

DNMT and HDAC inhibitors induce cryptic transcription start sites encoded in long terminal repeats

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Several mechanisms of action have been proposed for DNA methyltransferase and histone deacetylase inhibitors (DNMTi and HDACi), primarily based on candidate-gene approaches. However, less is known about their genome-wide transcriptional and epigenomic consequences. By mapping global transcription start site (TSS) and chromatin dynamics, we observed the cryptic transcription of thousands of treatment-induced non-annotated TSSs (TINATs) following DNMTi and HDACi treatment. The resulting transcripts frequently splice into protein-coding exons and encode truncated or chimeric ORFs translated into products with predicted abnormal or immunogenic functions. TINAT transcription after DNMTi treatment coincided with DNA hypomethylation and gain of classical promoter histone marks, while HDACi specifically induced a subset of TINATs in association with H2AK9ac, H3K14ac, and H3K23ac. Despite this mechanistic difference, both inhibitors convergently induced transcription from identical sites, as we found TINATs to be encoded in solitary long terminal repeats of the ERV9/LTR12 family, which are epigenetically repressed in virtually all normal cells.

In contrast to genetic mutations, epigenetic changes are potentially reversible, making them an attractive target for cancer treatment. Inhibitors directed against DNA methyltransferases and histone deacetylases—DNMTi and HDACi, respectively—are used for the treatment of several hematopoietic malignancies^{1,2}. Although these compounds have been in clinical use for several years, there is still a lack of knowledge regarding their mode of action³. Two previous studies on DNMTi in cancer cell lines reported the upregulation of double-stranded RNA (dsRNA) molecules, originating from codogenic endogenous retroviruses (ERV), followed by an interferon response and the induction of viral defense genes^{4,5}. However, it remains unclear how other classes of epigenetic drugs integrate into these findings and whether there are additional effects potentially missed by candidate gene approaches. Here, we globally mapped DNMTi- and HDACi-induced transcriptomic and epigenomic changes by using

whole-genome profiling technologies (Supplementary Fig. 1 and Supplementary Table 1) and show that the vast majority of TSSs that transcriptionally responded toward epigenetic modulation were cryptic, currently non-annotated TSSs encoded in solitary long-terminal repeats (LTRs).

RESULTS

Epigenetic drugs activate cryptic TSSs in the *DAPK1* gene

To efficiently measure the effects of epigenetic drugs on endogenous gene expression, we engineered the lung cancer cell line NCI-H1299 by introducing a dual fluorescence/resistance reporter (EGFP-NEO) into intron 3 of *DAPK1*, which is epigenetically silenced in association with CpG-island hypermethylation (Fig. 1a and Supplementary Fig. 2a,b). Upon treatment with the DNMTi 5-aza-2'-deoxycytidine (DAC) or with siRNAs or shRNAs targeting *DNMT1* mRNA, the

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DAPK1 promoter loses methylation, and a fusion transcript consisting of exons 1–3 and the EGFP-NEO reporter is expressed (Supplementary Fig. 2c–f). Consequently, *DAPK1*-reactivated cells can be further enriched and quantified by G418 selection (for NEO) or FACS sorting (for EGFP) (Fig. 1b). To determine the suitability of this cell line to screen for epigenetically active substances, we tested several compounds that are known to affect various epigenetic enzyme classes. Epigenetic reactivation was read out in a G418-resistance screen in which cell viability increased mainly following treatments with DNMTi and HDACi (Fig. 1c and Supplementary Fig. 2g). We confirmed reporter gene expression after DNMTi or HDACi treatment by qRT-PCR (Fig. 1d, left). To our surprise, the canonical *DAPK1* mRNA was induced only upon DAC treatment but not after HDACi treatment (Fig. 1d, right). We hypothesized that HDACi activates alternative TSSs located upstream of the EGFP-NEO sequence, thus giving rise to a truncated transcript lacking the 5' region of the *DAPK1* mRNA. By performing 5' rapid amplification of cDNA ends (5' RACE) on RNA extracted from treated cells, we identified three distinct transcript isoforms originating from cryptic (currently non-annotated) TSSs located within *DAPK1* intron 2 (TSSs α , β , and γ), all of which were spliced into *DAPK1* exon 3 (Fig. 1e and Supplementary Fig. 2h). These transcripts contain novel sequences toward their 5' ends (α , β , or γ) in place of the canonical first two exons that harbor the regular *DAPK1* start codon, and they thus comprise alternative ORFs. We confirmed the existence of these transcripts by qRT-PCR (Fig. 1f). In response to DNMTi and HDACi, the γ transcript was also found in wild-type NCI-H1299 cells, as well as in various other cancer cell lines (Fig. 1g), indicating that its activation is neither cell line specific nor a consequence of genomic editing.

Global transcription from cryptic TSSs after treatment

We hypothesized that the aberrant activation of cryptic TSSs is not restricted to the *DAPK1* locus but is a global phenomenon following treatment with epigenetic drugs. By using cap analysis of gene expression (CAGE), we mapped the genome-wide TSS usage of NCI-H1299 reporter cells treated with DNMTi (DAC), HDACi (SAHA or SB939), or both DAC and SB939 (DAC+SB) (Supplementary Data 1). CAGE overcomes technical bottlenecks associated with standard RNA-seq, such as low coverage of transcript 5' ends and difficulties in distinguishing multiple isoforms and splice variants that often overlap with reference transcripts. As a proof of concept, the CAGE data recapitulated our previous observations in that only DAC treatment, and not HDACi alone, reactivated the canonical *DAPK1* TSSs (Fig. 2a, left). Moreover, we found several CAGE tags that supported the induction of the *DAPK1* γ transcript and its respective splicing into exon 3 after treatment (Fig. 2a, right).

Globally, epigenetic treatment substantially changed the TSS usage of cells, with the combinatorial treatment (DAC+SB) showing the strongest effects (Fig. 2b and Supplementary Fig. 3a). We performed differential TSS expression analysis using a fourfold expression change and a false discovery rate (FDR) <0.05 as minimal thresholds for differential expression. Epigenetic treatment caused both quantitative and qualitative expression changes at annotated TSSs (Fig. 2c, left). In line with previous reports, DAC or DAC+SB treatment significantly upregulated cancer testis antigens (CTAs) as well as Aza-induced immune and viral defense genes (AIMs)⁶, which was accompanied by transcription from codogenic ERVs that have the capability to form dsRNAs and trigger an interferon response (Supplementary Fig. 3b–d). It is important to note that neither SB939 nor SAHA significantly induced AIM expression, suggesting that HDACi exert their function independently.

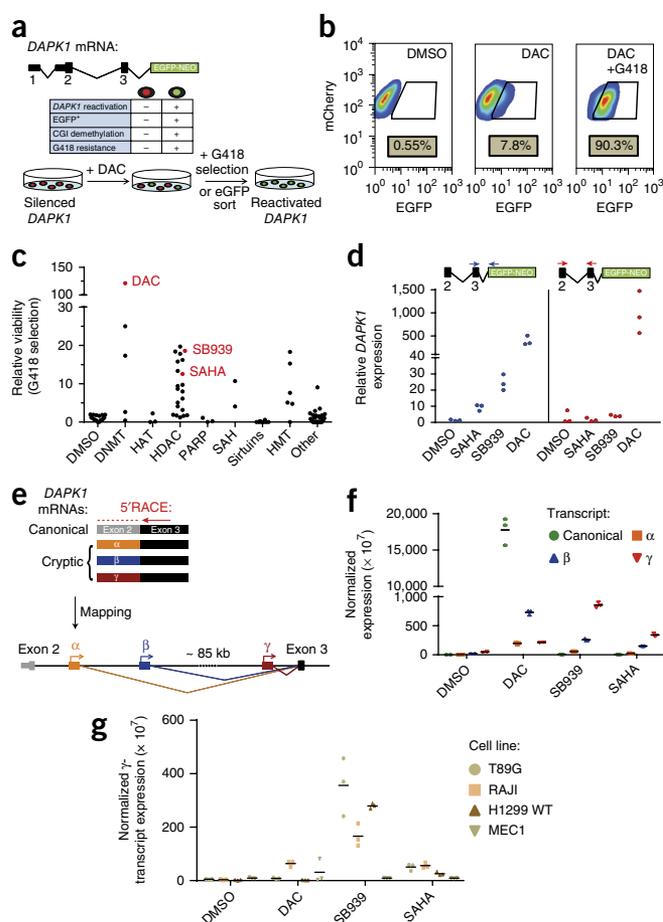


Figure 1 Novel *DAPK1* intronic TSSs arise upon epigenetic drug treatment. (a) A fluorescence and resistance marker was introduced into one allele of the *DAPK1* locus epigenetically silenced in NCI-H1299 cells. Administration of DAC reactivates a subpopulation of cells (green). The key characteristics of *DAPK1* silenced (red) and reactivated (green) cells are shown in the central table. CGI, CpG island. (b) FACS analysis showing the percentage of EGFP positive reporter cells before (left) and after DAC treatment with (right) or without (middle) additional G418 selection. (c) NCI-H1299 reporter cell viability after epigenetic compound treatment and G418 selection relative to DMSO controls. Data is sorted by inhibitor class: DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; PARP, poly(ADP-ribose)-polymerase; SAH, S-adenosyl-L-homocysteine; SIRT, sirtuins; HMT, histone methyltransferase. (d) *DAPK1* expression after DNMTi and HDACi treatment of NCI-H1299 reporter cells relative to DMSO. qRT-PCR analysis was performed using primers located either in *DAPK1* exon 2 and 3 (red) and the fluorescence/resistance marker (blue). (e) Three cryptic 5' exons (α , β , and γ) were identified by 5' RACE performed on RNA from HDACi treated cells. All cryptic transcripts spliced to the canonical *DAPK1* exon 3. γ , chr9 90219272–90219341; β , chr9 90134907–90135007; α , chr9 90125477–90125599. (f) qRT-PCR expression analysis of canonical *DAPK1* or cryptic transcripts (α , β , and γ) across treatments relative to housekeeping genes. Horizontal line represents the mean from three independent experiments. (g) Expression of the *DAPK1* γ transcript relative to housekeeping genes in untreated and treated cell lines. Horizontal line represents the mean from three independent experiments.

However, we observed that all investigated drug regimens predominantly induced *de novo* transcription from currently non-annotated TSSs, termed here as treatment-induced non-annotated TSSs (TINATs) (Fig. 2c, right). Although DNMTi and HDACi target distinct epigenetic pathways, inhibitor treatment mostly converged on the activation of identical TINATs (Fisher's exact test, $P < 2.2 \times 10^{-16}$) (Fig. 2d, top).

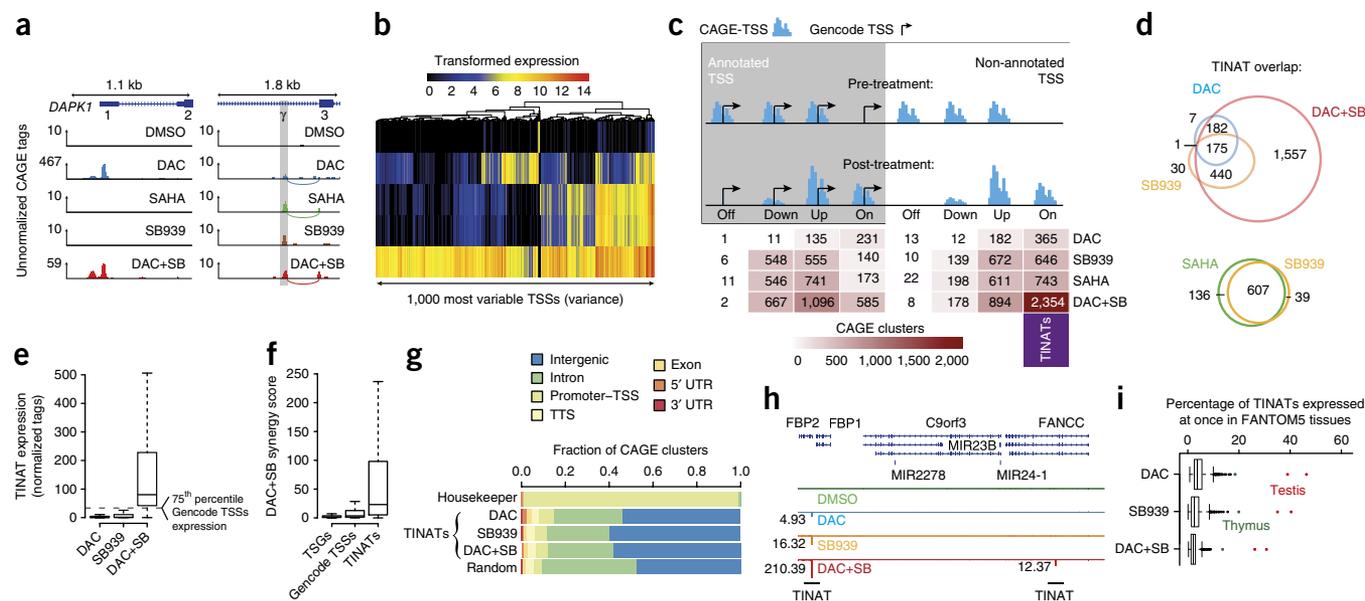


Figure 2 CAGE-seq identifies genome-wide activation of non-annotated TSSs upon treatment. **(a)** CAGE coverage at the canonical *DAPK1* TSS (left) and the intronic γ TSS (right, gray coloring). Curved lines indicate split CAGE tags. Numbers above vertical axis line denote the scale. **(b)** Variance stabilized expression values⁴⁸ of the 1,000 most variable TSSs across treatment conditions. **(c)** CAGE clusters categorized into annotated (left) or non-annotated (right) peaks. CAGE TSSs <150 bp away from the nearest Gencode TSS were considered annotated. Silenced (Off), repressed (Down), induced (Up), and *de novo*-transcribed (On) CAGE peaks were quantified and are shown in the table. TINATs are highlighted (rightmost column). Arrow, Gencode TSS; blue bars, CAGE TSS. **(d)** TINAT overlap between DAC, SB939, and DAC+SB treatment (top) as well as SB939 and SAHA treatment (bottom). TINATs were considered overlapping if expression was significantly different from DMSO control in more than one condition. **(e)** Normalized DAC, SB939, and DAC+SB TINAT expression. **(f)** Synergy score for TSSs associated with lung adenocarcinoma TSGs⁴⁹, *de novo*-induced genes (Gencode TSSs), and the union of TINATs. Synergy score was calculated as follows: expression_{DAC+SB} / (expression_{DAC} + expression_{SB939}). Data points beyond the extremes of the whiskers are not shown. **(g)** Genomic distribution of CAGE TSS. HOMER⁵⁰ was used to annotate TINATs and housekeepers (100 least variable TSSs) to genomic features. TTS, transcription termination site; UTR, untranslated region. **(h)** TINAT expression in the introns of the *FBP2* and *FANCC* across treatments. Numbers next to the bar indicate normalized CAGE-tag counts. **(i)** Percentage of simultaneously expressed TINATs in FANTOM5 samples.

Moreover, TSS activity after SAHA and SB939 treatment was highly similar ($r = 0.99$) (Fig. 2d, bottom, and Supplementary Fig. 3e, right). Thus, we focused on SB939 as a representative of HDACi for further analyses. In line with previous findings of the synergistic effect on gene expression by combined demethylation and HDACi⁷, we found multiple TINATs exclusively expressed after DAC+SB treatment. Moreover, the level of expression after combinatorial treatment was stronger than expected for the additive effect of DNMTi and HDACi alone (Fig. 2e). This synergistic effect was significantly stronger at TINATs (median synergy score = 23.2) than at the TSSs of *de novo*-induced annotated genes (median = 3.5) or tumor suppressor genes (TSGs, median = 1.1) (Wilcoxon and Mann–Whitney two-sided test, $P < 2.2 \times 10^{-16}$; Fig. 2f). The majority of TINATs were located in either intergenic (~60%) or intronic (~20%) regions (Fig. 2g and Supplementary Fig. 4) with a median distance to the nearest annotated TSS of 9.3, 9.0, and 11.6 kb for DAC, SB939, and DAC+SB, respectively. Genes in the vicinity of DAC-induced TINATs were neither enriched for any biological process nor influenced by TINAT expression (correlation between TINAT expression and expression of nearby genes, $r = 0$). In contrast, genes most proximal to SB939- or DAC+SB-induced TINATs were enriched for neuronal and developmental processes (Supplementary Fig. 3f), and TINAT expression was positively correlated with the expression of nearby genes (SB939, $r = 0.4$ ($P = 2.2 \times 10^{-9}$); DAC+SB, $r = 0.21$ ($P = 3.9 \times 10^{-29}$); Supplementary Fig. 3g). However, unlike active enhancer sites that are transcribed bidirectionally⁸, TINATs displayed unidirectional transcription (Supplementary Fig. 3h).

As an example, Figure 2h depicts expression of two TINATs located in the introns of *FBP2* and *FANCC*. We further confirmed

the treatment specificity of TINATs by analyzing their expression across the FANTOM5 expression atlas⁸. The transcripts were generally not expressed under physiologic conditions, with the notable exception of testicular and fetal thymic tissues, which concurrently expressed up to ~40% and ~20% of all TINATs, respectively (Fig. 2i and Supplementary Fig. 3i).

TINAT–exon fusion transcripts encode aberrant proteins

Based on our initial observations at the *DAPK1* locus, we analyzed whether TINATs generally spliced into genic exons. Approximately 50–60% of all TINATs generated spliced transcripts, of which another ~30% were spliced into protein-coding exons (Fig. 3a). These observations are exemplified at the *FBP2* locus, where TINAT-proximal splice sites join the cryptic TSS, with exon 2 located downstream of the canonical *FBP2* translation start site (Fig. 3b). We confirmed the existence of 15 TINAT–exon fusion transcript candidates in different cell lines by qPCR (Fig. 3c). Fusion candidates were manually selected based on their expression level and the number of CAGE tags supporting the splicing event. Using StringTie⁹, we reconstructed 453, 744, and 3,627 TINAT–exon transcript isoforms for DAC-, SB939-, and DAC+SB-treated cells, respectively (Supplementary Table 2 and Supplementary Data 2). The exon–intron structure of the reconstructed transcripts closely matched the annotation of reference genes, as illustrated for *FBP2*. However, they often lacked the 3' end of the canonical mRNA, because CAGE-tag density is strongly skewed toward the 5' end and TSS of a transcript. Around 14–21% of TINAT transcripts overlapped protein-coding genes, and around 33–40% overlapped with long noncoding RNAs (lncRNAs)¹⁰. Although lncRNAs initiating

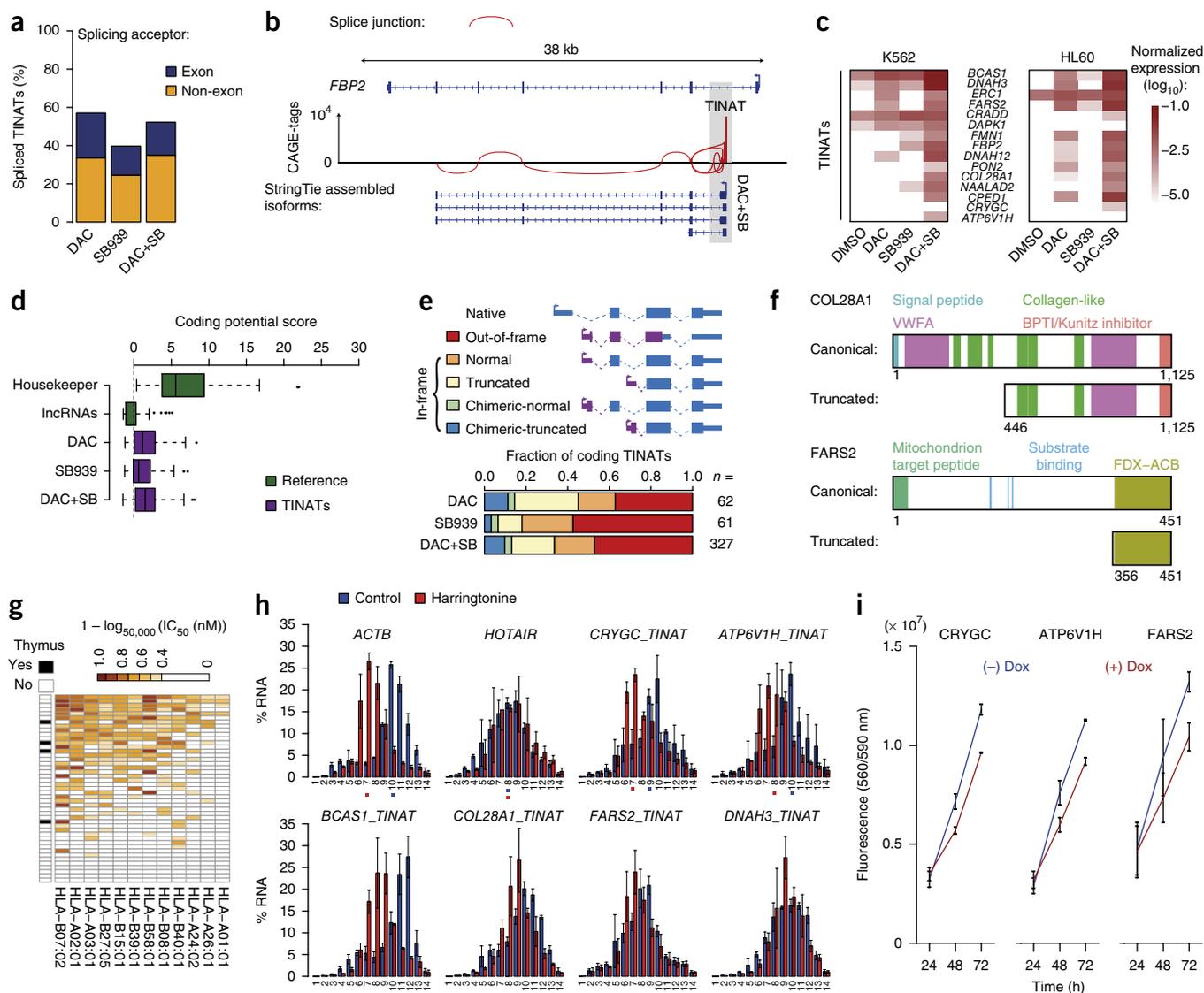


Figure 3 TINAT–exon fusion transcripts encode novel protein isoforms with abnormal functions. (a) Fraction of TINATs having >1% split CAGE-seq reads. (b) Splice junctions at the *FBP2* locus based on TINAT-derived CAGE tags of DAC+SB treated NCI-H1299 cells. (c) TINAT–exon fusion transcript expression in K562 (left) and HL60 (right) cells. The \log_{10} of the mean expression from three independent experiments relative to housekeepers is shown. (d) The coding potential of 100 housekeeping genes, 100 randomly selected noncoding RNAs, and TINATs was assessed using the coding-potential calculator⁵¹. Dashed line denotes the threshold for protein-coding transcripts. (e) Schematic representation of the different scenarios for the translation of TINAT–exon fusion transcripts (top). ORFs were categorized based on the criteria described in Online Methods. The canonical (blue) and the novel TINAT-derived sequence (purple) are shown schematically. The fraction of TINATs in each category is shown below. (f) COL28A1 and FARS2 protein domains for the canonical and truncated isoform are illustrated. Numbers below proteins indicate amino acid positions. (g) NetMHCpan⁵² was used to predict the binding affinity of 12 major HLA alleles (columns) for 45 DAC+SB chimeric peptide sequences (rows). The presence of a TINAT within the adult thymus is displayed. (h) Distribution of *ACTB*, *HOTAIR*, and six TINAT–exon fusion transcripts along polysome fractions. Colored squares below horizontal axis line indicate the fraction where half of the mRNAs have accumulated. (i) Cell viability of NCI-H1299 reporter cells transduced with DOX-inducible TINAT-derived ORFs with or without DOX. Data from two independent experiments are shown.

from TINATs included transcripts with known function in disease, such as *SCHLAP1* (ref. 11), we focused our further analyses on the protein-coding potential of TINATs. Most of the assembled fusion transcripts that contain a cryptic sequence at their 5' end and a native protein-coding exon sequence downstream were predicted to be coding transcripts (Fig. 3d). *In silico* translation showed that about half of the candidates encode in-frame isoforms relative to the coding DNA sequence of the canonical mRNA, whereas the other half generates out-of-frame, and thereby completely novel, peptide sequences (Fig. 3e). Fusion transcripts that are translated in-frame with the native coding DNA sequences give rise to either the original, truncated, or chimeric

isoforms, depending on whether the canonical or variant in-frame start codons are used. The truncated isoforms often lacked domains or peptide sequences important for proper protein function, localization, or binding, whereas other functional regions remained unaffected (Fig. 3f). TINAT fusion transcripts encoding the canonical full-length sequence comprised genes with products of diverse biological functions, including those for transcription factors (*TFEC*, *TBX4*, *GTF2H5*), DNA damage repair and apoptosis (*RAD50*, *SESN1*, *TNFRSF10B*), epigenetic modifiers (*HDAC4*), and CTAs (*MAGEB10*, *BRDT*). Several other CTAs were expressed from TINATs (*PRSS55*, *MAGEB2*, and *XAGE5*), but the resulting fusion transcripts were predicted

to encode out-of-frame peptides. While these out-of-frame transcripts are likely subjected to nonsense-mediated decay, chimeric peptide sequences encoded in TINAT fusion transcripts are potentially immunogenic, based on their foreign sequence and their capability of being presented on MHC class I molecules (Fig. 3g and Supplementary Fig. 5a). Furthermore, most of these transcripts were not expressed in the adult thymus and hence would not be expected to contribute to central tolerance. Notably, none of the potentially immunogenic peptides corresponded to known CTAs¹².

To confirm the translational capacity of selected fusion transcripts, we translated the canonical *CRYGC* mRNA and three TINAT–exon transcripts (*CRYGC* (chimeric-truncated), *BCAS1* (normal), and *FBP2* (truncated)) *in vitro*. For all RNA templates, we observed translation products with the predicted sizes (Supplementary Fig. 5b). We further compared polysomal association of 15 TINAT–exon fusion candidates in DAC+SB-treated cells incubated in the absence or presence of the translation inhibitor harringtonine, to deplete elongating ribosomes from mRNAs¹³ (Supplementary Fig. 5c). As a positive control, β -actin (*ACTB*) mRNA showed highest abundance in heavy polysome fractions and strongest release upon harringtonine treatment (Fig. 3h; others shown in Supplementary Fig. 5d). In contrast, the lncRNA HOTAIR was barely associated with polysomes and did not respond to harringtonine. All candidates were more strongly associated with heavy polysomes than HOTAIR, and we observed a harringtonine shift for *CRYGC*, *ATP6V1H* (out-of-frame), *BCAS1*, and *COL28A1* (truncated), indicating their active translation. *FARS2* (chimeric-truncated) and *DNAH3* (truncated) displayed a weak shift across only some replicates, thus precluding confident confirmation of their translational capacity. Additional testing of 28 TINAT–exon fusion candidates along fewer polysomal fractions identified nine additional candidates that reacted to harringtonine treatment (Supplementary Fig. 5e). Concurrently, polysome fractionation of untreated colorectal cancer cells¹⁴ showed that sporadically expressed transcripts overlapping with TINAT coordinates are preferably associated with heavy polyribosomes (Supplementary Fig. 5f). To test the impact of translated fusion transcripts on cellular fitness, we overexpressed 11 candidate ORFs (Supplementary Table 3) in NCI-H1299 reporter cells and measured cellular proliferation. Overexpression of *CRYGC*, *ATP6V1H*, and *FARS2* resulted in decreased cell growth (Fig. 3i), whereas overexpression of the other ORFs had no effect (data not shown). Together, these observations suggest that TINATs frequently splice into protein-coding exons to create fusion transcripts that become translated into aberration protein isoforms.

DNMTi and HDACi activate TINATs via distinct mechanisms

To investigate the epigenetic reprogramming accompanying TINAT activation, we generated genome-wide maps of DNA methylation and 15 histone modifications before and after treatment (Supplementary Table 1). As expected, DAC treatment reduced global DNA methylation levels (Fig. 4a), whereas HDACi rapidly increased the acetylation of histone tails at various positions (Fig. 4b and Supplementary Fig. 6).

In untreated NCI-H1299 reporter cells, TINATs were silenced in association with DNA methylation and H3K9me3 around their TSS (Fig. 4c) but not with H3K27me3 (Supplementary Fig. 7a). Treatment with DAC or DAC+SB ubiquitously decreased DNA methylation, which was partially compensated by increased levels of H3K9me3 (Fig. 4c). Loss in DNA methylation was accompanied by an active promoter signature around TINATs, as suggested by the presence of various active histone modifications (Fig. 4c and Supplementary Fig. 7a). In stark contrast to DAC or DAC+SB treatment, SB939 or SAHA treatment alone did not induce a classical promoter signature

around TINATs, as indicated by the lack of demethylation and H3K4me3, H3K9ac, or H3K27ac histone marks (Fig. 4c and Supplementary Fig. 7b). Rather, SB939 induced TINATs in association with H3K14ac, H2AK9ac, and H3K23ac (Fig. 4c and Supplementary Fig. 7a). Although these marks were centered on TINATs, their signal intensity was low, potentially reflecting that only a small fraction of cells or alleles in the population responded to HDACi. Of note, most activating histone modifications displayed the strongest mean signal around TINATs after DAC+SB treatment, and some modifications, such as H2BK5ac and H3K4ac, were exclusively found after combinatorial inhibition, thereby providing a potential foundation for the synergistic effects of DAC+SB treatment on TINAT expression.

To gain further insights into the interplay of chromatin modulation and TINAT expression, we clustered TINATs based on their surrounding DNA methylation and histone modification profiles. We identified three distinct clusters (Supplementary Fig. 7c) that differed in their respective epigenetic makeup (Fig. 4d and Supplementary Fig. 7d). Cluster 1 was devoid of any of the investigated chromatin modifications in the vicinity of TINATs and was characterized by relatively low TINAT expression. TINATs of the second cluster harbored the most focal chromatin modifications that were strongly enriched in proximity to the TSS. Cluster 3 TINATs had lower levels of repressive DNA methylation and H3K9me3 than TINATs of cluster 2 and more widely distributed active chromatin marks after treatment. This chromatin signature was associated with the highest TINAT expression among the clusters. Together, these findings suggest that DNMTi and HDACi drive TINAT expression via distinct mechanisms, and that TINATs are associated with a heterogeneous group of TSS classes.

TINATs arise from LTRs of the LTR12 family

Although DNMTi and HDACi target different epigenetic pathways and are observed to employ different mechanisms of TINAT activation, both inhibitor classes converge on activating identical TINATs. We therefore hypothesized that these regions harbor some universal sequence commonality. Since it has been shown that transposable elements (TEs) play significant roles in regulating gene networks through novel promoter, enhancer, and splicing mechanisms^{15,16}, we explored whether sequence-specific features of TEs explain TINAT activation.

Indeed, more than 80% of TINATs overlapped with TEs (Fig. 5a), and, specifically, the LTR class was more frequently associated with TINATs than expected by chance (Fig. 5b, top). Following combination treatment, expression levels from LTR-derived TINATs were higher than from other TINATs (Supplementary Fig. 8a). LTRs belonging to the LTR12 family, whose members are more frequently found at the promoter–TSS of genes than are other LTRs (Fisher's exact test, $P < 2.2 \times 10^{-16}$; Supplementary Fig. 8b), were strongly enriched for TINATs (Fig. 5b, bottom). Moreover, certain TE families were enriched for the different chromatin clusters identified previously (Supplementary Fig. 8c), suggesting that different epigenetic mechanisms are preferred for activation of certain TE families. Within the LTR12 family, LTR12C had the highest enrichment value (associated with ~50% of all TINATs, Supplementary Fig. 8d and Supplementary Table 4). Analysis of public RNA-seq data from cells treated with SAHA¹⁷ confirmed the selective transcriptional activation of LTR12C copies after HDACi (Supplementary Fig. 8e). Moreover, we observed increased LTR12C transcription after SAHA treatment in a neuroblastoma mouse xenograft model (Wilcoxon and Mann–Whitney two-sided test, $P = 0.0079$; Fig. 5c). Treatment with several chemotherapeutic agents did not affect LTR12C transcript levels (Supplementary Fig. 8f), suggesting that their induction is a specific effect of epigenetic modulation. Next, we anchored the start

positions from TINATs to the LTR12C consensus sequence to identify if any sequence-specific context in LTR12C was contributing to the generation of TINATs (**Supplementary Fig. 8g**). This analysis uncov-

ered two intriguing results. First, all TSS activity originated from the second half of the sense strand, suggesting that LTR12C encodes uni-directional transcriptional regulation, similar to promoter function.

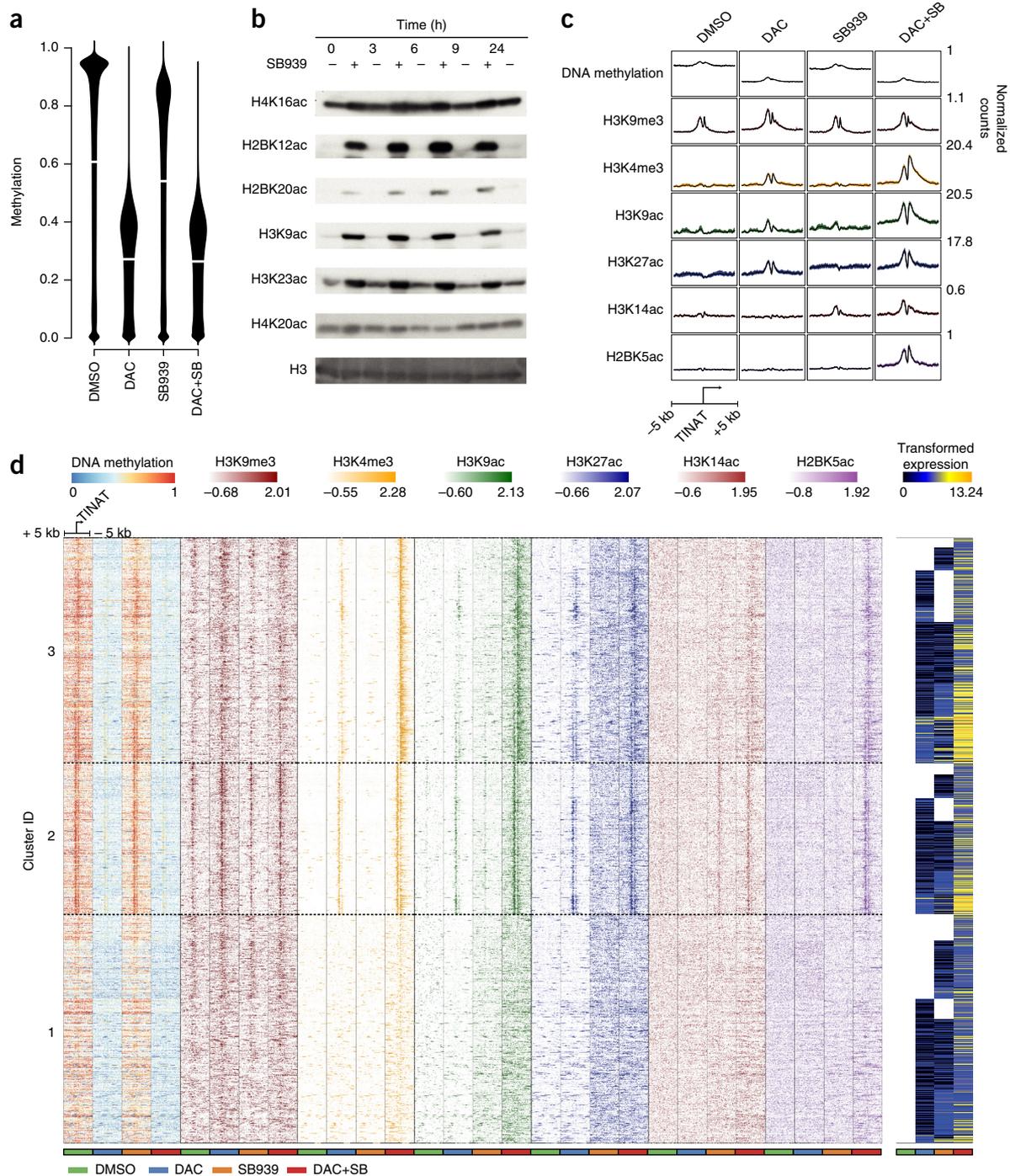


Figure 4 DNMTi and HDACi use distinct mechanisms to activate TINATs. **(a)** Beanplots showing the distribution of DNA methylation in untreated and treated NCI-H1299 cells based on whole-genome bisulfite sequencing. **(b)** Protein blot analysis of post-translational modifications of histones extracted from NCI-H1299 cells at different time points following treatment with DMSO or SB939. Gel images were cropped and mirrored. Original blots are shown in **Supplementary Figure 6**. **(c)** ChIP-seq occupancy plots showing the average level of DNA methylation (gray), H3K9me3 (red), H3K4me3 (orange), H3K9ac (green), H3K27ac (blue), H3K14ac (brown), and H2BK5ac (purple) 5 kb up- and downstream of all identified TINATs. Colored areas indicate the 95% confidence interval, and numbers indicate the normalized read counts. **(d)** DNA methylation, H3K9me3 (red), H3K4me3 (orange), H3K9ac (green), H3K27ac (blue), H3K14ac (brown), and H2BK5ac (purple) levels around TINATs after DMSO (green bar), DAC (blue bar), SB939 (orange bar), or DAC+SB (red bar) treatment. Color intensity of the histone modifications represents Z scores. Variance-stabilized TINAT expression⁴⁸ is shown to the right. TINATs were categorized into three groups using *k*-means clustering on the Z scores of DNA methylation and histone modification levels relative to DMSO.

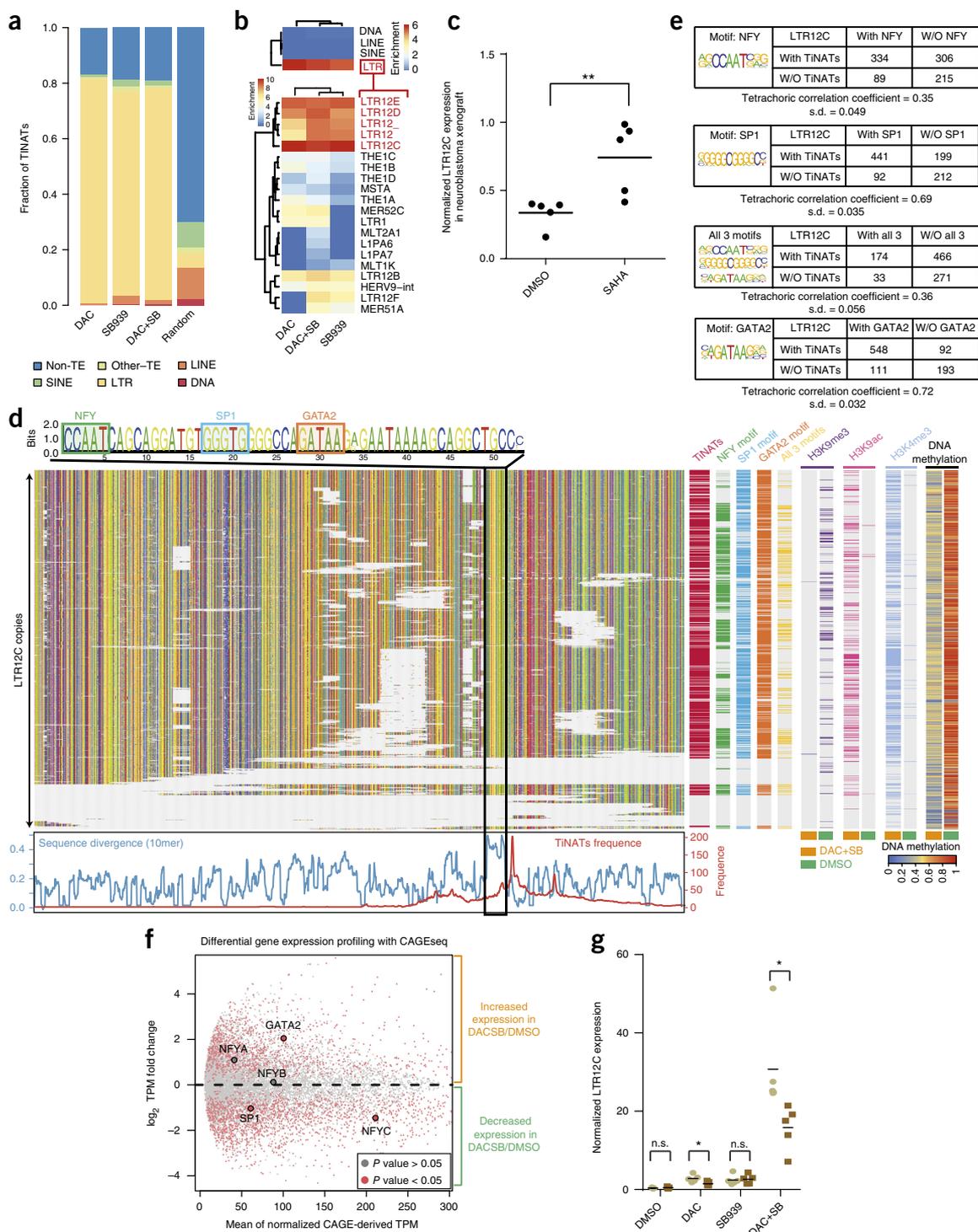


Figure 5 TINATs arise from LTRs, especially of the LTR12 family. **(a)** TINATs overlapping with TE classes. **(b)** Cluster analysis of enrichment scores for TINATs across TE classes (top) and LTR families (bottom). **(c)** qRT-PCR expression analysis of LTR12C copies relative to housekeepers in BE(2)-C neuroblastoma cells xenotransplanted into mice treated with DMSO or SAHA. Vertical lines represent the mean. **(d)** Sequence alignment of LTR12C copies. G, A, T, C, nucleotides are colored yellow, green, red, and blue, respectively (top left). TF motifs are highlighted. TINAT frequency and sequence divergence between LTR12C copies with and without TINATs are shown below. The presence of TINATs, TF motifs, histone modifications, and DNA methylation are shown (right). **(e)** Association between TINAT expression and the presence of NF-Y, SP1, and GATA2 motifs. Enrichment of SP1 and GATA2 sites in LTR12C with TINATs was significant (Pearson's χ^2 test, $P < 2.2 \times 10^{-16}$). **(f)** Differential gene expression between DAC+SB and DMSO using CAGE peaks. NF-Y, Sp1, and GATA2 are highlighted. NF-Y transcription factor is a trimeric complex of NFYA, NFYB and NFYC. Genes with significant expression differences are labeled in red (Student's t test P value < 0.05 , not corrected for multiple hypothesis testing). **(g)** Expression of LTR12C copies relative to housekeepers in the presence (brown) and absence (gray) of siRNAs targeting GATA2. Data from five independent experiments are shown. Horizontal lines show mean from five independent experiments.

Second, there was one major summit around position 1,165 bp that was activated synergistically by combination treatment, which corresponded to the previously identified ERV9 provirus TSS^{18–20}. The broad range of CAGE peaks in LTR12C corresponds to the promoter region of the solitary LTR, thus supporting the notion that TINATs derive from cryptic silenced promoters²¹. This observation is in line with the previously described cases of LTR12C copies that harbor promoter activity (Supplementary Table 5). We therefore predicted that promoter-specific histone modifications increase around expressed LTR12C copies when treated with epigenetic drugs. As expected, we observed a significant increase in H3K4me3 and H3K9ac around expressed LTR12C but not for LTR12C copies without TINATs after DAC+SB treatment (Supplementary Fig. 8h).

Next, we asked whether LTR12C elements harbor TF-binding sites that provide insight into master TFs that mediate *de novo* transcription and whether there are sequence features that discriminate transcribed and non-transcribed LTR12C elements. Multiple-sequence alignment comparison of TINAT-producing and non-TINAT LTR12C copies showed that the most prominent sequence feature that segregated both LTR12C groups mapped immediately upstream of the 1,165-bp TINAT summit (Fig. 5d). The ERV9/LTR12 U3 enhancer and promoter region harbors several TF-binding sites, such as NF- κ B, Sp1 and GATA2²¹. We explored whether the presence of these three TF motifs directly upstream of the LTR12C summit correlated with TINAT presence. TINAT expression correlated with the presence of a GATA2 motif (tetrachoric correlation coefficient (cc) = 0.72) and Sp1 motif (cc = 0.69) (Fig. 5e). Then, we checked expression levels of the LTR12C promoter TFs after DAC+SB treatment to explore the potential mechanism for TINAT activation. Using CAGE signal as a surrogate for gene expression, we identified that only GATA2 had significantly higher expression (Student's t test, P = 0.03) in DAC+SB relative to DMSO (Fig. 5f). These findings suggest GATA2 is likely the upstream TF responsible for TINAT activation. Indeed, using siRNA-mediated knockdown, we validated the requirement of GATA2 for full TINAT activation (Fig. 5g and supplementary Fig. 8i).

DISCUSSION

We show that DNMTi and HDACi do not predominantly alter the expression of canonical genes but induce the *de novo* transcription of LTRs of the LTR12 family. Previous efforts to understand the transcriptional response toward epigenetic therapy were largely based on gene expression microarrays and thus were limited to the quantification of known transcripts and lacked information about their TSSs^{22,23}. Our findings extend recent reports that demonstrated the presence of dsRNA molecules upon DNMTi^{4,5}, originating from the bidirectional transcription of codogenic ERV envelope gene loci (for example, *Syncytin-1* and *env-Fc2*). While we confirmed *Syncytin-1* expression and the subsequent induction of AIMs⁶ upon DNMTi treatment, our data has important new implications. First, we show that HDACi must exert their function independently. Second, the unidirectional transcription from up to thousands of solitary LTRs is an additional effect to the bidirectional transcription from full-length ERV copies following treatment. Therefore, we provide a novel mechanism for the action of different classes of epigenetic inhibitors.

With the exception of *Syncytin-1* and a few other codogenic ERVs that produce functional proteins²⁴, most ERV genes became nonfunctional through various evolutionary forces²⁵. Most of the ~700,000 ERV copies within the human genome exist as solitary LTRs²⁶. Unlike other ERVs, the ~5,500 LTRs of the ERV9 family (LTR12s) carry several tandem repeats containing multiple TF-binding sites^{21,27}. LTRs of this family have been shown to shape the transcriptomic landscape through

enhancer-like and promoter-like mechanisms^{28,29}, which have been adopted for tissue-specific functions^{18,19}. Our data suggest that either the loss of DNA methylation or HDAC inhibition is sufficient to drive faint expression of LTR12C elements, but combinatorial inhibition is required for full activation. The loss in DNA methylation upon DNMT inhibition is global and also occurs at LTR12Cs and other subfamilies that do not show a transcriptional response. We therefore propose that the selectivity of LTR expression is conferred by the disruption of repressive chromatin structure followed by binding of TFs to the regulatory sequence of exposed LTR elements. In line with the reported recruitment of GATA2 to LTR12 elements³⁰, we show that GATA2 is required for full LTR12C expression. The selectivity of HDACi toward the activation of LTR12 family elements was also reported for multiple other cancer types based on candidate gene approaches^{31,32}, indicating that this is a universal mechanism. However, non-epigenetic treatment examples of EVR9/LTR12 reactivation have been discovered in viral-induced tumors²⁰ and in primary T cells infected with HIV³³.

Splicing of ERV-derived transcripts into their genomic vicinity has been observed during normal development¹⁶ and in tumors^{20,34}. There are reports from studies in mice or human cancer cells in which an LTR element gives rise to a chimeric protein by means of being spliced to a protein-coding gene^{34,35}. In line with these reports, we show that the treatment-induced expression of LTRs generates numerous fusion transcripts that encode novel protein isoforms, often lacking N-terminal peptide sequences important for proper protein function. Given that truncated protein isoforms affect cellular function and contribute to human disease^{36,37}, one expects that the simultaneous expression of aberrant peptides partially accounts for the clinical efficacy of these drugs.

So far, there are two major limitations to epigenetic therapy that could potentially be overcome by combining it with immunotherapy³⁸. First, efficacy of DNMTi in different tumor entities is still quite limited, and second, despite promising initial results in lung cancer³⁹, no phase 3 randomized trial has yet demonstrated therapeutic synergism between DNMTi and HDACi. The combination of epigenetic inhibitors with immunotherapy raises the hope that epigenetic therapy will demonstrate an antineoplastic effect in common cancer entities. Indeed, in preclinical cancer models, treatment with DNMTi or HDACi sensitizes tumors to the effects of immune checkpoint inhibition^{4,40}. Moreover, combining DNMTi with allogeneic T-cell infusions in the treatment of relapsed AML patients⁴¹ indicates a curative potential⁴². Our data provide an elegant explanation for this priming effect, as epigenetic therapy may induce the expression of LTR-derived immunogenic antigens presented on MHC class I molecules for recognition by cytolytic T cells. This would be of utmost importance for those cancer types with low mutational burden that respond poorly to immune therapy⁴³. The mechanism described here likely synergizes with other effects of epigenetic therapy, including the inhibition of nonsense-mediated decay⁴⁴, transcription of viral defense genes⁴, increased antigen processing and presentation⁴⁵, re-expression of epigenetically silenced inflammatory chemokines⁴⁶, and upregulation of CTAs⁴⁷. Future proteomic approaches combined with T-cell cytotoxicity assays will further shed light on the interaction between epigenetic and immune therapy and the role of ERV-derived antigen presentation.

URLs. FANTOM5, http://fantom.gsc.riken.jp/5/datafiles/latest/extra/CAGE_peaks/; EpiDesigner, <http://www.epidesigner.com/>.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the [online version of the paper](#).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

C.R.S., M.D., D.B., D.B.L., S.L., M.S.I., H.B., S.H., M.H., A.L., A.R., G.S., J.S., R.W., J.P.M., K.R., D.W., C.C.O., and C.P. designed the experiments and performed experimental work. D.B., C.S., M.D., D.L., J.L., H.S.J., N.M.S., Y.H., B.Z., Y.A., C.D.I., B.B., and T.W. performed data analysis. I.O., O.W., and M.L. provided clinical expertise and data. D.B., M.D., T.W., and C.P. prepared the manuscript and figures. T.W., C.G., B.B., M.E., C.C.O., and C.P. provided project leadership. All authors contributed to the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Engineering of the DAPK1 reporter cell line, 5' RACE, and the epigenetic compound screen are described in detail in **Supplementary Note 1** and **Supplementary Tables 6–9**.

Cell culture and treatment. RAJI (ACC-319, DSMZ), MEC1 (ACC-497, DSMZ), HL60 (ACC-3, DSMZ), K562 (ACC-10, DSMZ), NCI-H1299 (CRL-5803, ATCC) cells were grown in RPMI 1640 supplemented with 10% FCS. T89G human glioblastoma cells (CRL-1690, ATCC) were kept in DMEM containing 10% FCS. Cell line authenticity and purity was confirmed using the Multiplex Cell Authentication and Cell Contamination Test by Multiplexion. Cells were treated with 500-nM (250 nM for HL60) DAC, 500-nM SB939, 1500-nM SAHA, or 500-nM (250 nM for HL60) DAC + 500-nM SB939 for 72, 18, 18, or 72 + 18 h, respectively, and compound-containing media was refreshed every 24 h.

Cap analysis of gene expression (CAGE) sequencing. CAGE was performed in two independent experiments on normal and treated NCI-H1299 cells using the CAGE Preparation Kit from DNAFORM.jp, according to the manufacturer's instructions. Enrichment of capped RNAs versus uncapped ribosomal transcripts was used to assess sample quality. Samples with a minimum of 400-fold enrichment over ribosomal RNA were subjected to sequencing on the Illumina Hi-Seq 2000 system in 50-bp single-end (replicate 1) and 100-bp paired-end (replicate 2) mode by the DKFZ Genomics and Proteomics Core facility. Resulting raw sequencing data was processed as follows: multiplexed samples were separated by barcode, trimmed at the first position to remove nonspecific guanines⁵³ as well as to 50 bps in the case of the 100-bp paired-end reads, and aligned against the reference genome (hg19) using HISAT⁵⁴ version 0.1.6-beta. Only uniquely mapped reads were retained and in the case of SB939 and DAC+SB939, files were down-sampled to 25×10^6 aligned reads. The resulting BAM files were loaded into CAGER version 1.10.0 (ref. 55), and CTSS were called using the following parameters: sequencingQualityThreshold = 20, mappingQualityThreshold = 20. After simple tpm normalization, clusterCTSS were generated using the paraclu method (threshold = 0.1, nrPassThreshold = 2, thresholdIsTpm = TRUE, removeSingletons = TRUE, keepSingletonsAbove = 0.2, minStability = 2, maxLength = 100, reduceToNonoverlapping = TRUE). Finally, consensus TSSs across all conditions and replicates were created using the aggregateTagClusters function (tpmThreshold = 0.3, qLow = NULL, qUp = NULL, maxDist = 100, excludeSignalBelowThreshold = FALSE). Importantly, no confounding effects of the underlying sequencing protocol on TSS expression were observed (**Supplementary Fig. 3a**). Distance to the nearest Gencode GRCh37.p13 annotated TSS was calculated using HOMER⁵⁰ software and statistical analysis was performed in DESEQ version (1.18.0)⁴⁸. Size factors were calculated for the normalization of TSS expression and dispersion estimates for each gene were obtained using the estimateDispersions function with the following parameters (method = "per-condition", sharingMode = "maximum"). Differential expression between control and DAC, SB939, SAHA, and DAC+SB treated cells was assessed by testing the differences between the base means of two conditions (nbinomTest). Benjamini-Hochberg adjusted *q* values below 0.05 were considered as significantly differentially expressed.

RNA-sequencing analysis. RNA-seq data was obtained from the Gene Expression Omnibus under accession GSE54912 and from the European Nucleotide Archive under accession PRJEB5049. Illumina and ABI_SOLID reads were aligned against the human hg19 reference genome using HISAT version 0.1.6-beta with default parameters and bowtie version 1.0.0 with the parameters -C,-best, respectively. Overlap of aligned reads with TE subfamilies was counted using the summarizeOverlaps function of the GenomicAlignments R/Bioconductor package⁵⁶ with default parameters. Read counts were normalized in edgeR⁵⁷, using the total number of uniquely mapped reads as library size. After estimation of the dispersion, statistical significance was assessed by genewise exact tests for differences in the means between two groups of negative-binomially distributed counts.

Chromatin immunoprecipitation (ChIP). About 2×10^7 NCI-H1299 cells were crosslinked for 10 min using FCS-free RPMI 1640 containing 1.1% formaldehyde. After crosslinking, 1/20th volume of 2.5-M glycine was added,

incubating for 10 min to quench the crosslinking reaction. Cells were then washed three times with ice-cold PBS and scraped into a pre-chilled 15-ml polystyrene tube for subsequent centrifugation at 1,000 g at 4 °C. Cell pellets were carefully resuspended in 1 ml Lysisbuffer 1 (LB1: 50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100) supplemented with protease inhibitors (one tablet for 50 ml). Next, resuspended cells were incubated for 10 min at 4 °C on a rocker to permeabilize the cell membrane. After incubation, nuclei were centrifuged at 1,000 g at 4 °C and the supernatant was discarded. Hereafter, cells were washed in 1 ml cold Lysisbuffer 2 (LB2: 10 mM HEPES-KOH pH 8.0, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA). Finally, nuclei were washed twice in cold Lysisbuffer 3 (LB3: 10 mM HEPES-KOH pH 8.0, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% sodium deoxycholate, 0.5% sodium lauroyl sarcosine) then resuspended in 500–1000 μ l LB3. Sonication of chromatin was performed at 4 °C in 12 \times 24-mm glass tubes using the Covaris S220 Focused-ultrasonicator with the following settings: 30 min shearing, Duty Cycle 20%, Intensity 5, 200 Cycles per burst. Typically, this program resulted in fragment sizes between 150 bp and 450 bp. After shearing, cellular debris was removed by centrifugation at 16,000 g for 5 min, and the supernatant was aliquoted and stored at –80 °C. To assure sufficient shearing efficiency, a small fraction of each sample was digested with Proteinase K at 65 °C for 16 h and thereafter RNase-treated and QIAquick Gel column-purified. The concentration of purified DNA was assessed by Nanodrop measurement and gel-analyzed to analyze fragment size distribution. Only samples with average fragment sizes of 150–300 bp were subjected to further chromatin immunoprecipitation.

ChIP assays were performed using the SX-8G IP-Star Automated System in combination with the Auto ChIP kit according to the manufacturer's protocol (both Diagenode). IP reaction was carried out for 11 h using DiaMag protein A-coated magnetic beads (Diagenode) and the following antibodies: H3K4me3 (pAb-003-050, Diagenode), H3K27me3 (pAb-069-050, Diagenode), H3K9ac (17-658, Merck Millipore), H3K9me3 (ab8898, Abcam), H3K27ac (ab4729, Abcam), H3K4me1 (ab8895, Abcam), H3K36me3 (ab9050, Abcam), H3K23ac (39131, Active Motif), H4K8ac (61103, Active Motif), H3K14ac (ab52946, Abcam), H3K18ac (ab1191, Abcam), H4K12ac (ab46983, Abcam), H3K4ac (39381, Active Motif), H2AK9ac (ab177312, Abcam), H2BK5ac (ab40886, Abcam). After ChIP, DNA was isolated by Proteinase K digest at 65 °C for 4 h and subsequently purified using Agencourt AMPure XP beads. For Chip-Seq analysis, size-selected libraries were prepared with the NEB Next Ultra DNA Library Kit. Sequencing was performed on the Illumina Hi-Seq 2000 system in 50 bp single-end mode by the DKFZ Genomics and Proteomics Core facility.

Reads were aligned against the human reference genome (hg19) using BWA version 0.5.9-r16 (ref. 58) with default parameters and reads with a mapping quality <1 or putative PCR duplicates were removed. MACS2 (ref. 59) was used with default parameters to call peaks at a 1% FDR. Input-subtracted, whole-genome coverage tracks (bigWig files) of aligned reads were generated with a window size of 50 bps. To account for global differences in activating histone modifications after treatment (**Fig. 4b**), H3K4me3, H3K9ac, and H3K27ac tracks were multiplied by the multiplicative inverse of the mean signal intensity within 1.25 kp up- and downstream of the 100 TSSs with the lowest variance across treatments. The signal intensities of the other histone modifications were normalized to all aligned reads (RPM, reads per million) and used for down-stream analyses.

Whole-genome bisulfite sequencing. Whole-genome bisulfite sequencing of treated and untreated NCI-H1299 cells was performed as previously described⁶⁰. Libraries were sequenced on the Hi-Seq 2000 system in 100 bp paired-end mode. CpG methylation was calculated as previously described⁶¹ and the BSmooth algorithm⁶² was employed to estimate the sample-wise methylation levels using the bsseq R/Bioconductor package with default parameters.

siRNA transfection and shRNA transduction. siRNA transfection of cultured cell lines was carried out using DharmaFECT 1 (Thermo Scientific) according to the manufacturers recommendations. In brief, cells were transfected using 1 μ l transfection reagent per 0.02 pmol siRNA and all siRNAs (Dharmacon, siGenome series; DNMT1 – D-004605_1, _2, _4, _5; GATA2 – MU-009024-00

siRNA) were used as a pool of four individual sequences at a combined final concentration of 20 nM for DNMT1 and 10 nM for GATA2 knockdown. As a control, Dharmacon ON-Targetplus nontargeting siRNA #1 was used. In parallel to the siRNA-mediated GATA2 knockdown, epigenetic drug treatment was done as described above. Cells were harvested 96 h post transfection and used for downstream analyses. *DNMT1* and nontargeting (shLuciferase) shRNAs were cloned into the pRSI9 vector system (Collecta) and lentiviral particles were produced in HEK293T cells using psPAX2 and pMD2.G packaging vectors. Sequences used for shRNA cloning can be found in **Supplementary Table 10**. 24 h after transduction, transduced cells were enriched by treatment with 2 µg/ml puromycin for 48 h.

Comparison to FANTOM5 data. FANTOM5 CAGE-TSS expression across 625 tissues and primary cells was obtained using the hg19.cage_peak_phase1and2combined_tpm_ann.osc.txt file provided in the FANTOM5 website (URLs). Cell lines, universal references, and cancer samples were excluded for this analysis. A TINAT was considered expressed in a given cell type if the sample contained an active TSS (tags per million > 0) with a distance of <150 bps to the nearest TINAT.

HLA-binding prediction. Immunogenic peptides were predicted for DAC+SB induced chimeric and out-of-frame protein isoforms by defining all novel amino acid 8- to 11mers and modeling the binding affinity to various high-frequency HLA alleles using NetMHCpan (v2.8)⁵². For each novel protein, the kmer with the strongest binding affinity for a given HLA allele was selected.

In vitro transcription and translation. TINATs were *in vitro* translated by using the TNT Coupled Reticulocyte Lysate System (Promega) with T7 Polymerase, according to the manufacturer's instructions. In brief, cDNA of DAC+SB treated NCI-H1299 cells was used as a template in PCRs with primers amplifying full-length mRNA or TINAT sequences (**Supplementary Table 11**). A T7-promoter sequence was introduced by reamplification of the purified PCR products with the same reverse primers and forward primers harboring an extended T7-promoter sequence at the 5' end. PCR fragments were incubated with the Quick Coupled Transcription/Translation System (Promega) in the presence of [³⁵S]methionine.

Effect of TINATs ORFs on cell viability. Selected TINAT ORFs based on their potential capability to encode novel, so far not-described proteins were synthesized and cloned in vector pMK-RQ (Thermo Fisher Scientific) (further information in **Supplementary Table 3**). The ORFs were then Gateway-shuttled (Thermo Fisher Scientific) into the lentiviral vector rwpTRIPZ, a Gateway-compatible derivative of pTRIPZ (GE Healthcare) that allows doxycycline induction of the cloned gene. Lentiviral particles containing the diverse recombinant rwpTRIPZ constructs were generated in HEK293T cells using a second-generation packaging system. Particles were then transfected into H1299 reporter cells followed by puromycin selection. The expression of TINAT ORFs was induced in stable transfectants by addition of doxycycline (1 µg/ml final concentration) into the growth medium (RPMI 1640, Pan Biotech). Proper induction was monitored by qRT-PCR.

For *in vitro* proliferation assays, stably ORFs-overexpressing H1299 cells were plated into 96-well plates in technical triplicates at a number of 5×10^3 cells per well in a final volume of 100 µl complete RPMI with or without Doxycyclin. Cell proliferation was analyzed in technical triplicates 24, 48, and 72 h after induction with the Cell Titer-Blue Cell Viability Assay (Promega, cat. no. G8081) as described in the manual using Spectramax M5e (Molecular Devices) for the readout.

Mouse xenograft studies with HDAC inhibitor. 2×10^6 BE(2)-C viable neuroblastoma cells were resuspended in 100 µl Matrigel and 20 U/ml heparin and implanted into the subcutaneous tissue of right flank of 5- to 6-week-old female athymic nude mice (HsdCpb: NMRI-Foxn1nu). Mice were randomly assigned to groups of five individuals bearing similarly sized tumors without blinding. Group size was estimated by the DKFZ biometry core facility. HDAC inhibitor SAHA was dissolved in 100% DMSO and given by intraperitoneal injection at a concentration of 150 mg/kg per day for 2×5 days. At explanation, tumor material used for isolation of total RNA was shock frozen in

liquid nitrogen immediately after removal and stored at -80 °C. Total RNA was isolated with the RNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. All animal studies were approved by the German Cancer Research Center (DKFZ) Institutional Animal Care and Use Committee and the Regional Administrative Council Karlsruhe, Germany. All experiments were in accordance with the relevant regulatory standards.

qRT-PCR expression analysis. RNA was transcribed to cDNA using random hexamers and Superscript III Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. Unless stated otherwise, expression analysis was performed on the Roche Lightcycler 480 system and target-gene expression was normalized to the housekeeping genes *GAPDH*, *β-actin*, and *HPRT1* (primer sequences in **Supplementary Table 11**).

Western blot. Total protein or histone extracts were isolated followed by electrophoretic separation and transferred to a polyvinylidene fluoride membrane. Antibodies against the following antigens were applied: Pan-Ac H3 (06-599, Millipore), DNMT1 (D63A6, Cell Signaling Technology), *β-actin*-HRP conjugated (sc-47778, Santa Cruz Biotechnology), H3K9ac (17-658, Merck Millipore), H3K23ac (39131, Active Motif), H2BK20ac (ab52988, Abcam), H4K16ac (39167, Active Motif), H2BK12ac (ab40883, Abcam), H4K20ac (61531, Active Motif).

Analysis of transposable elements. The TINAT TE enrichment was computed based on Xie *et al.*¹⁵. Briefly, the enrichment score is the ratio between the observed and the expected number of transposable elements overlapping TINATs, assuming a genome-wide random distribution model. TINAT start positions in each LTR12C copy were aligned to relative locations on the LTR12C consensus sequence and the LTR12C TSS frequency was defined as the accumulated density. *De novo* motif analysis was performed using HOMER⁵⁰ on 640 LTR12Cs that fulfilled the following two criteria: (1) No CAGE signal (CTSS tags) in DMSO control and (2) TINAT expression in both CAGE-seq replicates after DAC+SB treatment. 304 LTR12C copies without any CAGE-seq signal (CTSS tags) before and after treatment were used as a background. EMBOSS Needle tool⁶³ was used to calculate the pairwise alignment between each LTR12C copy and the consensus sequence. The frequency of conserved 10mer DNA sequence in both LTR12C groups was calculated, and the sequence divergence was defined as the difference of 10mer sequence frequency between both groups.

EpiTYPER MassARRAY quantitative DNA methylation analysis. MassARRAY was used for high-resolution DNA methylation analysis as previously reported⁶⁴. For PCR amplification of target regions, tagged primers specific for bisulfite-converted DNA were designed with the EpiDesigner Software (URLs) and are listed in **Supplementary Table 11**.

Transcript assembly and *in silico* translation. For TINAT transcript assembly, only properly paired mates where the first in-pair read originated from a TINAT were used as input for Stringtie version 1.0.1 (-g 150)⁹. Only the longest isoform per TINAT that overlapped with at least one exon of an annotated gene (Gencode v19) was used for subsequent *in silico* translation. In case a TINAT gave rise to multiple isoforms with the same length, the isoform with the highest coverage was used (**Supplementary Table 2**). To discriminate the main protein coding from upstream ORFs that are present in about 50% of all human mRNAs⁶⁵, only the first ORF that initiates from a strong ATG start codon (>80% sequence similarity to the Kozak consensus sequence⁶⁶) and encodes >30 codons⁶⁷ was considered. Prior to this, ATGs with a cap-to-ORF distance greater than 721 bps (95th percentile of the length of all human 5' untranslated regions)⁶⁸ were removed. The resulting translation products were aligned against the RefSeq (GRCh37.75) protein sequence of the corresponding splicing-acceptor gene using the Smith-Waterman algorithm⁶⁹. Transcripts with no alignment for any isoform were classified as out-of-frame, whereas transcripts with alignment for at least one isoform were denoted as in-frame. In-frame peptides were further classified into normal, chimeric-normal, truncated, or chimeric-truncated based on the following criteria: Normal, ORF peptide and RefSeq align perfectly; Chimeric-normal, the ORF encodes novel in-frame N-terminal amino acids followed by the full-length canonical

RefSeq protein sequence; Truncated, the ORF lacks parts of the canonical N-terminal protein sequence; Chimeric-truncated, the ORF encodes novel, in place of canonical, N-terminal amino acids followed by the native peptide (Fig. 3e). If the classification was ambiguous for different protein isoforms of the same gene, the hierarchically highest state (in the order: normal > truncated > chimeric-normal > chimeric-truncated) was used to assign a final state for the affected protein.

Polysome fractionation. Sucrose density gradients were produced by consecutively adding layers (790 μ l per layer) of decreasing sucrose concentrations (50%, 41.9%, 33.8%, 25.6% and 17.5% in polysome buffer) into a Beckman Centrifuge Tube (11 \times 60 mm). After each step, the tubes were frozen at -80°C . On the day before the experiment, tubes were slowly thawed overnight at 4°C . Harringtonine (10 $\mu\text{g}/\text{ml}$) was added to DAC+SB treated cells for 15 min at 37°C to deplete elongating ribosomes from mRNA molecules. Cells were washed in ice-cold PBS containing 100 $\mu\text{g}/\text{ml}$ cycloheximide and lysed in 200 μ l Polysome lysis buffer (15 mM Tris-HCl pH 7.4, 15 mM MgCl_2 , 300 mM NaCl, 100 $\mu\text{g}/\text{ml}$ cycloheximide, 1% Triton-X-100, 0.1% β -mercaptoethanol, 200 U/ml RNasin (Promega), one complete Mini Protease Inhibitor Tablet (Roche) per 10 ml). Nuclei were removed by centrifugation (9,300 \times g, 4°C , 10 min) and the cytoplasmic lysate was loaded onto a sucrose density gradient (17.5–50% in 15 mM Tris-HCl pH 7.4, 15 mM MgCl_2 , 300 mM NaCl). After ultracentrifugation (2.5 h, 35,000 rpm at 4°C in a SW60Ti rotor), gradients were eluted with a Teledyne Isco Foxy Jr. system into 14 fractions of similar volume. A rabbit HBB2 *in vitro* transcript was added to each fraction as a spike-in control (25 fmol/fraction) (Supplementary Table 11) and RNA was purified using phenol chloroform extraction and analyzed via qPCR. To assess RNA quality and equal purification efficiency across all fractions, the HBB2 *in vitro* transcript and endogenous Ncl mRNA were detected through northern blotting.

Transcriptional directionality. Transcriptional directionality was calculated as previously described⁸ with modifications. The sum of CAGE tags mapping to the forward (Expf) or reverse (Expr) strand within ± 700 bps from the center position of TINAT or enhancer coordinates was used to calculate the directionality score $(\text{Expf} - \text{Expr})/(\text{Expf} + \text{Expr})$. Ubiquitous cell-line enhancer coordinates⁸ were used as a reference.

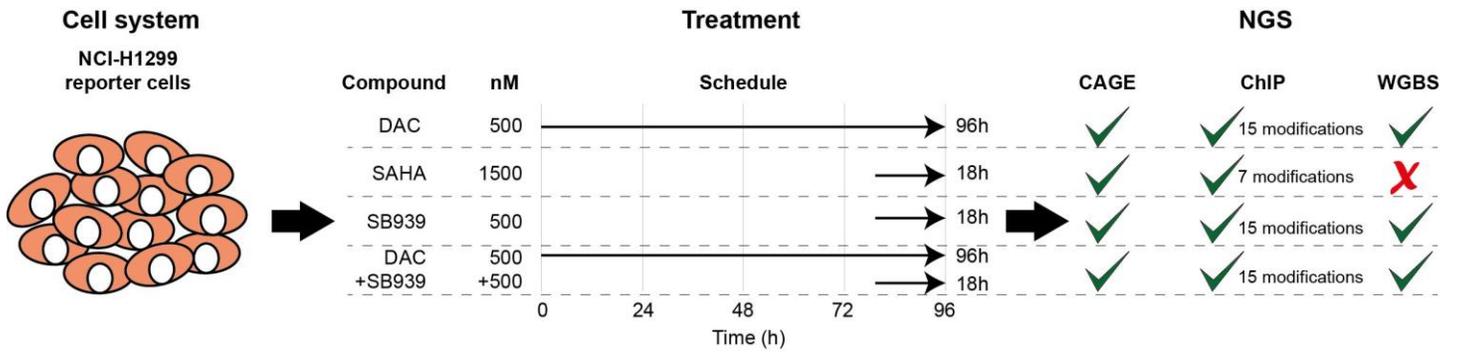
Statistical analysis. All statistical analyses were performed using the R statistical environment. Box plot center lines indicate data medians, box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, and outliers are shown by individual points. For group-wise comparison of two distributions from different samples or treatments, the two-tailed nonparametric Wilcoxon and Mann-Whitney

test was used. For experimental settings with replicates of paired treatments or samples, a two-tailed Student's *t* test was applied. *P* values < 0.05 were considered statistically significant and significance levels are depicted as follows: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Code availability. Scripts are available upon request.

Data availability. CAGE, ChIP, and WGB-sequencing data generated in this study have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession GSE81322.

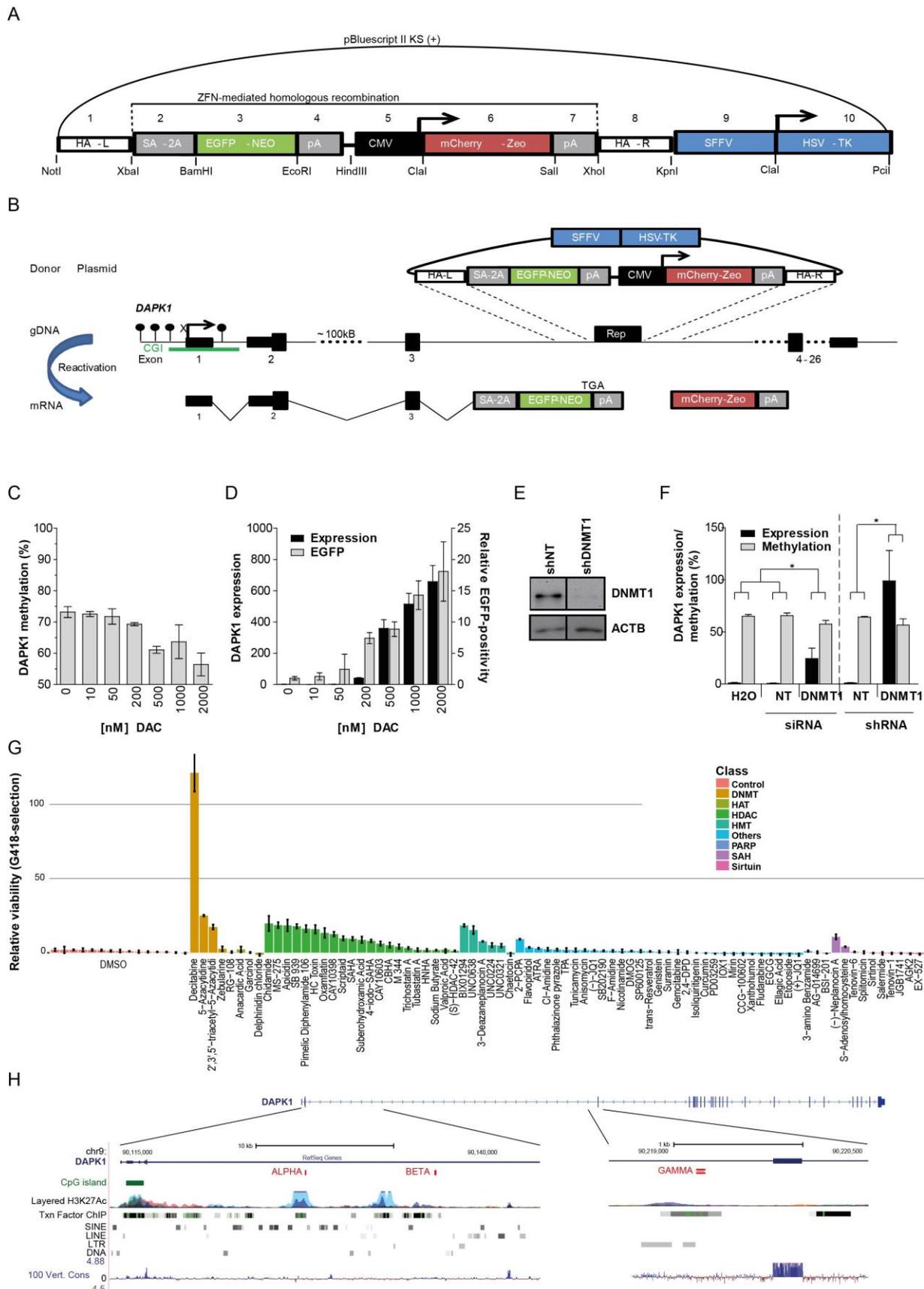
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Supplementary Figure 1

Schematic representation of the experimental approach used in this study.

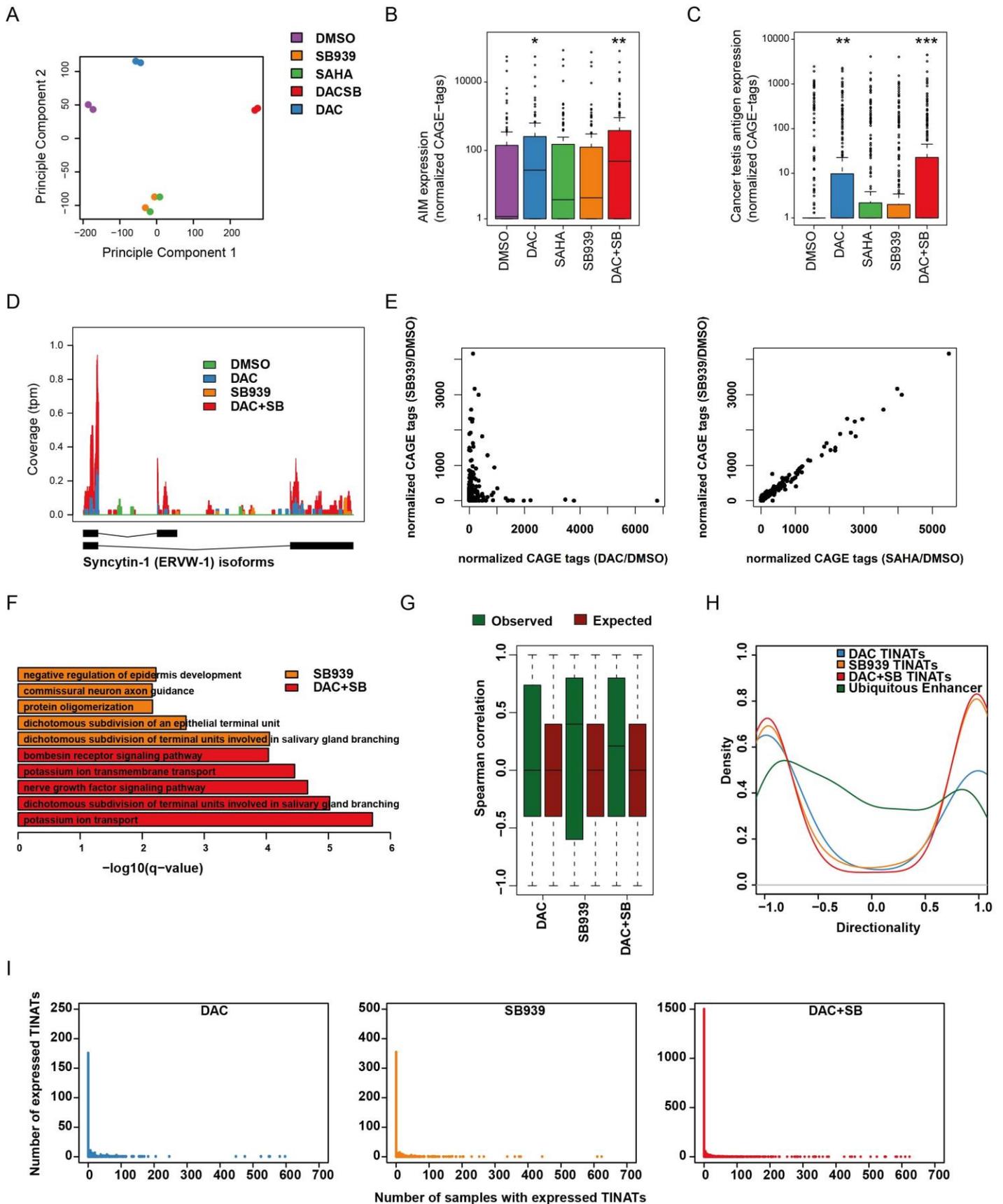
NCI-H1299 cells containing a dual fluorescence/resistance reporter at the epigenetically silenced *DAPK1* locus were treated with DAC, SAHA, SB939, or DAC+SB as indicated. After treatment, cells were harvested and subjected to downstream transcriptomic or epigenomic profiling. CAGE = Cap-analysis of gene expression; ChIP = Chromatin immunoprecipitation; WGBS = Whole-genome bisulfite sequencing.



Supplementary Figure 2

Engineering of reporter cell line, epigenetic compound screen, and cryptic transcripts.

- a) Schematic cloning overview of the dual-fluorescence/resistance reporter construct. Key components are depicted by the numbers 1-10. Arrows indicate activity of the CMV and SFFV promoter driving mCherry-Zeo and HSV-TK expression, respectively. HA-L/R = homology arm left/right; SA-2A = Splice-acceptor with T2A self-cleavage peptide; pA = polyA termination sequence; CMV = Cytomegalovirus promoter; SFFV = Spleen focus-forming virus promoter; HSV-TK = Herpes Simplex Virus Thymidine Kinase.
- b) Predicted model of the DAPK1 locus after successful reporter integration. The reporter integration (Rep) results in a novel fusion transcript containing DAPK1 exons 1-3, spliced to the EGFP-NEO cassette. The T2A cleavage peptide creates separate proteins for EGFP-NEO and a truncated DAPK1 protein upon translation. mCherry-ZEO is constitutively expressed from the CMV promoter.
- c) MassARRAY EpiTYPER quantitative DNA methylation analysis of the DAPK1 promoter CpG island in NCI-H1299 reporter cells after increasing DAC concentrations. DAPK1 methylation is calculated as average methylation value of all covered CpG-units.
- d) DAPK1 mRNA levels and EGFP fluorescence after increasing doses of DAC. Expression values were normalized to housekeeping genes and depicted relative to the individual mock treated sample (left axis). Percentage of EGFP positive cells relative to untreated cells (right axis).
- e) DNMT1 protein levels assessed via western blot after knockdown with a DNMT1-specific shRNA. ACTB = β -Actin.
- f) DAPK1 expression (black) and promoter methylation (grey) after knockdown of DNMT1. Expression is normalized to housekeeping genes and depicted relative to the individual NT-control treated sample. Promoter methylation was calculated as average methylation value of all CpG-units covered. NT = non-targeting.
- g) Relative viability of NCI-H1299 reporter cells after compound treatment and G418 selection. Cells were seeded 24 h prior to compound treatment in 96-well plates in triplicates. Compounds were refreshed every 24 h for 72 h and then G418-selection performed for 10 d. After selection, cell viability was quantified by Calcein-AM assay and normalized to DMSO controls. All bars represent mean values \pm SD (n=3). Results are displayed sorted by drug target and quantified viability.
- h) Genome browser view of the newly identified intragenic transcripts at the DAPK1 locus (ALPHA, BETA, and GAMMA). All three transcripts initiated in the intron between DAPK1 exons 2 and 3. Key functional annotations were derived from the ENCODE cell line data and visualized relative to the transcript start sites to characterize the genomic architecture in the vicinity of the cryptic TSSs: Enhancer mark H3K27Ac, transcription factor binding, transposable elements, and vertebrate conservation. LTR = long terminal repeat, SINE = short interspersed nuclear element, LINE = long interspersed nuclear element, DNA = DNA transposon.



Supplementary Figure 3

CAGE QC and characterization of TINATs

- a) Principle component analysis based on the variance stabilized expression¹ of the 1000 most variable CAGE-clusters. For each treatment condition (DMSO = purple; SB939 = orange; SAHA = green; DAC = blue; DAC+SB = red) both replicates are shown.
- b) Boxplot showing normalized CAGE-tags for TSSs associated with 116 AIMs previously described to be ubiquitously up-regulated after DNMTi across three cancer entities². AIM expression levels in NCI-H1299 cells are shown after DMSO, DAC, SAHA, SB939, and DAC+SB treatment. Statistical significance was calculated using the Wilcoxon and Mann-Whitney two-sided test.
- c) Boxplot showing normalized CAGE-tags for TSSs associated with 276 CTAs³. Statistical significance was calculated using the Wilcoxon and Mann-Whitney two-sided test.
- d) CAGE-tags mapping to Syncytin-1 (ERVW-1) before and after treatment in NCI-H1299 cells. Reads were aligned against the ERVW-1 sequence (hg38) using HISAT⁴ and coverage was calculated using the R/Bioconductor package GenomicAlignments⁵.
- e) Comparison of expression changes relative to DMSO for DAC against SB939 (left) and SB939 against SAHA (right).
- f) Gene ontology enrichment of SB939 (orange) and DAC+SB (red) TINAT proximal genes calculated by GREAT⁶. 224,491 TSS coordinates consisting of our and previously described TSSs (Gencode.v19.TSS.notlow.gff) were used as background regions. The top 5 most significantly enriched biological processes are shown. DAC TINATs showed no enrichment.
- g) TINAT expression correlates with expression of nearby genes. Spearman correlation for the expression of each TINAT – nearest gene pair was calculated across all conditions (green) and compared to the null distribution obtained by permuting sample labels (red).
- h) Density plot showing the difference in directionality based on CAGE tags located within 1400 bps of TINATs or 200 ubiquitous cell line enhancers (green)⁷. Directionality scores of ± 1 or 0 correspond to perfectly strand-specific or balanced transcription, respectively.
- i) TINAT expression across various human tissue and cell types from the FANTOM5⁷ project. TINATs less than 150 bps away from a FANTOM5 CAGE cluster supported by at least one tag were considered expressed in a given sample.

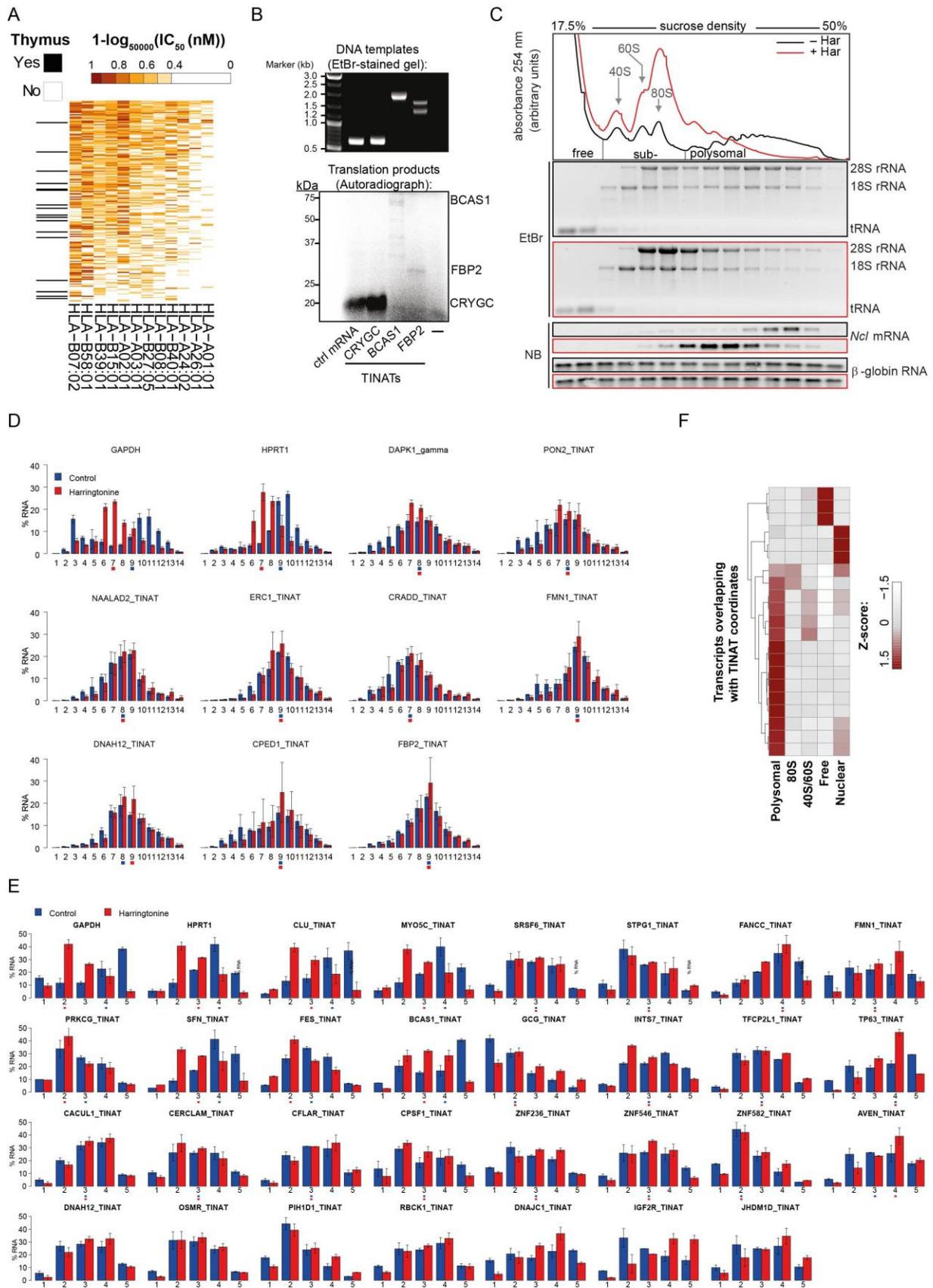
DAC+SB
SB939
DAC



Supplementary Figure 4

Genomic distribution of TINATs

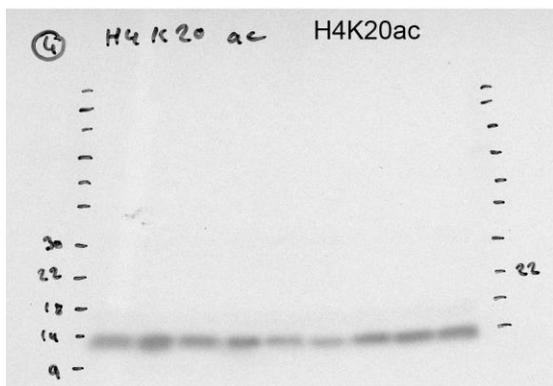
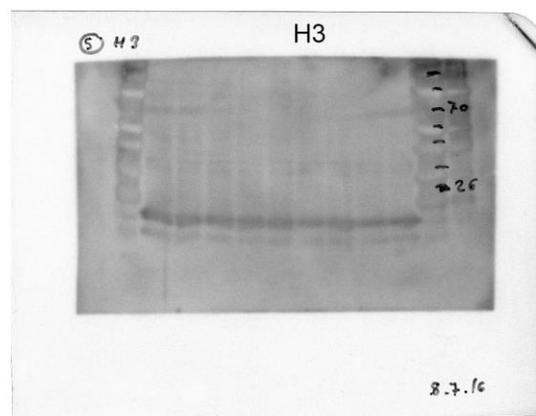
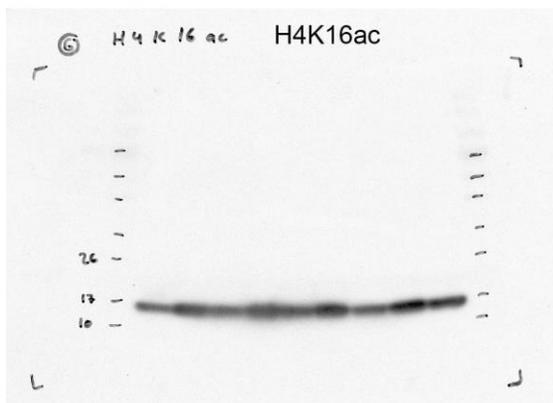
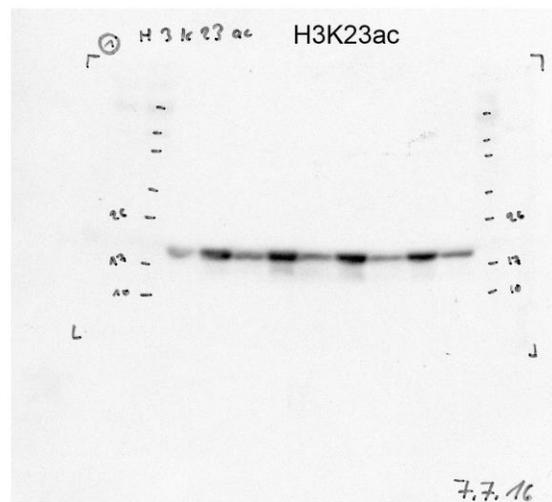
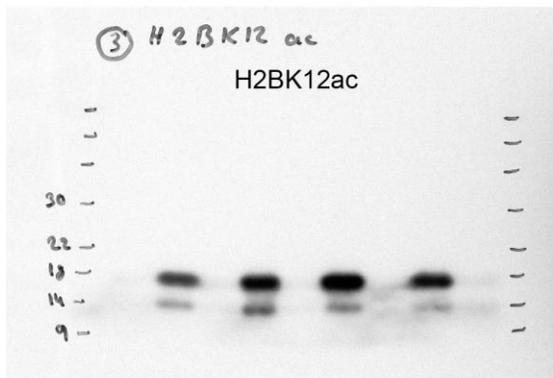
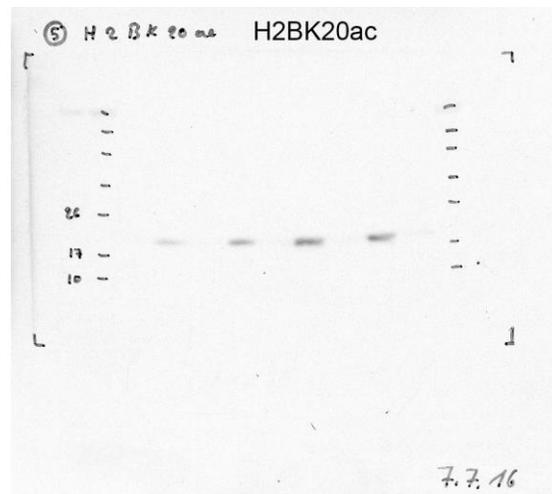
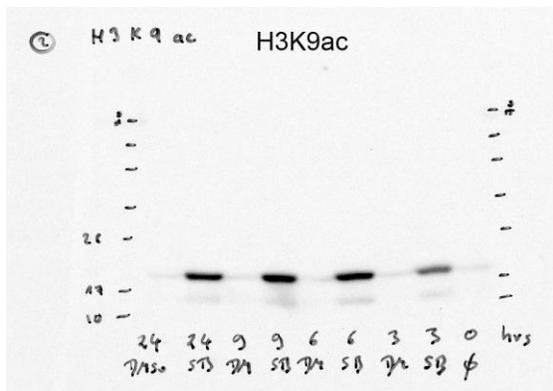
Chromosome-wise genomic distribution of TINATs identified after DAC (blue), SB939 (orange), and DAC+SB (red) treatment.



Supplementary Figure 5

Translation of TINATs into proteins

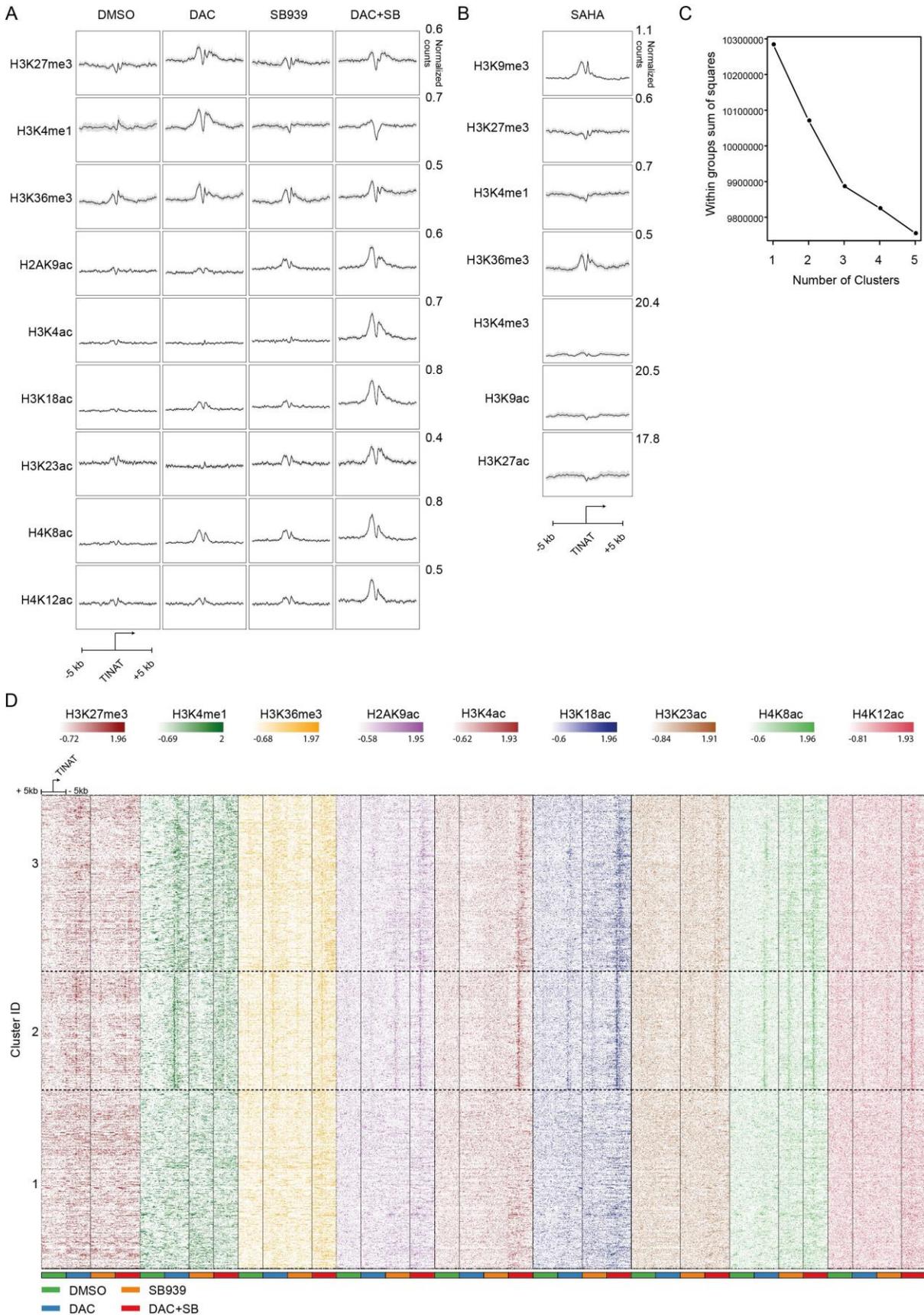
- a) NetMHCpan⁸ was used to predict the binding affinity of 12 major HLA alleles (columns) for 178 DAC+SB out-of-frame peptide sequences (rows). Shown are the log-transformed binding affinity IC50 values (values > 0.426 correspond to an IC50 value stronger than 500 nM). Binding affinity ranges from low (white) to high (brown). The presence of a TINAT within the adult thymus is displayed.
- b) Coupled in vitro transcription and translation of TINAT-exon fusion candidates. cDNAs of DAC+SB treated NCI-H1299 reporter cells was PCR-amplified with gene-specific primers introducing a T7 promoter at the 5' end (top panel). PCR fragments were transcribed and translated in the presence of radioactively labeled methionine. Translation products were size-separated and visualized by autoradiography (bottom panel). The full-length canonical CRYGC mRNA served as positive control.
- c) Polysome profiles of NCI-H1299 cells treated with (red) or without (black) harringtonine (top). 40S, 60S, and 80S subunits are highlighted. tRNA, 18S, and 28S rRNA levels in the presence (red) or absence (black) of harringtonine were measured across 14 fractions by ethidium bromide (EtBr) staining (middle). Lower panel shows Ncl mRNA levels as well as the spike-in control rabbit beta-globin as quantified by northern blot (NB).
- d) Distribution of GAPDH, HPRT1 mRNA as well as nine TINAT-exon fusion transcripts along 14 polysome fractions isolated from DAC+SB treated NCI-H1299 cells in the presence (red) or absence (blue) of harringtonine. Colored squares below horizontal axis line indicate the fraction where half of the mRNAs have accumulated. The mean of three biological replicates is shown and error bars denote standard deviation.
- e) Distribution of GAPDH, HPRT1 mRNA as well as 28 TINAT-exon fusion transcripts along 5 polysome fractions isolated from DAC+SB treated NCI-H1299 cells in the presence (red) or absence (blue) of harringtonine. Colored squares below horizontal axis line indicate the fraction where half of the mRNAs have accumulated. The mean of two biological replicates is shown and error bars denote standard deviation.
- f) Expression levels of transcripts overlapping TINAT coordinates across different polysome fractions in untreated LS-174 T-pTER- β -catenin cells⁹. Intronic or intergenic TINAT coordinates were extended downstream by 500 bps or to the next exon and the overlap with bowtie hg19 aligned reads was counted and normalized by the total number of uniquely mapped reads. Expression levels of 21 TINAT candidate transcripts with a normalized read count >1 in at least one fraction are shown as Z-scores from -1.5 (white) to 1.5 (red).



Supplementary Figure 6

Original gel images

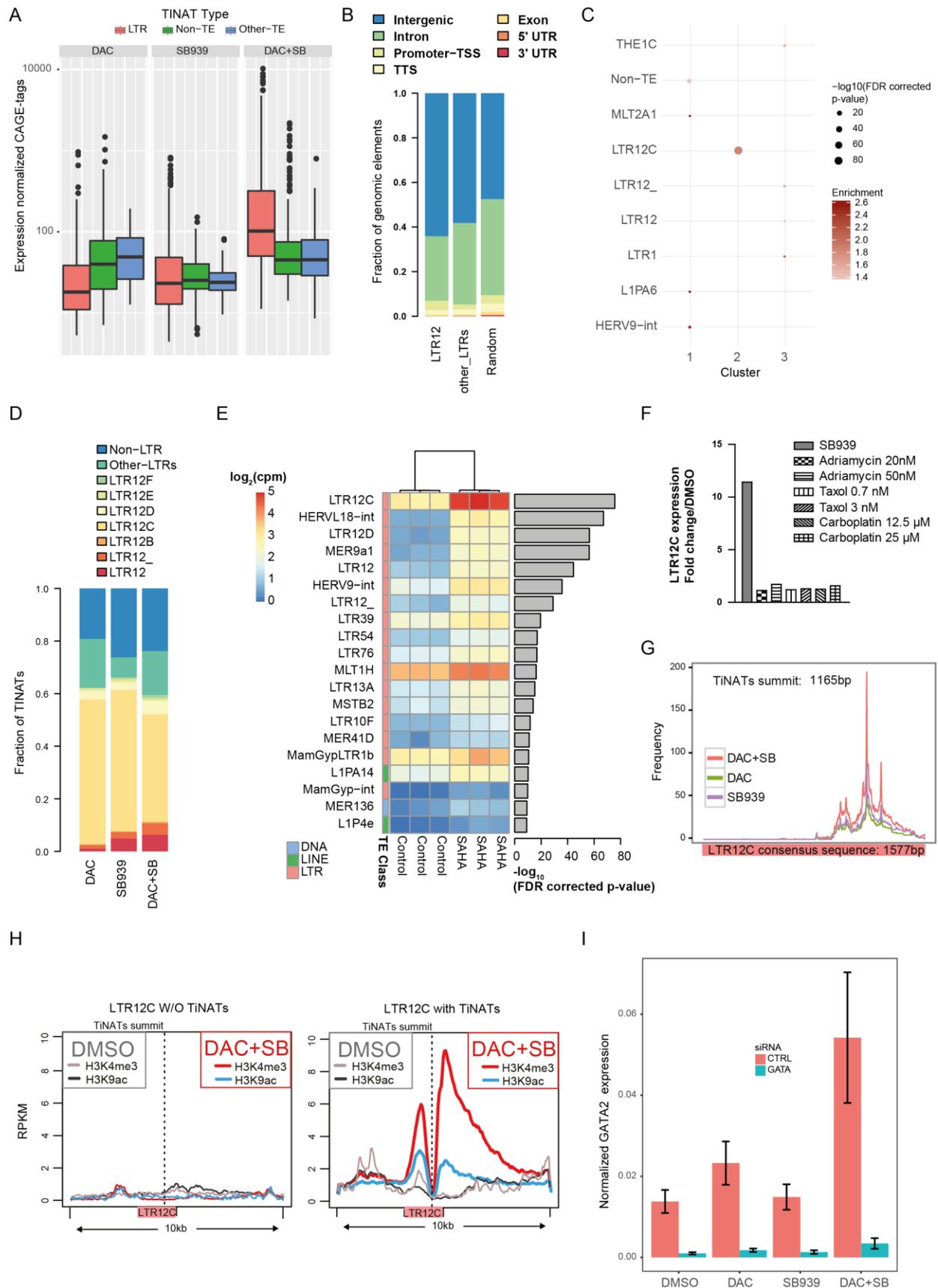
Western blot analysis of histone post-translational modifications at different time points following the treatment with SB939 or DMSO. Samples are arranged from left to right: DMSO 24h, SB939 24h, DMSO 9h, SB939 9h, DMSO 6h, SB939 6h, DMSO 3h, SB939 3h, DMSO 0h.



Supplementary Figure 7

Additional histone modifications around TINATs

- a) ChIP-seq occupancy plots showing the average level of the indicated histone modifications 5 kb up- and downstream of all identified TINATs. Shaded areas indicate the 95% confidence interval and numbers indicate the normalized read counts.
- b) Average level of the indicated histone modifications 5 kb up- and downstream of all identified TINATs after SAHA treatment. Shaded areas indicate the 95% confidence interval and numbers indicate the normalized read counts.
- c) Within group sum of squares as a function of the number of k-means clusters.
- d) H3K27me3 (darkred), H3K4me1 (green), H3K36ac (orange), H2AK9ac (violet), H3K4ac (red), H3K18ac (blue), H3K23ac (brown), H4K8ac (lightgreen), and H4K12ac (lightred) levels around TINATs after DMSO (green bar), DAC (blue bar), SB939 (orange bar), or DAC+SB (red bar) treatment. Color intensity of the histone modifications represents Z-scores. TINATs are arranged as in Fig 4d.



Supplementary Figure 8

Characterization of transposable elements associated with TINATs

- a) Normalized CAGE-tag expression levels of TINATs overlapping with LTRs (red), TEs other than LTRs (blue), or no TEs (green) after DAC (left), SB939 (middle), or DAC+SB (right) treatment. Y-axis is logarithmic.
- b) Genomic distribution of LTR12 copies, all other LTRs, as well as a random set. HOMER¹⁰ software was used to annotate genomic features.
- c) Enrichment of certain TE families across the three chromatin clusters identified in Figure 4d. Point size represents the significance of the overrepresentation which was calculated cluster-wise for each TE family using Fisher's exact test. Color represents fold-enrichment (observed vs expected).
- d) Fraction of TINATs overlapping with LTRs of the LTR12 subfamily, other LTRs, or non-LTR genomic regions.
- e) Heatmap of normalized read counts for the 20 most significant differentially expressed TE subfamilies between DMSO and SAHA treated primary vascular endothelial cells¹¹. The FDR corrected p-value for differential expression is shown in the bar plot to the right. CPM = counts per million
- f) LTR12C expression in NCI-H1299 reporter cells normalized to housekeepers after HDACi (SB939) or treatment with different chemotherapeutic agents relative to DMSO. The results from a single experiment are shown.
- g) Frequency of TINAT start positions along the 1577bp LTR12C consensus sequence. Height of the bar depicts TINAT frequency after DAC (green), SB939 (purple), and DACSB (red) treatment.
- h) ChIP-seq occupancy plots showing the average intensity [reads per kilobase per million mapped reads (RPKM)] of H3K4me3 and H3K9ac for LTR12Cs without (left panel) and with TINATs (right panel) relative to the TINAT coordinates. For both groups, histone signal intensities are shown before (black/grey) and after (colored) treatment with DAC+SB.
- i) qRT-PCR expression analysis of GATA2 transcript levels relative to housekeepers after different treatments in the presence (blue) or absence (red) of siRNAs targeting GATA2. Error bars represent standard deviation.

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Supplementary Note 1

***DAPK1* reporter cell line generation**

Context-dependent assembly (CoDA) of Zinc Finger Nucleases:

ZiFiT software version (4.215)¹ was used to design Genome Editing Nucleases targeting GACAGCCACCAATCAGTTGATTGT on human chr9:90225669-90225692, downstream of *DAPK1* exon3 according to the CoDA method². The underlined sequence indicates the 6nt spacer adjacent to the 2x9nt binding sequence. The predicted ZFN target sequence was checked by blast nucleotide search for lack of predicted additional targets.

ZFN-synthesis and reporter construct cloning:

Left and right ZFN-coding plasmid was synthesized by GeneArt® (Life technologies) into the standard cloning vector pANY (sequences are available upon request). Left and right ZFN-sequences were subcloned via BamHI + XhoI overhangs into the ZFN-expression vectors pAC.HA.nIL2RGL.hNeeai_1140 and pAC.HA.nIL2RGR.hNqkiv_1141, respectively, and sequence-verified by Sanger sequencing. pAC.HA.nIL2RGL.hNeeai_1140 and pAC.HA.nIL2RGR.hNqkiv_1141 were kindly provided by Prof. Cathomen (University Medical Centre, Freiburg). Standard molecular cloning techniques were used to create the reporter construct. The reporter was cloned into pBluescript II KS(-) as follows: First, PCR-amplification and subcloning of EGFP-Neo from LeGO-G/Neo-opt, mCherry-Zeo from LeGO-C/Zeo, SFFV-promoter from LeGO-C/Zeo (see Weber et al.³ for vector details), BGH-polyA from pcDNA™5/FRT/TO (Invitrogen), SV40 polyA and CMV-TO from pT-Rex-DEST30 (Invitrogen), Spliceacceptor+self-cleaving peptide (SA-2A, synthesized as in AAVS1-SA-2A-puro-pA donor 19) and HSV-TK (from PL253 20) into pBluescript II KS(-). Next, the sequence-verified elements were excised from the plasmids, gel purified, and then step-wise ligated by T4-ligase to create the full reporter construct. After cloning, the 9.7 kb full-size dual-fluorescence/resistance reporter construct was partially re-sequenced at critical components to assure sequence correctness. Finally, left (chr9:90224925+90225674) and right (>chr9:90225687+90226448) homology arms of ~750 bp were PCR-amplified from NCI-H1299 genomic DNA and adjacently cloned into the donor plasmid. For details on restriction enzymes and primers used and further cloning details, see **Supplementary Table 6** and **Supplementary Fig. 2a and B**.

ZFN-mediated targeting of the endogenous *DAPK1* locus:

NCI-H1299 cells grown in RPMI 1640 supplemented with 10% FCS were seeded in T75 flasks at a density of 12000 cells/cm² and expanded for 24 h. Thereafter, transfection with 2 µg of left and right ZFN-encoding plasmid and 16 µg of donor plasmid using Trans-IT LT1 (Mirus Bio) was performed. Fresh media containing 100 µg/ml Zeocin (Invitrogen) was applied 48 h after transfection. After 10 d of Zeocin selection, integration positive polyclonal cell pools were subjected to monoclonal cell line generation by FACS-mediated single-cell cloning. For this purpose, mCherry-positive cells were sorted into 96 well plates by the DKFZ FACS core facility using the FACS Aria III machine (BD Biosciences). Two weeks after initial seeding and expansion under positive (100 µg/ml Zeocin) and negative (10 µg/ml Ganciclovir, Sigma) selection pressure, surviving clones were further expanded. Correct reporter construct integration was validated by “In-Out” PCR and Southern-Blotting.

Genotyping polymerase chain reaction

Genomic DNA from polyclonal and monoclonal NCI-H1299 cell lines was isolated using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer’s protocol. Genotyping PCRs were performed with 50 ng template DNA in 96well format using Phusion High-Fidelity DNA-Polymerase (Thermo Scientific). One DAPK1-locus specific primer and one primer binding the reporter construct were applied for integration specific amplification. PCR-products of both 5` and 3` integration were visualized by Ethidium-Bromide stain on 1.5% Agarose gels to determine correct integration and sequence-verified in case of positively genotyped monoclonal cells. Primers used for cell line genotyping are listed in **Supplementary Table 7**.

Southern blotting

Genomic DNA (10 µg) was isolated by Phenol-chloroform extraction and separated on a 0.8% agarose gel after overnight digest with 100 U BamHI (NEB). Depurinated and denatured gel was then capillary transferred over-night to a nylon membrane (Amersham Hybond NX) and finally hybridized with ³²P-labeled (Megaprime DNA Labeling System, Amersham) probes against genomic DAPK1 loci upstream (chr9:90,224,318-90,224,820) or downstream (chr9:90,228,320-90,229,358) of the ZFN-mediated integration site. Probing was carried out in Church’s buffer (7% SDS, 1 mM EDTA, 0.5 M NaPi pH 7.2) supplemented with 100 µg/ml salmon sperm DNA (Invitrogen).

Epigenetic compound viability screen

Epigenetic compound screen in NCI-H1299 reporter cells was performed using the Epigenetics Screening Library (Cayman Chemical) in 96 well format with modifications. Compound concentrations were cross-referenced with commonly used cell-culture concentrations and adjusted to have maximum cytotoxic effects of 50 % viability reduction after 72 h of treatment. For details on

compounds and used concentrations, see **Supplementary Table 8**. Media containing the compound was refreshed every 24 h for 72h. G418-selection of DAPK1-reactivated cells was carried out in media containing 800 µg/ml G418 (Sigma) for 10 d and cell viability was quantified using the Calcein-AM assay. In brief, cells were grown in fresh media containing 0.8 µM Calcein-AM for 1 h and then lysed with PBS containing 3% Triton-X (Sigma) and fluorescent signal intensity was measured on a Spectramax M5e plate reader (at ex494 nm and em530 nm).

5' Rapid amplification of cDNA ends (5' RACE)

5'RACE was essentially performed as described previously⁴. Firstly, cDNA was synthesized using 10 pmol gene-specific primer 1 (GSP1) and Thermoscript Reverse Transcriptase with 1 µg DNA-free template RNA for 50 min at 61 °C in 20 µl reaction volume. After heat inactivation at 80 °C for 5 min, RNA from cDNA-RNA hybrids was specifically digested by adding 1 µl of RNase H for 30 min at 37 °C. Next, RNase H was heat inactivated at 70 °C for 10 min. To purify the now single-stranded cDNA, DNA was precipitated by addition of 0.1 vol. 3 M sodium acetate, 2 µg Glycogen and 2.5 vol. 100% ethanol. After centrifugation at 13,000 g, the DNA pellet was washed in 500 µl 70% ethanol and then air dried before it was resuspended in 50 µl of H₂O. To tail the 3' end of the newly synthesized cDNA, single-stranded cDNA was incubated with 30 U TdT and 100 pmol dATP at 37 °C for 15 min in a 20 µl reaction, followed by heat inactivation at 70 °C for 10 min and finally diluted by addition of 30 µl H₂O. The first amplification PCR was carried out using the High Fidelity PCR system with 3 pmol primer Q_T, 25 pmol primer Q₀ and 25 pmol primer GSP2 in a 50 µl reaction using 1.5 µl of tailed and diluted cDNA under the following cycling conditions: 2 min initial denaturation at 95 °C, 2 min annealing of primer Q_T, 40 min of linear amplification with primer Q_T at 68 °C. Next, linearly amplified and Q_T tagged cDNA was then exponentially amplified by 10 touchdown cycles of 15 s at 94 °C, 30 s at 60 °C (-0.5 °C per cycle) and 1 min extension at 68 °C followed by 30 PCR-cycles at 55 °C annealing temperature and 1 min extension time. For the second PCR, 1.5 µl of 1:20 diluted PCR product from the first amplification was amplified employing 10 pmol Primer Q₁ and 10 pmol GSP3 primer with the same PCR protocol (touchdown 10+30 cycles) as for the first PCR, but lacking the initial steps of linear amplification. Finally, the PCR products were separated on a 1.5 % agarose gel and then gel-purified for subcloning, utilizing the TOPO TA Cloning Kit according to the manufacturer's protocol (Invitrogen). Cloned PCR-products from single blue-white selected colonies were Sanger-sequenced (GATC, Konstanz, Germany) to identify the cloned transcripts. For details on the used primers see **Supplementary Table 9**.

Supplementary Tables

Supplementary Table 2: Filtering procedure of StringTie assembled transcripts

	DAC	SB939	DAC+SB
Total	453	744	3627
Overlap protein-coding exon	98	105	571
Longest isoform only	87	97	474
Highest coverage only	71	81	376
Translated (<i>in silico</i> prediction)	62	61	327

Supplementary Table 6: Oligonucleotides used for reporter construct and ZFN cloning

Primer/Target	Forward (5' -> 3')	Reverse (5' -> 3')	Details
HA_3'4_Left	CTAGCTAGGCGGCCG CACTGCCACTGACCC AAAAAG	CTAGCTAGTCTAGA GCTGTCCCTTCGGG GTGG	Cloning of the ~750 bp ZFN3'4 associated homology arm upstream (left) of the ZFN target site tagged with NotI (Forward, bold) and XbaI (Reverse, bold) recognition sites.
HA_3'4right	CTAGCTAGCTCGAGG ATTGTTCTGTGTGTA ATTTTGGAT	CTAGCTAGGGTACC TGAGCTATGATTGC ACCACTG	Cloning of the ~750 bp ZFN3'4 associated homology arm downstream (left) of the ZFN target site tagged with XhoI (Forward, bold) and KpnI (Reverse, bold) recognition sites.
SA-T2A	CTAGCTAGTCTAGAC TGACCTCTTCTTCC TCCCAC	CTAGCTAGGGATCC CTCGAGCCTAGGGC CGG	Cloning of the Spliceacceptor-T2A cassette from the synthesized ZFN vector tagged with XbaI (Forward, bold) and BamHI (Reverse, bold) recognition sites.
EGFP-NEO	AAAGGATCCATCAGT CAGCGTACGATGGTG AGCAAGGGCGA	AAAGAATTCAGT CTAGTCCGGATCAG AAGAACTCGTCCAG CAG	Cloning of the EGFP-NEO cassette from the LeGO-G/Neo-opt vector tagged with BamHI (Forward, bold) and EcoRI (Reverse, bold) recognition sites.
BGH-PolyA	CTAGCTAGGAATTCC CTCGACTGTGCCTTCT AGTT	CTAGCTAGAAGCTT CCATAGAGCCCACC GCAT	Cloning of the BGH-pA cassette from the pcDNA™5/FRT/TO vector tagged with EcoRI (Forward, bold) and HindIII (Reverse, bold) recognition sites.
CMV-TO	CTAGCTAGAAGCTTA TGCATGTCGTTACAT	CTAGCTAGATCGAT TCCCGGTGTCTTCT	Cloning of the CMV-TO cassette from the pT-Rex-DEST30 vector tagged with HindIII

	AACTTACG	ATGGAG	(Forward, bold) and ClaI (Reverse, bold) recognition sites.
Cherry-Zeo	AAAATCGATACTAGC TAGACGCGTAATCCC GCCACCATGGTGA	AAAGTCGACACTAG CTAGCCCGGGTCAG TCCTGCTCCTCGGC	Cloning of the Cherry-Zeo cassette from the LeGO-C/Zeo vector tagged with ClaI (Forward, bold) and Sall (Reverse, bold) recognition sites.
SV40-PolyA	CTAGCTAGGTCGACA ACTTGTTTATTGCAGC TTATAATGGT	CTAGCTAGCTCGAG GCCGATTCATTAAT GCAGGA	Cloning of the SV40-pA cassette from the pT-Rex-DEST30 vector tagged with Sall (Forward, bold) and XhoI (Reverse, bold) recognition sites.
SFFV	CTAGCTAGGGTACCC TGAAAGACCCACCT GTAGG	CTAGCTAGATCGAT GGGCGACTCAGTCA ATCG	Cloning of the SFFV promoter from the LeGO-C/Zeo vector tagged with KpnI (Forward, bold) and ClaI (Reverse, bold) recognition sites.
HSV-TK	CTAGATCGATAATCC CGCCACCATGGCTTC GTACCCCGG	CTAGACATGTTTCAG TTAGCCTCCCCATC T	Cloning of the HSV-TK cassette from the PL253 vector tagged with ClaI (Forward, bold) and PciI (Reverse, bold) recognition sites.
ZFN-left	GAAAAAATCTAGAC CCGGGG	CAGGAAACAGCTAT GACCGG	Cloning of the ZFN “left” pairs from the synthesized ZFN plasmids into pAC.HA.nIL2RGL.hNeeai_1140.
ZFN-right	GAAAAAATCTAGAC CCGGGG	GCAATGTAACATCA GAGATGGATC	Cloning of the ZFN “right” pairs from the synthesized ZFN plasmids and pAC.HA.nIL2RGR.hNqkiv_1141.

Supplementary Table 7: Genotyping primers

Primer/Target	Forward (5' -> 3')	Reverse (5' -> 3')	Details
ZFN3'4 genotyping_Left	GAACTTCCTCTGAGG GTTAGTG	GTGGGAGGAAGAGA AGAGGTCA	Forward primer binds genomic sequence upstream of ZFN3'4 target site. Reverse primer binds SA-2A sequence.
ZFN3'4 genotyping_Right	GACTTCGTGGAGGAC GACTT	AAAAAGGAAAAATTG AAATTCTGG	Reverse primer binds genomic sequence downstream of ZFN3'4 target site. Forward primer binds Zeo cassette.

Supplementary Table 8: Epigenetic compound library

Compound	Target group	Primary target proteins	IC50 in NCI-H1299 H2 cells	Concentration used [nM]
Zebularine	DNMT	DNMT1	>50μM	50000
RG-108	DNMT	DNMT1	>50μM	50000
2',3',5'-triacetyl-5-Azacytidine	DNMT	DNMT1	>50μM	50000
Decitabine	DNMT	DNMT1	>50μM	1000
5-Azacytidine	DNMT	DNMT1	7.5μM	5000
Delphinidin chloride	HATS	HATs,	25μM	50000
Anacardic Acid	HATS	HATs,p300;PCAF	>50μM	50000
Garcinol	HATS	HATs,p300;PCAF	6μM	5000
SB939	HDAC	HDAC pan	700nM	500
CAY10398	HDAC	HDAC1	7.5μM	5000
MS-275	HDAC	HDAC1	120nM	100
Suberohydroxamic Acid	HDAC	HDAC1 , HDAC3	20μM	10000
CBHA	HDAC	HDAC1, 3	4000nM	1000
M 344	HDAC	HDAC1, 3,6	1μM	500
Tubastatin A (trifluoroacetate salt)	HDAC	HDAC6	15μM	1000
CAY10603	HDAC	HDAC6	1μM	500
Valproic Acid (sodium salt)	HDAC	HDACs pan	>50μM	50000
Chidamide	HDAC	HDACs pan	5μM	5000
HNHA	HDAC	HDACs pan	>50μM	5000
(S)-HDAC-42	HDAC	HDACS pan	0.5μM	500
VPA	HDAC	HDACS pan	1600μM	50000
HC Toxin	HDAC	HDACs 1,2,3,8	40nM	50
Scriptaid	HDAC	HDACs 1,2,8	1μM	1000
Oxamflatin	HDAC	HDACs 3,6	100nM	50
Pimelic Diphenylamide 106	HDAC	HDACs Class I	16μM	10000
Sodium Butyrate	HDAC	HDACS Class I, lia	>50μM	50000
Apicidin	HDAC	HDACs Class I,II	200nM	100
Trichostatin A	HDAC	HDACs Class I,II	50nM	50

SAHA	HDAC	HDACs Class I+II, IV	3-4 μ M	1000
4-iodo-SAHA	HDAC	HDACs Class I+II, IV	0.6 μ M	500
Chaetocin	HMTS	SU(VAR)3-9; G9a/GLP	< 20nm	5000
3-Deazaneplanocin A	HMTS	EZH2, other HMTs	>50 μ M	10000
BIX01294 (hydrochloride hydrate)	HMTS	G9a	10 μ M	5000
UNC0321 (trifluoroacetate salt)	HMTS	G9a, GLP	>50 μ M	1000
UNC0638	HMTS	G9A, GLP	15 μ M	1000
UNC0224	HMTS	G9a, GLP	20 μ M	1000
2-PCPA (hydrochloride)	HDM	LSD1, MAOA, MAOB	>50 μ M	50000
Genistein	OTHER	various	7.5 μ M	5000
Nicotinamide	OTHER	various	>50 μ M	50000
Suramin (sodium salt)	OTHER	various	>50 μ M	50000
EGCG	OTHER	various	50 μ M	50000
IOX1	OTHER	2OG	10 μ M	10000
Gemcitabine	OTHER	Gadd45a	70nM	50
2,4-DPD	OTHER	HIF-PH	>50 μ M	5000
SP600125	OTHER	JNK	>5 μ M	5000
Phthalazinone pyrazole	OTHER	Aurora-A-Kinase	>50 μ M	1000
(+)-JQ1	OTHER	BETs, BRD4	500nM	1000
(-)-JQ1	OTHER	None, inactive	25 μ M	1000
Flavopiridol	OTHER	CDKs	70nM	50
Tunicamycin	OTHER	Cell cycle	200ng/ml	100ng/ml
trans-Resveratrol	OTHER	COX, Sirts	25 μ M	10000
Ellagic Acid	OTHER	CytP450	10 μ M	10000
Fludarabine	OTHER	DNA-Synthesis inhibition	30nM	50
DMOG	OTHER	HIF-PH	>50 μ M	50000

PD03259	OTHER	MAPK	4.1nM	1
Mirin	OTHER	MRN; ATM	50µM	50000
SB202190	OTHER	p38a , p38b	>5µM	5000
F-Amidine (trifluoroacetate salt)	OTHER	PAD4	>50µM	50000
Cl-Amidine	OTHER	PAD4	>50µM	50000
Phorbol-12-myristat-13-acetat (TPA)	OTHER	PKC activation	2µg/ml	1ng/ml
Isoliquiritigenin	OTHER	Quinone reductase-1	7.5µM	5000
ATRA	OTHER	RAR	25µM	10000
CCG-100602	OTHER	Rho signaling	4.5µM	5000
Anisomycin	OTHER	Ribosome	100nM	100
Etoposide	OTHER	Topoisomerase	800nM	1000
Curcumin	OTHER	various	3µM	1000
Xanthohumol	OTHER	various	6µM	3000
BSI-201	PARP	PARP-1	>50µM	50000
AG-014699	PARP	PARP-1	10µM	1000
3-amino Benzamide	PARP	PARP-1	>50µM	50000
(-)-Neplanocin A	SAH	SAH	600 nM	500
S-Adenosylhomocysteine	SAH	SAH	>50µM	50000
Splitomicin	SIRTUINS	Sir2p	>50µM	50000
EX-527	SIRTUINS	Sirt1	>50µM	50000
Sirtinol	SIRTUINS	SIRT1; SIRT2	10µM	5000
AGK2	SIRTUINS	SIRT2	>50µM	50000
JGB1741	SIRTUINS	SIRTs	50µM	50000
Salermide	SIRTUINS	SIRTs	5µM	5000
Tenovin-6	SIRTUINS	SIRTs	3µM	1000
Tenovin-1	SIRTUINS	Sirts	1.5µM	1000

Information on inhibited proteins for the individual compounds was derived from <http://www.caymanchem.com>

Supplementary Table 9: 5'RACE primers

Primer/Target	Forward (5' -> 3')	Details
Q _T	CCAGTGAGCAGAGTGACGAGGACTCGAGCT	5' RACE standard primer.

	CAAGCTTTTTTTTTTTTTTTTTT	
Q ₀	CCAGTGAGCAGAGTGACG	5' RACE standard primer.
Q ₁	GAGGACTCGAGCTCAAGC	5' RACE standard primer.
GSP1	GGTCAGGGTGGTCACGAG	Binds EGFP.
GSP2	GCCCTTGCTCACCATCGTA	Binds SA-2A sequence.
GSP3	CACGTCACCGCATGTTAGA	Binds SA-2A sequence.

Supplementary Table 10: Oligonucleotides used for shRNA cloning into pRS19

Primer/Target	Forward (5' -> 3')	Reverse (5' -> 3')
shDNMT1-3	accgggcccaatgagattgacattaagtaata ttcatagcttgatgtcagctcattgggctttt	cgaaaaagcccaatgagactgacatcaagctatgaatattaa cttaatgtcaatctcattgggcc
shLuc2	accggcttcgaaatgttcgtttggttgtaaatatt catagcaaccgaacggacatttcgaagtttt	cgaaaaacttcgaaatgtccgttcggttgctatgaatattaac aaccaaacgaacatttcgaagc

Supplementary Table 11: Primer sequences

Sequence 5' -> 3'	Target	Target	Orientati on	Application
GCAGGAAAACGTGGATGATT	DAPK1	Exon 2	Forward	qRT-PCR
CATTTCTCACAACCGCAA	DAPK1	Exon 3	Reverse	qRT-PCR
TGCAAGAAATACGAAGCCAGA	DAPK1	3' UTR	Forward	qRT-PCR
GGTTGGTCCATTGAGCTT	DAPK1	3' UTR	Reverse	qRT-PCR
ACGGACGTCATCCTGATCTT	DAPK1	Exon 3	Forward	qRT-PCR
CACGTCACCGCATGTTAGA	DAPK1	EGFP construct	Reverse	qRT-PCR
GGACTGTGAGTTTTCTGAGGGTA	DAPK1	α -transcript	Forward	qRT-PCR
CATTTCTCACAACCGCAA	DAPK1	α -transcript	Reverse	qRT-PCR
GGAAATTGGAGCTTCCAAAAG	DAPK1	β -transcript	Forward	qRT-PCR
CATTTCTCACAACCGCAA	DAPK1	β -transcript	Reverse	qRT-PCR
TACCTTGAGACGGGAGGAGA	DAPK1	γ -transcript	Forward	qRT-PCR
CATTTCTCACAACCGCAA	DAPK1	γ -transcript	Reverse	qRT-PCR
aggaagagagAGTTTAGTAATGTGTTAT AGGTG	DAPK1	Promoter CpG island	Forward	MassARRAY
cagtaatacgactcactataggagaaggctA	DAPK1 CGI	Promoter CpG	Reverse	MassARRAY

CCAATAAAAACCTACAAAC		island		
AGCCACATCGCTCAGACAC	GAPDH	Housekeeper	Forward	qRT-PCR
GCCCAATACGACCAAATCC	GAPDH	Housekeeper	Reverse	qRT-PCR
TGACCTTGATTTATTTGCATACC	HPRT1	Housekeeper	Forward	qRT-PCR
CGAGCAAGACGTTCAATCCT	HPRT1	Housekeeper	Reverse	qRT-PCR
CAACGAGTCTGGCTTTGAGA	DNMT1	Housekeeper	Forward	qRT-PCR
GACACAGGTGACCGTGCTTA	DNMT1	Housekeeper	Reverse	qRT-PCR
ATTGGCAATGAGCGGTTCC	beta-Actin	Housekeeper	Forward	qRT-PCR
GGATGCCACAGGACTCCAT	beta-Actin	Housekeeper	Reverse	qRT-PCR
ATGGGCCGCCTTAAGAGTAA	NAALAD2	TINAT	Forward	qRT-PCR
GCAATGCTTCTCTCCTGGA	NAALAD2	Exon	Reverse	qRT-PCR
TGATTTTCTCTTGCTGCCGC	ERC1	TINAT	Forward	qRT-PCR
CGCAATTCATCCTGGAGAGC	ERC1	Exon	Reverse	qRT-PCR
AGCTCTCATCTGGTCTGCTG	CRADD	TINAT	Forward	qRT-PCR
CTGGTTAATCTGCCGGTCTG	CRADD	Exon	Reverse	qRT-PCR
AACGCATCGGAAGCAAGA	FMN1	TINAT	Forward	qRT-PCR
CTCTGTTGGGAAGGTCTTTAGG	FMN1	Exon	Reverse	qRT-PCR
GAACAACCTCCAGACGCG	DNAH3	TINAT	Forward	qRT-PCR
TGGAAATGATATTCAGATGGCGA	DNAH3	Exon	Reverse	qRT-PCR
CTCCAACACATCCGACCAT	DNAH12	TINAT	Forward	qRT-PCR
TTTTCTGTGCCTGCTGG	DNAH12	Exon	Reverse	qRT-PCR
TCCTCATTTTCTTTGTACCAC	FARS2	TINAT	Forward	qRT-PCR
TGCGTAATTCTCAGAGGGCA	FARS2	Exon	Reverse	qRT-PCR
TCACTGTGAGGGTCCGCG	CPED1	TINAT	Forward	qRT-PCR
GTGGCTGCTGTATTTCTGGT	CPED1	Exon	Reverse	qRT-PCR
ACGCGCTGCCTTTTAGAAC	COL28A1	TINAT	Forward	qRT-PCR
AATCCTTCTCCGGGTAAGCC	COL28A1	Exon	Reverse	qRT-PCR
ACTGTCCTACGGGCTAGAGA	FBP2	TINAT	Forward	qRT-PCR
ACCATGTTGATCACCAGGGA	FBP2	Exon	Reverse	qRT-PCR
TTCTTCTGGTGTCTGTGGA	BCAS1	TINAT	Forward	qRT-PCR
TGGTAAGTCTCTGCTTCTGGT	BCAS1	Exon	Reverse	qRT-PCR
GCTCCATTCATGATCACCTTCT	CRYGC	TINAT	Forward	qRT-PCR
TTGGTAGTTGGGACGCTCAT	CRYGC	Exon	Reverse	qRT-PCR
GCTCCATTCATGATCACCTTCT	ATP6V1H	TINAT	Forward	qRT-PCR

TTGGTAGTTGGGACGCTCAT	ATP6V1H	Exon	Reverse	qRT-PCR
GGGTGTGTTTTCTTCCCCTT	PON2	TINAT	Forward	qRT-PCR
ATTCCCGTGCCCTTGGTTTT	PON2	Exon	Reverse	qRT-PCR
GGTCAGCAAATGCATGGAG	AVEN	TINAT	Forward	qRT-PCR
TCTGCATCGCTGTCATCTC	AVEN	Exon	Reverse	qRT-PCR
ACCAATTCTGGACACACTGG	CACUL1	TINAT	Forward	qRT-PCR
TGATCTATGGCACCATCCAA	CACUL1	Exon	Reverse	qRT-PCR
TGGGAGATGGAGATCTCTGG	CERCAM	TINAT	Forward	qRT-PCR
CTGATGGCACTGCTGTTGAG	CERCAM	Exon	Reverse	qRT-PCR
CATGGTTTTTGGCTTCACCT	CFLAR	TINAT	Forward	qRT-PCR
GCAAATTGGCCAAGAATCTG	CFLAR	Exon	Reverse	qRT-PCR
CGGATGAAGGACCAGTGTG	CLU	TINAT	Forward	qRT-PCR
TTCCTGGTCAACCTCTCAGC	CLU	Exon	Reverse	qRT-PCR
CATGGCTCCCCTCATCCT	CPSF1	TINAT	Forward	qRT-PCR
AAAGCGGACTTTGAGATTGC	CPSF1	Exon	Reverse	qRT-PCR
CCCAACACATCCGACCAT	DNAH12	TINAT	Forward	qRT-PCR
TAGCCAAGTCCTTGGTGGTT	DNAH12	Exon	Reverse	qRT-PCR
GGAAGATGCACTGACTAGAACTG	DNAJC1	TINAT	Forward	qRT-PCR
CAGGAAATTCAGGTTTTGGTTT	DNAJC1	Exon	Reverse	qRT-PCR
TATTCATCCATGGGCCAGAT	FANCC	TINAT	Forward	qRT-PCR
CCCAAGACCTTGAGTGAAAAG	FANCC	Exon	Reverse	qRT-PCR
GCATGGAGTACCTGGAGAGC	FES	TINAT	Forward	qRT-PCR
GGGACATCCCAAAGTCACTG	FES	Exon	Reverse	qRT-PCR
TCCGGACACAATGGTACAAA	FMN1	TINAT	Forward	qRT-PCR
TTCTTTGTGCTCCCTCTTCAA	FMN1	Exon	Reverse	qRT-PCR
GAATTCATTGCTTGGCTGGT	GCG	TINAT	Forward	qRT-PCR
CGGCCAAGTCTTCAACAAT	GCG	Exon	Reverse	qRT-PCR
ATGCACGACTTGAAGACACG	IGF2R	TINAT	Forward	qRT-PCR
CAATGCTGCTCTGGACTCTG	IGF2R	Exon	Reverse	qRT-PCR
TTGCTCCTCATTGCCTTCT	INTS7	TINAT	Forward	qRT-PCR

AAAGTCTGGGAAAGCGAACA	INTS7	Exon	Reverse	qRT-PCR
GGAGCAAACCTCCAGACACG	JHDM1D	TINAT	Forward	qRT-PCR
CAGCATGATGTTCTTCTACTCC	JHDM1D	Exon	Reverse	qRT-PCR
ATGTGCTCCTGCAGAAAAGG	MYO5C	TINAT	Forward	qRT-PCR
GAGAGAGGGGCTCCAAAGTT	MYO5C	Exon	Reverse	qRT-PCR
TCTGCCACCATGTGAAATGT	OSMR	TINAT	Forward	qRT-PCR
GGTAAGTCCTCAAGGACAGCA	OSMR	Exon	Reverse	qRT-PCR
GGAACCCTGACCTGTACGAT	PIH1D1	TINAT	Forward	qRT-PCR
ATGTTCTGCTGCGAGATGG	PIH1D1	Exon	Reverse	qRT-PCR
GTGAAGCGGTTGAGTTCCTT	PRKCG	TINAT	Forward	qRT-PCR
AACTTGCCCAGCTGTTGAAT	PRKCG	Exon	Reverse	qRT-PCR
CAGCAGGAGGGGAACCTACCT	RBCK1	TINAT	Forward	qRT-PCR
AAGGTGTGCAGACTCACG	RBCK1	Exon	Reverse	qRT-PCR
TCTCTCTGGCCAAGACCACT	SFN	TINAT	Forward	qRT-PCR
TGATGAGGGTGCTGTCTTTG	SFN	Exon	Reverse	qRT-PCR
AACCACCACCAAAAACCTCA	SRSF6	TINAT	Forward	qRT-PCR
CGAACAGGTGGTCCGTATTT	SRSF6	Exon	Reverse	qRT-PCR
AGAAGGACGTGTTTGCTTCC	STPG1	TINAT	Forward	qRT-PCR
GCCAGTGCGTTCATTTTTCT	STPG1	Exon	Reverse	qRT-PCR
TTGCCTGGGAGGTACAGTTC	TFCP2L1	TINAT	Forward	qRT-PCR
GCGAGCACATCACTGAGCTA	TFCP2L1	Exon	Reverse	qRT-PCR
TTCCGGACACCCTATCAGAG	TP63	TINAT	Forward	qRT-PCR
ATGAGCTGGGGTTTCTACGA	TP63	Exon	Reverse	qRT-PCR
CAGAAAGGGAATCTTCAGTCG	ZNF236	TINAT	Forward	qRT-PCR
ACCCATATGCATCTTGCTGA	ZNF236	Exon	Reverse	qRT-PCR
TCATGCAGAACTTGCTCGAC	ZNF546	TINAT	Forward	qRT-PCR
TTCCGTTTGAAGACGAAAGG	ZNF546	Exon	Reverse	qRT-PCR

GCCAGTGACAATGCCTTCAT	ZNF582	TINAT	Forward	qRT-PCR
TGGGTCAGCTTCTCTGAGGT	ZNF582	Exon	Reverse	qRT-PCR
TCACTCTTTGGGTCCACACT	LTR12C_1	Consensus	Forward	qRT-PCR
TGGAGTTGTTTCGTTCTCCC	LTR12C_1	Consensus	Reverse	qRT-PCR
GAAGGCTCATGGCAAGAAGG	Rabbit beta-Globin	Housekeeper	Forward	qRT-PCR
ATGATGAGACAGCACAATAACCAG	Rabbit beta-Globin	Housekeeper	Reverse	qRT-PCR
CTGAACTCGCATCATCCGTG	CRYGC	5'UTR	Forward	In vitro transcription
CTCACTGACTCACCCAGAGC	CRYGC	TINAT	Forward	In vitro transcription
TGGGAAATTGGTAGTGTTAAGCT	CRYGC	3'UTR	Reverse	In vitro transcription
CCGTTTGGGTCTCTTTCC	FBP2	TINAT	Forward	In vitro transcription
GCTTCCAAACCTGTCGTAAG	FBP2	3'UTR	Reverse	In vitro transcription
TCATTCTTCTGGTGTCTGTGG	BCAS1	TINAT	Forward	In vitro transcription
TTCCCTCCCTTCTCTGCTTG	BCAS1	3'UTR	Reverse	In vitro transcription

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