Cuellar et al., https://doi.org/10.1083/jcb.201612160

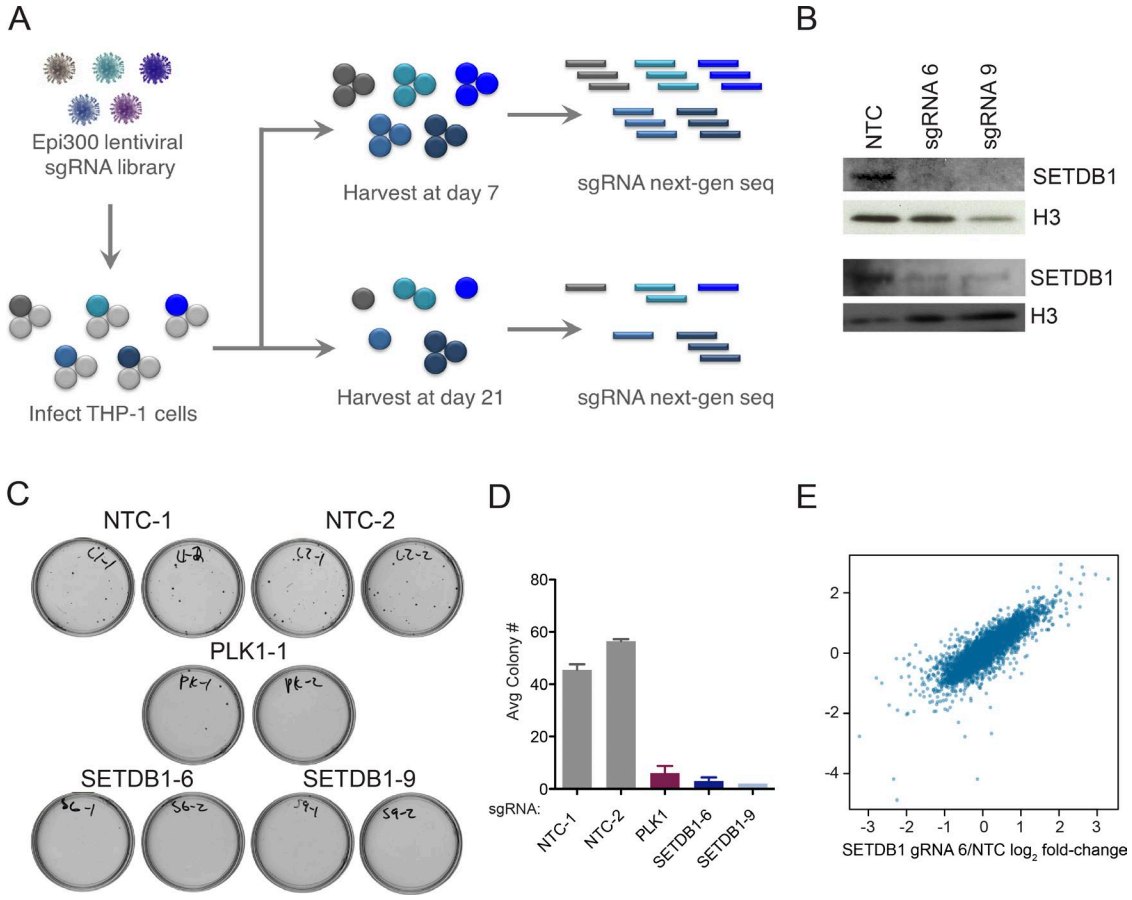

Figure S1. **CRISPR screen overview and validation of *SETDB1* as a target.** (A) CRISPR negative selection screen flowchart showing low MOI transduction with the sgRNA library into THP-1–Cas9 cells. Reference cells were collected at day 7, and cells were also analyzed at day 21 by next generation sequencing. (B) Western blotting for SETDB1 in THP-1–Cas9 cells after treatment with two different *SETDB1*-specific sgRNAs or NTC for 5 d. $n = 2$ blots shown. (C) THP-1–Cas9 colony growth after 28 d in methocult, after treatment with three different *SETDB1*-targeting sgRNAs, a positive control sgRNA against PLK1, or two different control NTC sgRNAs. (D) Quantification of colony numbers from C. $n = 2$ biological replicates, and error bars represent standard deviation. (E) RNA-seq correlation plot comparing gene expression fold change values at day 4 for cells treated with *SETDB1* sgRNA 6 or 9, relative to NTC sgRNA. $n = 3$ biological replicates for each treatment condition; $R^2 = 0.7814$.

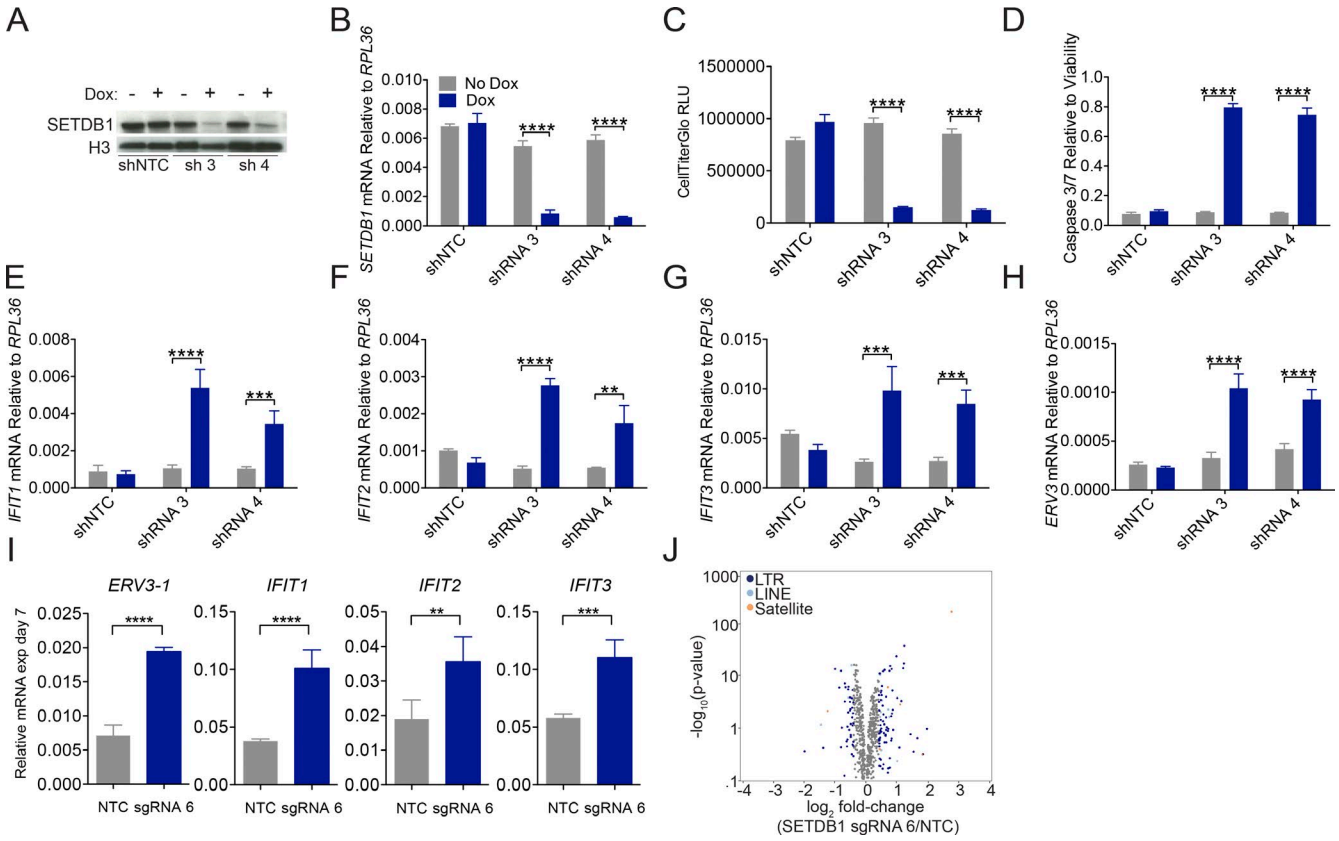

Figure S2. **Loss of *SETDB1* leads to loss of viability and induction of viral response genes and induction of TEs.** (A) Western blotting of THP-1 cells treated with *SETDB1*-specific or NTC-inducible shRNA THP-1 cells with or without 7 d of doxycycline treatment. (B) Taqman qRT-PCR assay for *SETDB1* mRNA levels with *SETDB1*-specfic or NTC shRNAs 5 d after treatment with doxycycline. (C) THP-1 viability as measured by CellTiter-Glo after 14 d of exposure to induced *SETDB1*-specific or NTC shRNAs. (D) Caspase 3/7 apoptosis assay on inducible shRNA cell lines treated with doxycycline for 14 d. (E–H) Expression of *ERV3-1* endogenous retrovirus and ISGs 5 d after treatment with doxycycline. (I) Taqman qRT-PCR assay for *ERV3-1* and *IFIT1-3* mRNA levels after treatment with *SETDB1*-specfic or NTC sgRNAs for 7 d. (B–I) $n = 3$ experiments, and Student's *t*-tests were performed. All error bars represent standard deviation. (B–I) **, $P < 0.01$; ***, $P < 0.001$; ****, $P \leq 0.0001$. (J) An RNA-seq volcano plot depicting expression changes of TEs in THP-1–Cas9 cells at day 4 after treatment with a representative *SETDB1*-specific sgRNA versus NTC. $n = 3$ for all samples; EdgeR GLM $\log_2$ fold change.

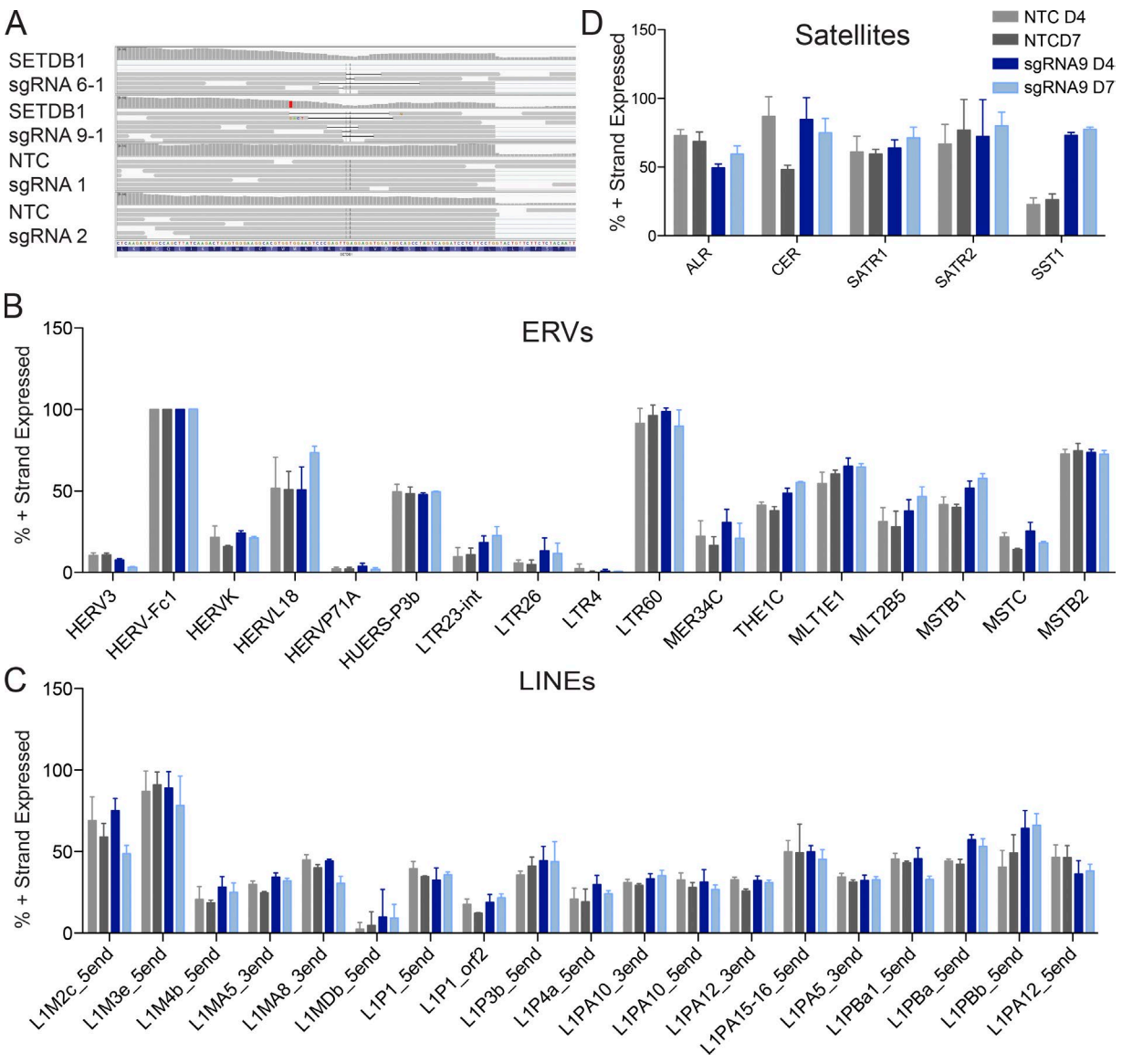

Figure S3. **Integrative genomics browser view of *SETDB1* deletions caused by sgRNA 6 and strand-specific analysis of TE transcripts.** (A) IGV view RNA-seq data confirm deletions in *SETDB1* transcripts caused by *SETDB1* sgRNA 6 at day 4 after treatment with *SETDB1* sgRNAs. (B–D) Percentage of strands expressed for significantly up-regulated ERV, LINE-1, and Satellite subfamily members to assess bidirectional transcription after treatment with NTC or *SETDB1* sgRNA after 4 and 7 d of treatment. *n* = 3 biological replicates, and all error bars represent standard deviation.

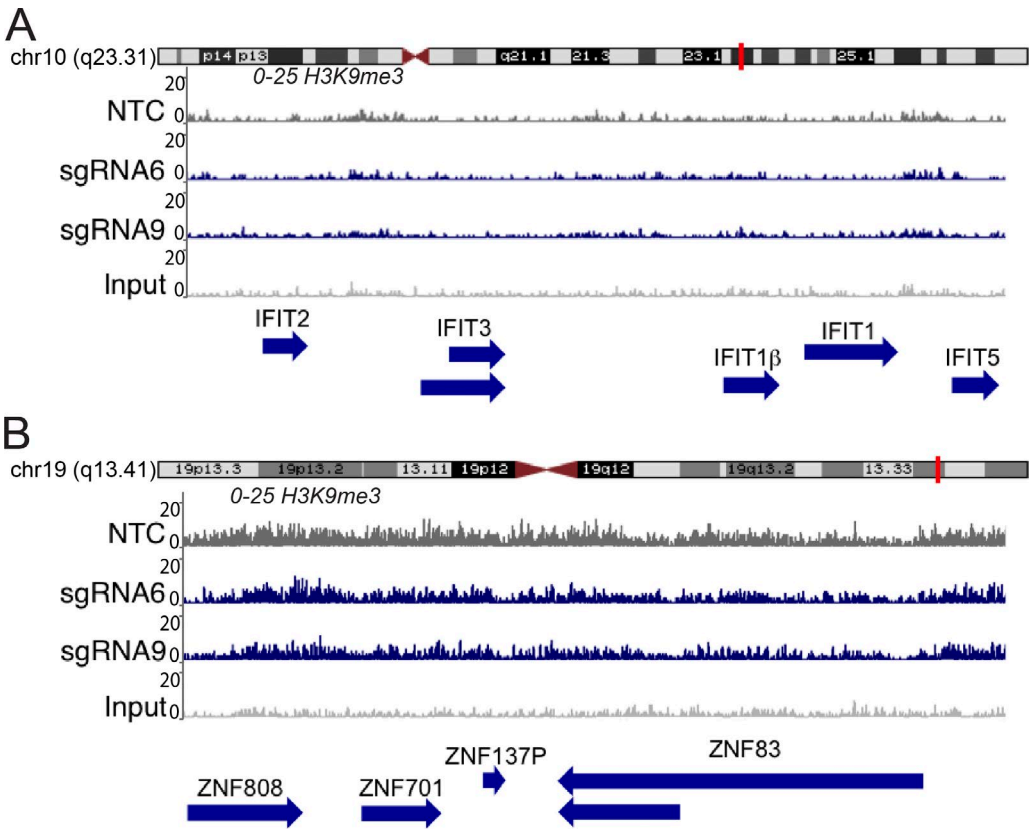

Figure S4. **ChIP-seq on *SETDB1* mutant cells shows some loss of H3K9me3 at zinc-finger genes, but not IFIT genes.** (A and B) H3K9me3 ChIP-seq density plots showing H3K9me3 peaks at IFIT (A) and zinc-finger genes (B) at 6 d after treatment with *SETDB1* sgRNAs or NTC. Y axes are 0–25, and regions are ~150 kb.

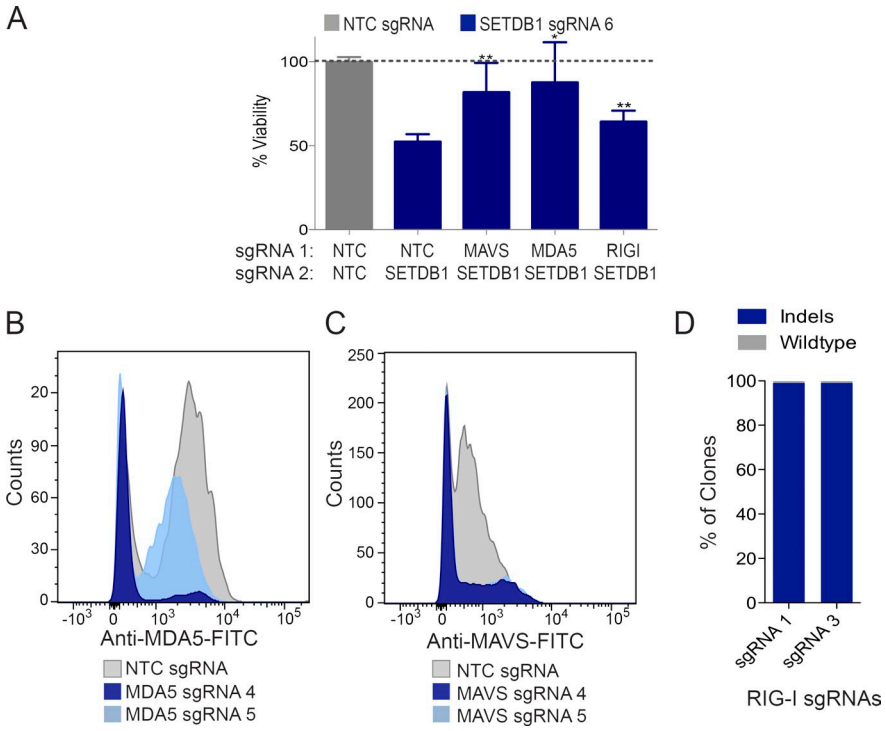

Figure S5. **Cell death in *SETDB1* mutant cells is dependent on the viral sensing machinery.** (A) Viability of cells treated with five different sgRNAs/target for *MDA5*, *MAVS*, *DDX58* (*RIG-I*), or NTC control for 2 d, followed by 7 d of treatment with *SETDB1* sgRNA 6 or an NTC sgRNA. Mean represents viability of cells treated with five different *MAVS* or *MDA5* sgRNAs and *SETDB1* sgRNA 6). *, $P < 0.05$; **, $P < 0.01$. (B and C) FACS assays confirming loss of MDA5 and MAVS with distinct, target-specific sgRNAs 14 d after transduction with *SETDB1* sgRNA 6. (D) Indel rate in THP-1–Cas9 cells treated with *RIG-I* sgRNAs 14 d after transduction with *SETDB1* sgRNAs.