 and EOL-1 lines were insensitive (Fig. 1 F). Our results are consistent with recent CRISPR/Cas9 screens that identified a cell line–dependent, but perhaps AML-specific requirement for SETDB1 (Hart et al., 2015; Tzelepis et al., 2016; Wang et al., 2017). Although reduced viability of SETDB1-depleted liver cancer cell lines has been shown to be driven by p53 mutation status, we could not identify an overt genetic signature that could explain sensitivity to SETDB1 loss in the evaluated AML lines (Fei et al., 2015). Therefore, we sought a deeper understanding of the mechanisms by which SETDB1 contributes to AML cell survival and death.
understanding of the molecular responses triggered upon loss of SETDB1 in distinct AML cell line contexts.

**Disruption of SETDB1 leads to induction of viral response genes**

To gain insight into the molecular mechanisms by which SETDB1 provides a survival advantage in AML, we generated individual SETDB1 mutant THP-1 lines with two distinct SETDB1-specific sgRNAs and compared their transcriptome changes relative to that of an NTC sgRNA–treated line. Total mRNA was collected from these cells and analyzed by RNA-seq at 4 and 7 d after transduction with the gene-specific or control sgRNAs. Though analyses were performed on both day 4 and 7 samples, here we focus on the early time point (day 4) to

**Figure 1.** A CRISPR/Cas9 genetic screen identifies SETDB1 as a critical regulator of the leukemic cell survival that is overexpressed in many cancers. (A) Median negative fold changes of sgRNA abundance at day 21 versus day 7, ranked by percentage of sgRNAs depleted for each gene in THP-1–Cas9 cells. n = 3 biological replicates for the day 7 reference and n = 2 biological replicates for day 21. The size of each circle corresponds to the fraction of depleted sgRNAs/target. Select regulators of AML cell growth are highlighted in black text. Significant fold changes were calculated with DESeq2. (B) Compiled data from TCGA RNA-seq. SETDB1-normalized RPKM (reads per kilobase of transcript per million mapped reads) values in cancer and normal tissues. Student’s t-tests were performed. (C and D) Viability and Caspase 3/7 levels, as measured by Caspase-Glo, in THP-1–Cas9 cells after 7 d of treatment with three different SETDB1-specific sgRNAs or an NTC sgRNA. Mean relative light units (RLU) are shown. n = 3 experiments. Error bars represent standard deviation. Student’s t-tests were performed. (B–D) *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P ≤ 0.0001. (E) Indel frequency at listed sgRNA target sites. Genomic DNA derived from THP-1–Cas9 cells 7 d after sgRNA infection. (F) Arrayed target validation screen in eight Cas9-stable AML lines. Mean viability at each day is shown. Screen performed in duplicate.
study gene expression changes before appreciable cell death and collapse of the cell population, which occurs by day 7. RNA-seq profiling of SETDB1 sgRNA–treated cells, relative to NTC sgRNA–treated cells, showed similar gene expression across these populations (Fig. S1 E). In addition, the RNA-seq reads derived from SETDB1 sgRNA–treated cells exhibited a nearly uniform presence of mutated alleles at the expected sgRNA target sites, confirming gene disruption (Fig. S3 A). RNA-seq data from SETDB1 mutant cells at day 4 demonstrated a robust activation of IFN-stimulated genes (ISGs) with both target-specific sgRNAs (Fig. 2, A and B). Accordingly, panther gene ontology analysis revealed that type I IFN signaling and antiviral response genes were identified as the most up-regulated pathways in these cells (Fig. 2, A–C; Mi et al., 2017). The most significant down-regulated pathways include genes that regulate gene expression (translation) and viral transcription. We validated the RNA-seq data by quantitative real-time PCR (qRT-PCR) analysis of the SETDB1 mutant cells, which confirmed increased expression of IFN-β and several ISGs, including IFIT1–3, RIG-I (DDX58), OAS3, and MDA5 (IFIH1; Fig. 2 D). ISG expression remained elevated at day 7 (Fig. S2 I). To exclude the possibility that DNA damage induced expression, ISG induction was further validated in THP-1 lines that express SETDB1-specific shRNAs (Fig. S2. E–G; Härtlova et al., 2015; Pépin et al., 2016). Consistent with the SETDB1 sgRNA–treated THP-1 cells, disruption of SETDB1 in three additional AML lines led to up-regulation of IFN-β at day 4 and increased ISG expression by day 7. Notably, though all three cell lines sensitive to SETDB1 mutation up-regulated IFN-β and IFIT2, they exhibited differential baseline and induced ISG expression levels (Fig. 2 E). Together, these results suggest that SETDB1 represses, directly or indirectly, type I IFN induction.

**Loss of SETDB1 leads to induction of retro-TEs**

Increased ERV expression has been observed after the deletion of SETDB1 in developing cells, including embryonic stem cells and B cells, although ERV suppression has not been reported as a function of SETDB1 in cancer cell lines (Matsui et al., 2010; Karimi et al., 2011; Collins et al., 2015; Tchasovnikarova et al., 2015). RNA-seq analysis of SETDB1-disrupted cells revealed striking up-regulation of the ERV ERV3-1 in THP-1, as well as three additional AML cell lines treated with SETDB1-specific sgRNAs or SETDB1-targeting shRNAs (Fig. 2, B, D, and E; and Fig. S2, H and I). Notably, repetitive elements may be hypomethylated in cancers, enabling them to mobilize and potentially destabilize the genome (Howard et al., 2008; Ehrlich, 2009). Therefore, we hypothesized that TEs are suppressed by SETDB1 in THP-1 cells, and removing this block would induce expression of TE transcripts, thus contributing to the observed antiviral response. ERV3-1 bears a unique nucleotide sequence and can be distinguished from ERVs within the same class. Although this transcript is listed in the standard genomic annotations (e.g., Ensembl) and evaluated in standard RNA-seq toolkits, such as that used for our initial expression analyses, retrotransposons can be present in the genome at many copies and are thus masked out (repeat masked) of the standard genome annotations as being multimappers or low-complexity sequences. Therefore, we re-analyzed our RNA-seq data using a pipeline that enables alignment of repetitive (repeat masked) reads (Criscone et al., 2014). Using this approach, we found that manyLTR-containing ERV subfamilies and non-LTR elements, including LINEs and satellite repeats, were consistently up-regulated in SETDB1 sgRNA–treated cells but not in the NTC controls (Fig. 4, A and B). Some LTRs are down-regulated, possibly because of induction of KRAB-ZNFs. Notable for their coevolution with and ability to silence ERVs, KRAB-ZNF genes are known to be enriched for the H3K9me3 mark (Schultz et al., 2002; Jacobs et al., 2014). These data suggest SETDB1 plays a critical role in repressing retro-TEs in AML cells.

It has previously been shown that both LINEs and ERVs exhibit bidirectional transcription and that these overlapping transcripts can pair to form dsRNAs (Dunn et al., 2006; Faulkner et al., 2009; Liu et al., 2016). Therefore, we reasoned that an increase in the homoeostatic levels of dsRNAs, formed by expressed TEs, could trigger the IFN response we observed in SETDB1–disrupted AML cell lines. To determine whether there was an increase in overlapping sense and antisense transcripts in the up-regulated TEs, we performed strand-specific RNA sequencing of THP-1–Cas9 cells treated with an NTC or a representative SETDB1–specific sgRNA. The most up-regulated TE subfamily members (genes shown in Fig. 4 B) were aligned to the Dfam consensus sequence (Hubley et al., 2016). Our analysis, which is consistent with previously published work, shows that whereas a few ERVs are unidirectionally transcribed (e.g., LTR4; Fig. 4 C), many of the ERV subfamily members and all LINE-1 and satellites surveyed exhibited increased concurrent sense and antisense transcription (e.g., L1P1 and L1PA10).
up upon SETDB1 disruption (Fig. 4, C and D; Walsh et al., 1998; Domansky et al., 2000; Kanellopoulou et al., 2005; Dunn et al., 2006; Liu et al., 2016). Interestingly, for many of these elements, the ratio of sense to antisense transcripts did not change (Fig. S3, B-D). To visualize whether the increase in transcription from both orientations occurred over the same regions for each element, thus increasing the probability that they could pair and form dsRNAs, we generated RNA-seq read density plots. As predicted, an increased yield of overlapping transcripts was observed at select loci. Representative examples of uni- and bidirectionally transcribed TEs, induced upon SETDB1 disruption, are shown in Fig. 4 D.

To experimentally determine directly whether there was an increase in the abundance of dsRNAs in cells after depletion of SETDB1, we stained THP-1 cells treated with either SETDB1- or CD81-specific synthetic gRNAs with the J2 antibody, which detects dsRNA (Weber et al., 2006). In these experiments, the CD81 gRNA was used to confirm CRISPR/Cas9 activity after gRNA nucleofection and control for the possibility that DNA
breaks themselves may induce dsRNA expression. We observed induction of dsRNAs in cells that have lost SETDB1 (as verified based on the mutation status of SETDB1 from an aliquot of the cells used for image analysis; see Fig. 6 C) but not in the control cells, consistent with our stranded RNA-seq data (Fig. 5 A). We then sought to determine whether this increase in dsRNA content was correlated with up-regulation of dsRNA derived from derepressed TEs. To test this, we performed an RNase protection assay (Roulois et al., 2015). In brief, total RNA was collected from THP-1 cells treated with distinct SETDB1-specific or NTC sgRNAs, and the RNA was digested with a cocktail of RNase A and T1 to remove single-stranded RNA, suggesting the evaluated TE loci can produce stable dsRNAs (Fig. 5 C).

Cell death induced by SETDB1 disruption is dependent on the viral sensing machinery
Because we observed concordant loss of H3K9me3 and up-regulated transcription of TE loci, in SETDB1 disrupted cells, we reasoned that one possible trigger of the IFN antiviral response could be the increased output of aberrant, TE-specific transcripts and their subsequent detection by the nucleic acid-sensing innate immune receptors. To determine whether this machinery was responsible for inducing type I IFN and, consequently, cell death, we used CRISPR to knock out cytosolic RNA sensors of the innate immune system, including IFIH1 (MDA5), DDX58 (RIG-I), and MAVS, followed by disruption of SETDB1. MDA5, MAVS, and RIG-I-specific sgRNA were >100–1,000-fold resistant to RNase digestion compared with single-stranded RNA, suggesting the evaluated TE loci can produce stable dsRNAs (Fig. 5 C).
expression conferred protection of viability in the presence of the SETDB1-specific sgRNAs (Fig. S5 A). The loss of MDA5 and MAVS in the combinatorial sgRNA-treated cells was confirmed by FACS analysis of THP-1–Cas9 cells treated with two different sgRNAs/gene (Fig. S5, B and C). RIG-I disruption was confirmed by sequencing the targeted loci for mutations, which demonstrated that >99% disruption occurred with two separate sgRNAs (Fig. S5 D).

As shown in Fig. 2 (D and E), expression of the ISG, IFIT2, provided a sensitive, robust readout of the IFN response within distinct SETDB1-disrupted AML lines. To evaluate whether activation of the cytosolic sensors contributed to IFIT2 induction, we generated single-cell THP-1–Cas9 KO clones for MDA5 as well as KO and hypomorphic clones for RIG-I by way of nucleofected gRNAs (Fig. 6). Western blotting was performed on each clone after overnight stimulation with IFN-β, and mutations were confirmed by sequencing (Fig. 6 G). IFIT2 induction and the negative effects on viability were significantly reduced when KO clones were nucleofected with two distinct gRNAs targeting SETDB1 compared with control THP-1–Cas9 cells (Fig. 6, A, B, D, and E). These effects were less pronounced in the RIG-I hypomorphic clone relative to the complete KO. SETDB1 gRNA activities were confirmed by DNA sequencing of the target sites, which show ~70–100% disruptive mutation rates across the treated cell populations (Fig. 6, C and F). Together, the combination of expression changes and functional data provided in these studies suggests a complex mixture of RNAs become expressed in the absence of SETDB1, which triggers a cytosolic, nucleic acid–sensing cascade and IFN-mediated cell death (Fig. 6 H).

Discussion

Using an unbiased LOF screen and cross validation in human AML cell lines, we uncovered a novel axis for stimulating an innate immune response and reducing cell viability through the disruption of SETDB1. Specifically, the mutation of SETDB1
leads to rapid desilencing of TEs, including ERVs, satellite repeats, and LINEs, many of which exhibit bidirectional transcription. Expression of these elements is coincident with an increase in dsRNA content, activation of the cytosolic RNA-sensing pathway, and IFN-induced apoptosis (Fig. 6 H).

Intriguingly, the means by which SETDB1 silences TEs may differ between cancerous or normal adult tissues and the germline, owing to cell type– or context-specific differences in SETDB1-dependent H3K9me3 deposition. Similar to studies in developmental contexts, SETDB1 KO in THP-1 cells resulted in modest global depletion of H3K9me3, and increased transcription was likely caused by localized turnover of this mark at TE loci (Collins et al., 2015; Koide et al., 2016). Furthermore, despite their presence in our library, we did not observe the dropout of sgRNAs targeting HP1 family members or TRIM28/KAP1, factors known to be required for TE silencing in embryonic cell types (Rowe et al., 2013). This is consistent with the retrovirus-silencing properties of SETDB1 that occur independent of its known germline-associated cofactors (Fig. 1 A; Maksakova et al., 2011; Tchasovnikarova et al., 2015).

Here, we have defined a unique avenue for inducing apoptosis in AML contexts and identified a previously uncharacterized role for SETDB1 in suppressing innate immunity by limiting the overall abundance of TE expression in cancer cells. Because SETDB1 is amplified and/or overexpressed in many human cancers and its expression is induced upon lethal chemo-

therapeutic drug exposure, further work to determine whether up-regulation of this gene is a common mechanism to evade the host innate immune response, thus acting as a cancer cell immunity cloak, should be pursued (Guler et al., 2017). Additionally, while we and others have found that the KO and knockdown of SETDB1 reduces AML cell viability in vitro and in vivo, it remains to be seen whether chemical inhibition of this enzyme is feasible and can induce a similar molecular phenotype (Shi et al., 2015; Wang et al., 2015, 2017; Koide et al., 2016; Tzelepis et al., 2016). The impact of TE expression may extend beyond the cell-intrinsic growth defects evaluated in our study. Indeed, TE-derived antigens have been considered as targets of antitumor immunotherapy, and induction of these elements via inhibition of DNA methylation has been linked to sensitization of cells to checkpoint blockade in preclinical settings (Cherkasova and Selyatitskaya, 2013; Chiappinelli et al., 2015; Roulois et al., 2015; Kassiotis and Stoye, 2016).

Moreover, repeat-associated dsRNAs have been shown to trigger production of siRNAs and silencing by an RNAi-dependent mechanism (Yang and Kazazian, 2006). Curiously, several components of the RNAi machinery, including DICER1, AGO1, and AGO4, are up-regulated in SETDB1-mutated THP-1 cells (unpublished data). Therefore, in light of evidence that the KO of DICER leads to an accumulation of repeat-associated dsRNAs and induction of IFN in both mouse and human cultured cells, it is tempting to speculate that SETDB1

Figure 5. Loss of SETDB1 leads to the induction of dsRNAs. (A) Confocal microscopy images of representative THP-1–Cas9 cells 5 d after nucleofection with synthetic gRNAs targeting CD81 (negative control, top) or SETDB1 (target sequence 6, bottom). Cells were stained with J2 antibody to label dsRNA and DAPI to mark nuclei. (B) qRT-PCR validated expression of bidirectionally transcribed LINEs and ERVs in THP-1–Cas9 cells after 5 d of treatment with SETDB1 sgRNAs or an NTC control sgRNA (n = 3 biological replicates). (C) RNase protection assay showing expression of dsRNAs in THP-1–Cas9 cells after 5 d of treatment with synthetic SETDB1-specific gRNAs after digestion of total RNA with RNase A/T1. ssRNA, single-stranded RNA. Enrichments are relative to NTC control sgRNA (n = 3 biological replicates, and Student’s t-tests were performed). Error bars represent standard deviation.
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contributes to a potential network of overlapping mechanisms aimed at silencing inappropriate somatic TE transcription (Murchison et al., 2005; Yang and Kazazian, 2006).

Materials and methods

CRI{SPR} library generation

A panel of \( \sim 350 \) known or predicted epigenetic regulators was identified, and \( \sim 15–25 \) sgRNAs were designed to target each gene. sgRNAs were designed to target coding regions common to the most isoforms, with the 5′ end of the CDS favored to maximize disruption. sgRNAs with the best off-target score based on the number and location of mismatches were used. Selected sgRNAs were formatted into a standard expression context (Mali et al., 2013) and cloned en masse into pLKO (SHC-201; Sigma-Aldrich) by Cellecta, Inc., creating what we term the Epi300 sgRNA library. Illumina sequencing confirmed that >99% of the designated sgRNAs were represented in the cloned plasmid library.

CRI{SPR}/Cas9 sgRNA pooled screen and analysis

THP-1 cells (ECACC; Sigma-Aldrich) were transduced with a lentiviral vector (pLenti7.3; ThermoFisher) engineered to coexpress a human codon-optimized \( S. \) pyogenes Cas9 along with emerald GFP (emGFP). emGFP-expressing cells were collected after fluorescence-activated cell sorting with a flow cytometer (FACS Aria, BD). \( 20 \times 10^6 \) THP-1–Cas9–emGFP cells per biological replicate were seeded into a 50-ml conical tube containing 8 \( \mu \)g/ml polybrene and Epi300 sgRNA viral pool (MOI 0.3). Cells were dispensed into six 6-well plates and spin transduced for 45 min at 1,800 rpm at 20°C. The next day, cells were pooled and transferred to flasks (T-175; Corning). 2 d after transduction, the cells were placed under selection with media containing 1 \( \mu \)g/ml puromycin. Cells were maintained at a minimum of \( 10 \times 10^6 \) cells at all times to maintain over 100× representation. Reference samples were taken at day 7, and competitive growth was assessed at day 21. At each time point, \( 10 \times 10^6 \) cells were pelleted and flash frozen. Genomic DNA was prepared with a blood and tissue kit (DNeasy; Qiagen) and quantified using a fluorimeter (Qubit; ThermoFisher). To determine the sgRNAs that were enriched or depleted, we amplified sgRNA insertions from genomic DNA by PCR. The mass of DNA required to maintain representation of each sgRNA was determined by multiplying the number of cells with integrants by nanogram/genome. PCR was performed with 500 ng DNA per PCR reaction with polymerase (Phusion; NEB). Primer sequences were forward, 5′-TCT TGT GGA AAG GAC GAG GTA CCG-3′, and reverse, 5′-CTA CTA TTC TTT CCC CTG CAC TGT-3′. \( \sim 221 \) bp PCR products were isolated. Next-generation sequencing libraries were prepared with the TruSeq Nano DNA library (Illumina) with 5% PhiX spiked in or NuGen Ovation low-complexity kits followed by PCR amplification for six cycles. Samples were sequenced on a MiSeq system (Illumina) with reagent kit v3 for 150 cycles. Raw FASTQ files were aligned with the genome-indexed nucleotide alignment program (GSNAP), and differential analysis was performed using DESeq2 (Love et al., 2014; Wu et al., 2016).

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TCGA data
For TCGA analysis, the data were based in part on data generated by the TCGA Research Network. Genotype-Tissue Expression Project expression data for normal blood samples were obtained from https://www.genome.gov/gtex/.

Arrayed sgRNA transduction
As detailed in the CRISPR/Cas9 sgRNA pooled screen and analysis section, MV-4-11 (ATCC), MOLM-13 (DSMZ), OCI-AML-3 (DSMZ), UKE-1 (Coriell Institute), HL-60 (ATCC), ML-2 (DSMZ), and EOL-1 (DSMZ) cells were transduced with pLenti7.3-Cas9 and selected based on emGFP expression. Individual sgRNAs were cloned into pLKO, and lentivirus was prepared in 96-well plates. For each cell line, 35 × 10⁶ Cas9-emGFP cells were seeded into round-bottom 96-well plates and transduced with virus in 8 µg/ml polybrene. Cells were spin transduced for 30 min at 1,800 rpm at 20°C. Screen was performed in duplicate. Viability was measured every 2–3 d for a total of 18 d using CellTiter-Glo (Promega). For caspase detection, the Caspase-Glo 3/7 assay kit (Promega) was used, and data were normalized to viability derived from CellTiter-Glo assays.

Inducible SETDB1 shRNA cell line generation
Individual shRNAs targeting human SETDB1 were designed using the DSIR algorithm. The Ren.713 non-targeting control was used in a previous study (Fellmann et al., 2013). All targeting sequences were converted into 125-nt DNA oligonucleotides, annealed, and cloned into the XhoI–EcoRI restriction sites of the lentiviral, doxycycline-inducible shRNA expression vector pMinDUCERv1-dRFP-miRE-ET2P, enabling expression of optimized miR-30-based shRNAs along with a turboRFP (Evrogen) marker.

shRNA oligo sequences were SETDB1_3_FOR, 5′-TCGAGAAGGTATATTGCTTGACATGAGCCGATAGCTGAGACCAAACGCTCTAGTGAAAGCAAGATGATGTACCTTTGCTGTCGACTAGTCCGCTCTGCTCAGTCTGAGTACCAACGCAATAATCTCCT-3′; SETDB1_4REV, 5′-TCGAGAAGGTATATTGCTTGACATGAGCCGATAGCTGAGACCAAACGCTCTAGTGAAAGCAAGATGATGTACCTTTGCTGTCGACTAGTCCGCTCTGCTCAGTCTGAGTACCAACGCAATAATCTCCT-3′; SETDB1_3_REV, 5′-AAATCTGAGCCCTTATGAGTCCGAGAGCAGTAGACGGCCTAGCTGAGACAAACGCTCTAGTGAAAGCAAGATGATGTACCTTTGCTGTCGACTAGTCCGCTCTGCTCAGTCTGAGTACCAACGCAATAATCTCCT-3′; SETDB1_4_REV, 5′-AAATCTGAGCCCTTATGAGTCCGAGAGCAGTAGACGGCCTAGCTGAGACAAACGCTCTAGTGAAAGCAAGATGATGTACCTTTGCTGTCGACTAGTCCGCTCTGCTCAGTCTGAGTACCAACGCAATAATCTCCT-3′; Ren.713_FOR, 5′-TCGAGAAGGTATATTGCTTGACATGAGCCGATAGCTGAGACCAAACGCTCTAGTGAAAGCAAGATGATGTACCTTTGCTGTCGACTAGTCCGCTCTGCTCAGTCTGAGTACCAACGCAATAATCTCCT-3′; and Ren.713_REV, 5′-AAATCTGAGCCCTTATGAGTCCGAGAGCAGTAGACGGCCTAGCTGAGACAAACGCTCTAGTGAAAGCAAGATGATGTACCTTTGCTGTCGACTAGTCCGCTCTGCTCAGTCTGAGTACCAACGCAATAATCTCCT-3′.

THP-1 cells (ECACC; Sigma-Aldrich) were transduced with lentiviral particles as described in the CRISPR/Cas9 sgRNA pooled screen and analysis section. After transduction, stable, transgenic cells were selected with 1 µg/ml puromycin. Stable lines were treated with 500 ng/ml doxycycline to induce the expression of shRNAs for various assays.

RNA-seq and analysis
THP-1–Cas9 cells were transduced with either SETDB1 sgRNA 6 or 9 or an NTC sgRNA and collected at various time points. Total RNA was isolated with the RNAeasy micro kit (Qiagen). The concentration of total RNA samples was determined using a NanoDrop 8000 (Thermo Scientific). The integrity of RNA samples was determined using a bioanalyzer (2100; Agilent Technologies). 0.5 µg of total RNA was used as an input material for library preparation using an RNA sample preparation kit v2 (TruSeq; Illumina). For stranded RNA-seq, 100 ng of total RNA for each sample was used for library preparation with a TruSeq Stranded total RNA library prep kit. The size of the libraries was confirmed using a 2200 TapeStation and high-sensitivity screen tape (D1K; Agilent Technologies), and their concentration was determined via a qPCR library quantification kit (KAPA). The libraries were multiplexed and then sequenced on an HiSeq2500 system (Illumina); we generated 30 M single-end 100-bp reads for TruSeq RNA libraries and 30 M paired-end 75-bp reads for TruSeq Stranded RNA libraries. Resultant FASTQ files were aligned to GSNAP, and differential gene expression analysis was performed with DESeq2 after removing genes with less than five reads in any of the samples (Love et al., 2014; Wu et al., 2016).

TE analysis
RNA-seq and ChIP-seq FASTQ files were aligned to the human genome (hg19) using Bowtie version 0.12.9 (Langmead et al., 2009). Unique mapping and multimapping reads were separated and analyzed using RepEnrich (; Criscione et al., 2014). Differential enrichment analysis was performed with EdgeR GLM on the fractional counts data (Robinson et al., 2010). Library size was determined based on Bowtie, (reads processed) – (reads that failed to align).

Bidirectional transcript analysis
Consensus sequences for elements for up-regulated TEs were downloaded from the Dfam database (Hubley et al., 2016). Stranded RNA-seq data were then aligned to consensus sequences using BWA-MEM and quantified using HTseq-count (Li, 2013; Anders et al., 2015). Reads were visualized using the Integrative Genomics Viewer (IGV) genome browser and a custom pseudogenome that was generated from the Dfam consensus sequences (Robinson et al., 2011; Thorvaldsdottir et al., 2013).

ChIP-seq and analysis
Native ChIP-seq on SETDB1 mutant cells (generated with SETDB1 sgRNA 6 or 9) or NTC-treated cells was performed as described in Brind’Amour et al. (2015). 10% of 300 µM Nase-digested chromatin was removed and saved as input/unenriched chromatin, and the rest was immunoprecipitated with an H3K9me3 antibody (49-1008; Thermofisher). 30 µl of input chromatin was decross linked in the presence of 5 µl of 5 M NaCl and 2 µl proteinase K at 65°C overnight on a thermocycler. The input was then purified over a column (miniElute; Qiagen) in 20 µl TE buffer (Qiagen). 50-bp single-end reads derived from immunoprecipitated and input samples were generated on a HiSeq2500 system. Reads were aligned to hg19 with Bowtie 2.2.4 and processed with MACS2 (Zhang et al., 2008; Langmead et al., 2009). Peak calling was performed with both broad- and narrow-peak functions using a bandwidth of 150, tag size of 50, and q-value cut-off of 0.05. The RepEnrich pipeline was used to determine H3K9me3 levels over TEs. This pipeline generates fractional counts for each element. Fractional counts are generated by quantifying reads that map exclusively to a single repetitive subfamily in addition to counting reads that map to multiple subfamilies using a fractional value (1/N), with N defined as the number of repetitive element subfamilies to which that read maps, as previously described (Criscione et al., 2014). The fractional counts generated by RepEnrich for each sgRNA were normalized to fractional counts from the input sample to generate the relative levels shown in the heat maps in Fig. 3 (relative value = SETDB1 sgRNA 6/9 or sgRNA NTC H3K9me3 fractional counts/input counts).
qRT-PCR assays: ChiP–qPCR assay and Taqman assays

The ChiP-PCR primer sequences were L1 5′UTR forward, 5′-ACG GAATTCGCTTGTTCGA-3′; and reverse, 5′-TGACTGCTACAAAAGG CTTG-3′; and reverse, 5′-GGGATTGATCCCAAGG-3′; HERV-Rev forward, 5′-AGTTGCGATCCACCAAGA-3′; and reverse, 5′- CTGATTGCTGCTTTCTCA-3′. A starting input of 10% was used for qRT-PCR analysis.

PCR was quantified via SYBR-green (biomake). Data were analyzed using the percent input method (100 × adjusted input – Ct (IP)). Input was adjusted by taking Ct input – 3.32. For Taqman qRT-PCR experiments, total RNA was isolated with RNeasy micro kits (Qiagen), including an on-column DNase digestion step. Reverse transcription was performed with 250–1,000 ng of total RNA (N8080234; Applied Biosystems). All Taqman qPCRs were run with 5 μL cDNA (diluted 1:5 first), 5 μL of 2× Taqman Universal master mix (4304437; Applied Biosystems), and 0.5 μL probe per reaction. Taqman gene expression probes were obtained from ThermoFisher, ACTB Hs0353733_g1, ERV3-1 Hs04184598_g1, GAPDH Hs00178991_g1, IFN-β Hs00779581_s1, IFIT1 Hs00327069_s1, IFIT2 Hs01927387_s1, IFIT3 Hs01922752_s1, MDA5 (IFIH1) Hs00223420_m1, MAVS Hs00920075_m1, OAS3 Hs00196324_m1, RIG-I (DDX58) Hs01061436_m1, RPL36 Hs00306633_g1, and SETDB1 Hs01048361_m1.

J2 antibody staining

THP-1–Cas9 cells nucleofected with CD81- or SETDB1-specific sgRNAs were attached to slides using a cytospin centrifuge at 800 rpm for 5 min after 5 d of treatment. Cells were fixed with 3% paraformaldehyde in PBS for 10 min and permeabilized in 0.5% Triton X-100 in PBS for 2 min. Slides were treated with 3% hydrogen peroxide solution to quench endogenous peroxidase activity for 60 min followed by blocking for 60 min with blocking buffer (B40913; ThermoFisher). Slides were incubated with the J2 primary antibody (10010200; Sci- cons) at 1:200 in PBS overnight at 4°C. The next day, the slides were processed with the Tyramide SuperBoost kit (B40913; ThermoFisher) according to the manufacturer’s protocol. Images were acquired using a confocal microscope (SP8; Leica) equipped with a 100× oil immersion lens (HC PL APO CS2 100×/1.4 oil), a pinhole of 0.5, and a Z step size of 0.19 μm, displayed as maximum intensity projections. DAPI was excited with a 405-nm laser diode at 2% and detected from 410 to 483 nm, and the tyramide Alexa Fluor 555–amplified J2 staining was excited using a white light laser at 50% output at 553 nm at 2% and acquired from 558 to 633 nm using HyD detectors in standard mode with 5× line averaging.

Histone purification and mass spectrometry

Core histones (H2A, H2B, H3, and H4) were purified from frozen cell pellets by acid extraction, ion exchange, and perchloric acid precipitation using a commercial kit (Histone Purification Mini kit; 40026; Active Motif) according to the manufacturer’s instructions. The purified histones were resuspended in deionized distilled water to a final concentration of 0.5–1.0 μg/ml and stored at −80°C until use. 2 μg aliquots of endogenous histones was mixed with equal amounts of purified stable isotope–labeled core histones, purified from PC9 cells, and grown in media supplemented with 13C6,N2 lysine and 15N6 arginine that served as internal standards. Samples were prepared for mass spectrometry by propionylation of lysines, digestion with trypsin, and derivatization of peptide N termini with phenyl isocyanate as previously described (Maile et al., 2015). Histone peptides were quantified by capillary reverse phase liquid chromatography nanoelectrospray tandem mass spectrometry on a hybrid quadrupole-orbitrap mass spectrometer (Q-Exactive HF; ThermoFisher) in a parallel reaction monitoring experiment. Quantitative data on 40 distinct posttranslational modifications of histones H3 and H4 in 78 combinations were extracted via Skyline software and normalized via internal standards as previously described (Vinogradova et al., 2016).

Viral sensor sgRNA and FACS assays

Cells were transduced with viral sensor sgRNAs (sgRNAs are in Table 2). 2 d after transduction, cells were subjected to a second transduction with SETDB1-specific sgRNAs or an NTC control sgRNA. Cells were selected with 1 μg/ml puromycin (viral sensor sgRNAs) and 5 μg/ml blasticidin (SETDB1 or NTC sgRNAs). Cells were harvested for FACS analysis 14 d after the second transduction. In brief, cells were fixed with 4% paraformaldehyde for 15 min at room temperature and then washed with 1 ml PBS. Cells were permeabilized with PBS + Tween 0.1% for 15 min. Primary antibodies were used at a 1:100 dilution (MABS; ab31334; Abcam; MDA5; ab69983; Abcam) in 150 μL FACS buffer (PBS with 0.5% BSA and 2 mM EDTA) at 4°C for 30 min. The cells were washed twice with 1 mL FACS buffer and analyzed on a LSRII analyzer (BD).

Western blotting

To detect SETDB1 protein, 5–10 × 10^6 THP–1–Cas9 cells treated with the respective sgRNAs were lysed in 150 μL radio-immunoprecipitation assay buffer plus protease inhibitors (4693159001; Roche). Samples were placed on ice and allowed to incubate for 30 min (with vortexing briefly every 5 min). Samples were then sonicated with a Bioruptor for 11 cycles of 30 s on and 30 s off. Samples were spun down at 13,000 rpm for 10 min at 4°C. 50–100 μg of protein was prepared in 1× SDS loading buffer (ThermoFisher) and 1× reducing agent (ThermoFisher), heated to 70°C for 10 min, and loaded into 4–12% Bis-Tris gels (ThermoFisher). MOPS buffer was chilled to 4°C, and gels were run at 75 V for ~3 h. Gels were transferred to polyvinylidene fluoride membranes using an iblot2 (ThermoFisher) for 7 min at 23 V. Membranes were blocked with 5% nonfat milk diluted in TBS with 0.1% Tween 20 (TBS-T) for 1 h and were rinsed briefly three times with TBS-T and incubated with primary antibodies overnight in TBS-T with 5% nonfat milk. Blots were washed three times for 15 s and then three times for 10 min in PBS-T and then incubated with secondary antibodies in TBS-T with 5% nonfat milk. Blots were washed as previously described, and detection was performed with Supersignal Femto (Pierce). The antibodies were 1:500 rabbit anti–SET DB1 (PA5-29101; ThermoFisher), 1:2,000 mouse antihistone H3 (3638; CST), 1:10,000 antihistone MRP (1721011; BIO-RAD), and 1:10,000 antirabbit HRP (1721019; BIO-RAD). To detect MDA5 and RIG-I, 1 × 10^6 THP-1 cells were lysed in 150 μL radio-immunoprecipitation assay buffer plus protease inhibitors. Samples were placed on ice and allowed to incubate for 30 min (with vortexing briefly every 5 min). Samples were spun down at 13,000 rpm for 10 min at 4°C. 10–20 μg of protein was loaded into 4–12% Bis-Tris gels (ThermoFisher) and run at 200 V in MOPS buffer for 50 min. Gels were transferred to polyvinylidene fluoride membranes using an iblot2 with program P0. Samples were blocked, washed, and developed as described earlier in this section. Antibodies were 1:1,000 rabbit anti-MDA5 (ALX-210-935; Enzo), 1:1,000 rabbit anti–RIG-I (ag-20b-0009; Adipogen), and 1:1,000 mouse anti–β-actin (47778; Santa Cruz Biotechnology).

RNase protection assay

RNA protection assays were performed as described in Roulois et al. (2015) with modifications. Total RNA from THP–1–Cas9 cells transduced with SETDB1 sgRNA 6 or 9 or NTC control sgRNA was purified

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with TRIzol (ThermoFisher) according to the manufacturer’s protocol. 3 μg of total RNA was digested with RNase A/T1 mix (EN0551; Thermofisher) with 3 μl of enzymes in 150 μl RNase protection buffer (10 mM Tris-HCl, pH 7.5, 300 mM NaCl, and 5 mM EDTA). Control samples were mock digested without the addition of RNases. RNA was digested for 30 min at 37°C, followed by the addition of 5 μg glycogen and purification with TRIzol. Reverse transcription was performed as described in the qRT-PCR assays section, and 5 µl cDNA (pre-diluted to 1:5 in water) was used for SYBR-green qPCRs. Thermocycling conditions were 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min, and then a melt curve of 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s, according to the manufacturer’s protocol. dsRNA enrichment was determined by normalizing to β-actin. Primers were designed based on Dfam consensus sequences for each element and focused on predicted regions of dsRNAs inferred from our stranded RNA-seq data. Primer sequences are listed in Table 1.

Methocult assay

THP-1–Cas9 cells were transduced with sgRNAs in 96-well plates as described in the Arrayed sgRNA transduction section. 2 d after transduction, cells were selected with 1 μg/ml puromycin. 5 d after transduction and before appreciable cell death, 1,000 cells were seeded into 1.1 ml methocult H4535 in 35-mm plates. Colonies were counted using a colony counter (GelCount; Oxford Optronix) after 28 d of growth.

Indel analysis

To identify the mutations generated with SETDB1-, RIG-I-, or MDA5-specific sgRNAs, cells were transduced or nucleofected with sgRNAs or gRNAs, respectively. Guide sequences are described in Table 2. After 5–7 d of treatment, genomic DNA was isolated with a DNeasy blood and tissue kit. Targeted loci were amplified via the Methocult assay. Colonies were counted using a colony counter (GelCount; Oxford Optronix) after 28 d of growth.

Nucleofection assay

To generate THP-1–Cas9 RIG-I, MDA5, and SETDB1 KO cells, nucleofections were performed with Alt-R gRNAs from IDT with the P4 Primary Cell 4D-Nuclease X kit S (V4XP-4032; Lonza). 10 nM gene-specific CRISPR RNAs (crRNAs) and tracrRNAs (1072534; IDT) were reconstituted to 100 μM in IDT duplexing buffer. tracrRNA and crRNA were mixed 1:1 and hybridized (95°C for 5 min followed by cooling to 4°C). For each nucleofection, 2 × 10⁵ THP-1–Cas9 cells were spun down and resuspended in 20 μl P4 primary cell buffer with supplement added. 4 μl of hybridized tracrRNA/crRNAs was added to each cuvette, and cells were layered on top, mixed, and allowed to incubate for 5 min at room temperature. Cells were nucleofected with protocol CM-138 using the Amaxa 4D system (Lonza). After nucleofection, 100 μl of prewarmed medium (RPMI + 10% FBS) was added to the cuvette and immediately pipetted into 24-well plate containing 1 ml of medium. For phenotypic assays, cells were allowed to incubate for 5 d before dilution to generate single-cell clones. For single-cell clone generation, cells were sorted with a FACSAria Fusion cell sorter 1 d after nucleofection crRNA sequences (Table 2).

Table 1. Primer sequences for TE qPCRs

| Element    | Forward primer | Reverse primer |
|------------|----------------|----------------|
| HERV3      | 5'-CTCTGCTATGACTCACCTGGA-3' | 5'-CTTCCCTGATATTCTCAAGC-3' |
| L1P1.5'end | 5'-TGCGCTAAAGAGCTCTCGA-3'  | 5'-TGGTTTGGACTGCGTGTA-3'  |
| HUER5P3b   | 5'-TCAGATTGTTTCTCTGCTGCA-3' | 5'-GTTTAGTTCGCCAGCACCAT-3' |
| L1PA10.5'end | 5'-TGAGAACAGGTGGAACAA-3' | 5'-TTGAGCTCTGCTGAGTGTT-3' |
| L1PA12.5'end | 5'-TCTGATCAATCTCCCAAC-3' | 5'-TCTCTGATTTCTGCAGTTGACTG-3' |
| L1Pba.5'end | 5'-CGTACGAGACCTCCACAG-3'  | 5'-GCTTAGCGACCCACACAG-3'  |
| L1Pba1.5'end | 5'-TGGTCAGCGCTGCACT-3' | 5'-TGCTAGCTCAGTCGGTGTT-3' |
| MSTB2      | 5'-CTGCAAGACATGACTGAATAT-3' | 5'-AACAGATCTGCAGCTGGTAA-3'  |
| LTR26       | 5'-ATGAGCTTTCACATCCTAGA-3' | 5'-ATTGAGCTATGATTTGCTGC-3'  |

Table 2. sgRNA sequences used for lentiviral vectors and Alt-R crRNA generation

| Gene         | sgRNA 6          | sgRNA 7          | sgRNA 8          | sgRNA 9          | sgRNA 10         | NTC (luciferase) | PLK1           | MAVS sgRNA 1   | MAVS sgRNA 2 | MAVS sgRNA 3 | MAVS sgRNA 4 | MAVS sgRNA 5 | MDA5 sgRNA 1 | MDA5 sgRNA 2 | MDA5 sgRNA 3 | MDA5 sgRNA 4 | MDA5 sgRNA 5 | MDA5 sgRNA 6 | RIG-I sgRNA 1 | RIG-I sgRNA 2 | RIG-I sgRNA 3 | RIG-I sgRNA 4 | RIG-I sgRNA 5 | CD81 sgRNA 2 (control) | Primary Cell 4D-Nuclease X kit S (V4XP-4032; Lonza). 10 nM gene-specific CRISPR RNAs (crRNAs) and tracrRNAs (1072534; IDT) were reconstituted to 100 μM in IDT duplexing buffer. tracrRNA and crRNA were mixed 1:1 and hybridized (95°C for 5 min followed by cooling to 4°C). For each nucleofection, 2 × 10⁵ THP-1–Cas9 cells were spun down and resuspended in 20 μl P4 primary cell buffer with supplement added. 4 μl of hybridized tracrRNA/crRNAs was added to each cuvette, and cells were layered on top, mixed, and allowed to incubate for 5 min at room temperature. Cells were nucleofected with protocol CM-138 using the Amaxa 4D system (Lonza). After nucleofection, 100 μl of prewarmed medium (RPMI + 10% FBS) was added to the cuvette and immediately pipetted into 24-well plate containing 1 ml of medium. For phenotypic assays, cells were allowed to incubate for 5 d before dilution to generate single-cell clones. For single-cell clone generation, cells were sorted with a FACSAria Fusion cell sorter 1 d after nucleofection crRNA sequences (Table 2).

Data deposition

The sequences reported in this paper have been deposited at the Gene Expression Omnibus as a SuperSeries and can be found under accession no. GSE103411.
Fig. S1 depicts a cartoon overview of the screen, verification of SET DB1 protein knockdown with sgRNAs, colony growth assays of THP-1 cells treated with SET DB1 sgRNAs, and a correlation plot showing the RNA-seq expression changes between two different SET DB1 sgRNAs used after 4 d of treatment. Fig. S2 depicts shRNA validation of SET DB1 KO phenotypes, including verification of protein knockdown with SET DB1 shRNAs, qRT-PCR validation of mRNA knockdown, viability and apoptosis data after SET DB1 knockdown, qRT-PCR analysis of ISG induction after SET DB1 knockdown, and additional time point analysis demonstrating that ISGs and an ERV are induced after SET DB1 KO. Fig. S3 depicts the verification of SET DB1 disruption mediated by CRISPR/Cas9 via an IGV view of the RNA-seq data and depiction of TE expression as a percentage of each strand expressed to assess bidirectional transcription of TEs after treatment with SET DB1 sgRNAs. Fig. S4 depicts ChiP-seq data from SET DB1 mutant cells across select IFTT and zinc-finger genes. Fig. S5 depicts the assessment of cell death after SET DB1 mutation and its dependence on viral sensing genes.

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