SI Materials and Methods

Participants. 24 patients diagnosed with hematological malignancies from Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Denmark, were randomly selected for measurement of plasma vitamin C levels (details are listed in Table S1). The study was approved by the Regional Ethical Committee, Capital Region of Denmark, and all participants provided written informed consent before enrollment.

Plasma vitamin C concentration measurement. Peripheral blood samples drawn with Na-heparin as anticoagulant by vein puncture were protected from light and centrifuged within 30 min at 4 °C for 10 min at 2000 x *g*. The plasma was isolated and immediately afterwards frozen at –80 °C protected from light. Plasma vitamin C concentration was determined within 5 d using the method of high performance liquid chromatography (HPLC) with UV detection.

Cell culture. Human carcinoma cell lines were obtained from the American Type Culture Collection (ATCC; www.atcc.org) and cultured according to standard mammalian tissue culture protocols and sterile technique. HCT116 (ATCC no. CCL-247), HL60 (ATCC no. CCL-240), MCF7 (ATCC no. HTB-22), SNU398 (ATCC no. CRL-2233), and HepG2 (ATCC no. HB-8065) cells were maintained in McCoy's 5A medium, Roswell Park Memorial Institute (RPMI) 1640 Medium with GlutaMAX Supplement, Eagle's Minimum Essential Medium, RPMI 1640 Medium, and Dulbecco's Modified Eagle Medium, respectively. Wild type A2780 and TET2 KO cells (established by CRISPR/Cas9 technology) were kindly provided by Dr. Stephen Baylin and were

maintained in McCoy's 5A medium. All media (from GIBCO) were supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 1% penicillin/streptomycin (GIBCO). 5-Aza-CdR and vitamin C (L-ascorbic acid) were purchased from Sigma-Aldrich.

Cell doubling time measurement. HCT116 and HL60 cells were untreated or treated with a daily dose of 57 μM vitamin C, with 300 nM 5-aza-CdR for 24 h, or with the combination of daily vitamin C at 57 μM with transient exposure of 5-aza-CdR for 24 h. For MCF7, SNU398, and HepG2 cells, 5-aza-CdR treatment was performed with three daily consecutive doses at 100 nM (72 h exposure). At subsequent time points indicated at Fig.1, cells were harvested, re-suspended, and counted using a Coulter counter analyzer (Beckman Coulter, Inc.), and $3x10^5$ cells were reseeded into fresh 100 mm culture dishes. Doubling time (in days) was calculated as follows: Doubling time = A*LOG(2)/(LOG(B) – LOG(C)), where A is the number of days since the cells were plated, B is the total number of cells when the cells were harvested, and C is the number of cells seeded.

Cell viability assay. To test for dose-dependent response, 1000 HCT116 cells were plated in each well of the 96-well plates 24 h prior to the treatment. On d 0, cells were treated or mock treated with serial 2-fold dilutions of 5-aza-CdR (from 37.5 to 4800 nM), vitamin C (from 7.1 to 912 μM), and their combinations. Cells were washed 24 h after treatment and replenished with fresh media. Daily doses of vitamin C were added to the culture media until 10 d after the treatment. Cells were then incubated with CellTiter-Glo

assay reagent (Promega) for 10 min and luminescence was measured using a Synergy HT multi-mode microplate reader (BioTek).

Evaluation of combination effect. Cell viability data were normalized to their corresponding untreated controls for each treatment condition and were expressed as percentage fractional affect (Fa). CompuSyn software (ComboSyn, Inc.) was use to calculate combination index (CI) values of Fa under different conditions using the Chou-Talalay equation: $CI = (D)_{Vc}/((D_m)_{Vc}\,(Fa/(1\text{-}Fa))^{1/m1}) + (D)_{Aza}/((D_m)_{Aza}\,(Fa/(1\text{-}Fa))^{1/m2})$, where D is the concentration of vitamin C and 5-aza-CdR either alone or in combination to achieve a given Fa (1). The median effect dose (D_m), m1 (vitamin C), and m2 (5-aza-CdR) values were determined using the median-effect equation: $(Fa)/(1\text{-}Fa) = ((D)/(D_m))^m$ (2) for vitamin C and 5-aza-CdR treatment alone. The CI values define synergistic effect when $CI < 1$, additive effect when $CI = 1$, and antagonism when $CI > 1$ (3).

Protein Extraction and Western Blot Analysis. HCT116 cells (3×10^5) were untreated or treated with 57 μM vitamin C, 300 nM 5-aza-CdR, or a combination of 57 μM vitamin C and 300 nM 5-aza-CdR for 24 h. Cells treated with higher concentration of vitamin C (570 μM) and 5-aza-CdR (3 μM) were used as positive controls. Cells were then trypsinized, washed with PBS and resuspend in RIPA buffer (50 mM Tris-HCl, ph 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) with protease inhibitors. The cells were then sonicated on ice and cellular debris was removed by centrifugation. Ten microgram of protein was mixed with SDS/β-mercaptoethanol

loading buffer and resolved on a Biorad 4-15% gradient SDS/PAGE gel. Antibodies against the γ-H2AX (Cell Signaling, #9718), H2AX (Cell Signaling, #2595), p53 (Santa Cruz Biotechnology, sc-126), and β-actin (Sigma-Aldrich, A2228) were used. For TET1 and TET2 western blots, 30 microgram of cell extract was prepared from SNU398, A2780 and TET2KO cells. Antibodies against TET1 (GeneTex, GTX124207) and TET2 (Sigma-Aldrich, HPA043135) were used. Proteins were visualized using the ECL detection system (Thermo Scientific) and ChemiDoc™ system (BioRad).

Microarray gene expression analysis. 5-Aza-CdR and combination treatment for HCT116, HL60, and SNU398 cells was performed as described for cell doubling time measurement. At day 5 after the treatment, all cells were harvested and RNA was extracted with Trizol reagent (Invitrogen), followed by clean-up and DNase I treatment with Zymo Direct-Zol RNA mini prep kit (Zymo Research) according to the manufacturer's protocol. RNA quality was assessed using Agilent 2100 bioanalyzer with RNA Nano chips (Agilent Technologies, Inc.). Expression analysis was performed at Sanford-Burnham Medical Institution (La Jolla, CA) using the Illumina genome-wide expression BeadChip (HumanHT-12_V4.0_R1) (Illumina). Gene expression data were normalized using quantile normalization method with lumi package (4), and differential expression analysis were performed using limma package (5) in R.

Pathway enrichment analysis. For each cell line, the differentially expressed genes between combination and 5-aza-CdR treatment were searched in the MsigDB v5.1 against gene sets compiled from published biomedical literature, including the Kyoto

Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg), Reactome (http://www.reactome.org), and Gene Ontology (GO, www.geneontology.org) (6, 7). Pathways with extreme numbers of genes (i.e., 10th percentile of pathway size distribution, less than 10 or more than 380) were removed from analysis to avoid stochastic bias or testing overly general biological processes.

RNA sequencing. HCT116 cells were untreated or treated with vitamin C, 5-aza-CdR, or the combination, as described for cell doubling time measurement in two replicates. RNA was prepared as described for microarray gene expression analysis. For total RNA-seq with ribosomal RNA (rRNA) reduction, libraries were prepared and sequenced as single-end 75 bases on a NextSeq 500 instrument (Illumina) at the Van Andel Research Institute Genomics Core. For directional RNA-seq with rRNA reduction and strand specificity retained, libraries were prepared and sequenced as paired-end 50 bases on a HiSeq 2500 instrument (Illumina) at HudsonAlpha Institute for Biotechnology Genomic Services Lab. The RNA-seq reads were mapped to the human transcriptome using TopHat version 2.1.0 with NCBI RefSeq as the reference annotation of transcripts (8). The transcripts were assembled and quantified using Cufflinks version 2.2.1.(9). Differential expression is measured using Cuffdiff program from the Cufflinks package (9).

Identification of bidirectionally transcribed ERVs. To quantify the transcription of repetitive element at a specific locus, we used Repeatmasker (10) (http://www.repeatmasker.org) annotation as our input and considered only uniquely mapped reads (with mapping quality threshold 10). To quantify transcription of a

repetitive element category, we combined all the repeats from the same family. Previous study showed that such approach accurately quantifies the transcription for most LTR repetitive elements (11). For each transcript, we separated reads mapped to the two strands. We considered a transcript as bidirectionally transcribed if the smaller read count divided by the greater reads count is over 0.5. We quantified the number of bidirectionally transcribed loci in each category for each repeat family to characterize whether the repeat family forms double-stranded RNA. The most upregulated transposable elements were selected based on FPKM greater than 100 in any of the treatment conditions.

J2 antibody pulldown followed by RNA-seq to identify dsRNA. After treatment, HCT116 cells were lysed with lysis buffer [30 mM HEPES-KOH at pH 7.4, 20 mM MgOAc, 5 mM DTT, 150 mM KOAc, 0.1% NP-40, and protease inhibitors (Roche)]. The cell lysates were next used for dsRNA pull-down with 3 μg of J2 antibody (Scicons) on Dynabeads Protein G (Invitrogen) for 2 h at 4 °C. The beads were washed with wash buffer [30 mM HEPES-KOH at pH 7.4, 20 mM MgOAc, 5 mM DTT, 150 mM KOAc, 0.1% NP-40, and protease inhibitors (Roche)] and digested again with RNase T1 (Roche) and TURBO DNase (Thermo Fisher) to further eliminate single-stranded RNA and DNA. dsRNA was purified and fragmented into 1200 bp by sonication using a Covaris E220 focused ultrasonicator (Covaris Inc.). Sequencing libraries were prepared using NEBNext multiplex small RNA library prep Kit (New England BioLabs Inc.) and sequenced as single-end 75 bases on a NextSeq 500 instrument (Illumina) at the Van Andel Research

Institute Genomics Core. Analysis of repeated elements were performed as described above.

Poly(I:C) transfection. HCT116 cells (5×10^5) were seeded in a 6-well plate and transiently transfected with 1 μg of poly(I:C) (Invitrogen) or mock-transfected using Lipofectamine LTX with Plus reagents (Invitrogen) according to the manufacturer's instructions. After 24 h, the medium was replaced and cells were maintained in fresh medium until the fourth day after transfection, when total RNA was extracted using Trizol reagent (Invitrogen). The expression of TBP and viral defense genes were measured by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) using KAPA SYBR FAST universal 2× qPCR master mix (KAPA) with primers listed in the Table S2.

Illumina Infinium HM450 DNA methylation assay. Genomic DNA from HCT116 cells was extracted using phenol–chloroform and ethanol precipitation after the treatment, and bisulfite treatment was performed using EZ DNA methylation kit (Zymo Research). The Infinium HM450 DNA methylation assay was performed at the University of Southern California Epigenome Center according to the manufacturer's specifications (Illumina). The DNA methylation level for each interrogated CpG site was reported as a beta value, ranging from 0 (not methylated) to 1 (fully methylated). Downstream processing and beta value calculations were performed as previously described (12).

Antioxidant and dehydroascorbic acid treatment. HCT116 (3 × 105) cells were seeded, untreated or treated with daily doses of vitamin C or L-dehydroascorbic acid (DHA, Sigma-Aldrich, 261556) at 57 μM, daily doses of L-Glutathione reduced (GSH, Sigma-Aldrich, G6529) at 4.9 mM or dithiothreitol (DTT, Sigma-Aldrich, 43815) at 500 μM. Combinations with 5-aza-CdR treatment were exposed to 300 nM 5-aza-CdR for 24 h together with daily doses of vitamin C, DHA, GSH and DTT indicated above. At day 5 after the treatment, cells were harvested, and genomic DNA and RNA were extracted for Dot blot analysis or qRT-PCR analysis with primers listed in the Table S2.

Dot blot analysis of 5hmC. HCT116, HL60, MCF7, SNU398, HepG2, A2780 and TET2 KO cells were untreated or treated with vitamin C, 5-aza-CdR, or the combination as described for cell doubling time measurement. Cells were harvested at day 5 after the treatment and genomic DNA was extracted using phenol–chloroform and ethanol precipitation. 5hmC dot blot analysis was performed as described previously (13). Briefly, 1 μg of genomic DNA was denatured, spotted on a nitrocellulose membrane, which was then baked, blocked, and incubated with 5hmC antibody (1:10,000, Active Motif, A39769) and secondary antibodies. The same blot was stained with 0.02% methylene blue in 0.3 M sodium acetate (pH 5.3) as DNA loading controls.

Bisulfite sequencing. On day 5 after treatment, HCT116 cells were harvested and genomic DNA was purified by phenol–chloroform extraction and ethanol precipitation. PCRs were performed using bisulfite-converted DNA with primers listed in Table S2,

and the products were cloned using the TOPO TA Cloning Kit (Invitrogen). Individual colonies were sequenced and analyzed as previously described (12).

Chemical pulldown of 5hmC. On days 1, 3, and 5 after treatment, HCT116 cells were harvested and genomic DNA was purified by phenol–chloroform extraction and ethanol precipitation. Chemical capture of 5hmC were performed using Hydroxymethyl Collector-seq Kit (Active Motif, # 55013) according to the manufacturers' protocol with minor modifications. Genomic DNA was sonicated to a mean size of 300 bp, and 500 pg of "spiked" 5hmC fragments (supplied in the Hydroxymethyl Collector-seq Kit) was added per 10 μg of genomic DNA. After setting aside 100 ng DNA for input, 10 μg of fragmented DNA containing the spike-in 5hmC control was then glucosylated through incubation with modified dUTP containing an azide glucose group with 100 units of T4 phage β-glucosyltransferase enzyme (New England BioLabs Inc. Cat# M0357L) at 37 °C overnight. Following glucosylation, the modified glucose group was then biotinylated through incorporation of biotin conjugation solution for 4 h further at 37 °C. Fragments of DNA containing modified biotin-azide-glucose-5hmC were then purified through binding to magnetic streptavidin beads following five washes with a wash buffer and a final wash in elution buffer for 30 min at room temperature. The enriched DNA was then eluted in elution buffer and passed through purification columns prior to qPCR analysis using primers listed in Table S2, or used for library preparation.

5hmC-seq and data analysis. Sequencing libraries were prepared using TruSeq ChIP Library Prep Kit (Illumina) and sequenced as single-end 75 bases on a NextSeq 500

instrument (Illumina) at the Van Andel Research Institute Genomics Core. We used bedtools (14) to count numbers of reads mapped to ERVs annotated in the Repeatmasker (10) as assayed in RNA-seq experiment. We selected the top 100 ERVs upregulated in combination treatment compared to untreated cells. To compare the expression levels of these ERVs across the treated samples, we used the natural logarithm (ln) for the read counts of each ERV loci and plotted in a heatmap shown in the top panel of Fig. 4B. To compare 5hmC levels on these ERVs after treatment, we studied each ERV loci and its +/- 3kb flanking region as shown in the lower panel of Fig. 4B. We first evenly sampled 100 positions inside each ERV loci and within +/- 3kb of its flanking regions, and counted the number of bases from 50bp bins using the raw read counts from the 5hmC-seq experiments. All the base counts were then normalized by the read counts of the spike-in control. Next, the normalized base counts were averaged across all the upregulated ERVs as the average 5hmC level, and plotted against the scaled genomic loci as shown in the lower panel of Fig. 4B.

Statistical analysis. Results were expressed as means ± standard deviation (SD) for quantitative variables. Statistical analyses were performed using two-tailed Student's *t*-test or paired Student's *t*-test for comparing the means of two groups as specified in Figure Legends. For expression microarray and RNA-seq data, the Pearson product-moment correlation coefficient of $\log_2$ fold change between two biological replicates and p-values were calculated using R program.

Accession codes and reviewer access links. All data have been deposited at the Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/) with accession codes GSE77031 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=elelmqsqrhijfyl&acc=GSE77031) for dsRNA sequencing data, GSE77032 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=ejipkqegnrkttyr&acc=GSE77032) for directional total RNA sequencing data, GSE77034 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=mbadogggndkjzmh&acc=GSE77034) for total RNA sequencing data, GSE77035 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=mbonkewobzilnmb&acc=GSE77035) for gene expression microarray data, and GSE77036 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=ibsfckkkxbgzlkt&acc=GSE77036) for HM450 DNA methylation data.

SI References

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SI Figure Legends

Fig. S1 The combination of 5-aza-CdR plus low doses of vitamin C act synergistically to inhibit cell growth. (A) Dosing schedules for the combination treatment. (B) Dose-dependent inhibition of cell growth by 5-aza-CdR plus vitamin C combination treatment. HCT116 cells were treated with one dose of 5-aza-CdR for 24 h and daily doses of vitamin C at various concentrations. Inhibition of cell growth was measured by CellTiter-Glo luminescent cell viability assay at day 10 after the treatment. Values are mean ± SD of three independent experiments. (C) Chou-Talalay model of the effects of combination treatment in HCT116 cells. Combination index (CI) across the fraction affected (Fa) values were calculated by CompuSyn software (ComboSyn, Inc.). CI < 1, CI = 1, and CI >1 indicate synergism, additive effect, and antagonism, respectively. Values are mean ± SD of three independent experiments. (D) Bar graph comparing the additive effects of vitamin C and 5-aza-CdR to the combinatorial effects in causing apoptosis of cancer cells (related to data in Fig. 1B). The additive effects of vitamin C and 5-aza-CdR treatment alone are shown as orange bars, and the effects of the combination treatment as determined by the apoptosis assay are shown as purple bars. Since the combinatorial effects are greater than the additive effects of vitamin C and 5-aza-CdR treatment alone, the two compounds exhibit synergistic effects in causing cellular apoptosis.

Fig. S2 Combination treatment with physiological levels of vitamin C and a low dose of 5-Aza-CdR did not increase DNA damage response. Western blot analysis on the levels of phosphorylated histone H2A.X (γ-H2AX), H2AX, p53 and β-Actin in HCT116 cells. Cells were either untreated (PBS) or treated with 57 μM vitamin C, 300 nM 5-aza-CdR,

combination of 57 μM vitamin C and 300 nM 5-aza-CdR, 570 μM vitamin C, or 3 μM 5-aza-CdR for 24 h.

Fig. S3 Combination treatment upregulated genes responsive to interferon alpha and gamma compared to 5-aza-CdR treatment alone. Scatter plots (left) show correlation of gene expression in two replicates of microarray data for HCT116 (panel A), HL60 (panel B) and SNU398 (panel C) cells. HCT116 and HL60 cells were treated with one dose of 300 nM 5-aza-CdR for 24 h; whereas SNU398 cells were treated with three consecutive daily doses of 5-aza-CdR at 100 nM, with drug withdrawal at 72 h. Five days after the treatment, RNA from the cells were collected and the transcript levels were assayed using microarray. Axes represent $\log_2$ fold change values comparing combination treatment to 5-aza-CdR treatment alone. Genes responsive to interferon alpha and gamma are represented by red dots. Pearson correlation and p-values at the lower right corner show a substantial degree of correlation in two replicates for these genes in all cell lines. The rest of the genes are shown as gray dots. Pink shade highlights the genes commonly upregulated in both replicates, whereas blue shade highlights genes commonly downregulated. Pathway enrichment analysis for genes commonly upregulated are shown to the right of the plot.

Fig. S4 Scatter plots comparing two replicates of RNA sequencing data 5 days after the treatments in HCT116 cells. Axes represent $\log_2$ fold change values of the conditions listed above the plots. Genes responsive to interferon alpha and gamma are represented by red dots and the rest of the genes are shown as gray dots. Pink shade highlights the

genes commonly upregulated in both replicates, whereas blue shade highlights genes commonly downregulated.

Fig. S5 Expression of (A) house keeping genes, (B) cytosolic sensors for DNA and toll-like receptors, and (C) DNMTs and TETs after vitamin C, 5-aza-CdR and combination treatment compared to untreated HCT116 cells. Values are calculated from fragments per kilobase per million and averaged from two independent RNA sequencing data sets.

Fig. S6 Total sequencing reads mapped to (A) all repetitive elements, and (B) ERV families after vitamin C, 5-aza-CdR and combination treatment compared to untreated HCT116 cells. Percentage of transcripts relative to total reads were quantified by Repeat Masker from two replicates of the RNA sequencing data. Replicate 1 represents the total RNA-seq with ribosomal RNA (rRNA) reduction without information on transcript direction. Replicate 2 represents the directional RNA-seq with rRNA reduction with strand specificity retained. (C) Screen shots of IGV sequence alignments from directional RNA-seq experiment for reads uniquely mapped to HERV-Fc1 and LTR12C loci after treatment, showing unidirectional and bidirectional transcription.

Fig. S7 Log2 fold change of dsRNA defense gene expression in HCT116 cells after poly(I:C) transfection compared to mock transfected cells by qRT-PCR. Values are mean ± SD of three independent experiments.

Fig. S8 Expression of (A) TET1, (B) TET2, and (C) TET3 transcripts in HCT116, HL60, MCF7, SNU398 and HepG2 cells at day 5 after untreated (PBS) or treated with vitamin C, 5-aza-CdR and combination relative to TBP levels by qRT-PCR. Values are mean ± SD of three independent experiments. (D) Western blot analysis on the levels of TET1 protein in SNU398 cells at day 5 after treatment with vitamin C, 5-aza-CdR and combinations. Loading controls of β-Actin are also shown.

Fig. S9 Cytosine modification status in HCT116 cells after treatments. (A) Density plots of HM450 DNA methylation data in HCT116 cells untreated (PBS) or treated with vitamin C, 5-aza-CdR and combination across all CpG probes. The x-axis represents beta values ranging from 0 (not methylated) to 1 (highly methylated). (B) Bisulfite sequencing of the 5′ LTR of HERV-Fc1 at day 5 after treatment. Black circles represent methylated or hydroxymethylated CpG sites; white circles represent unmethylated sites. The percentage of unmethylated CpG sites is shown to the right of each plot.

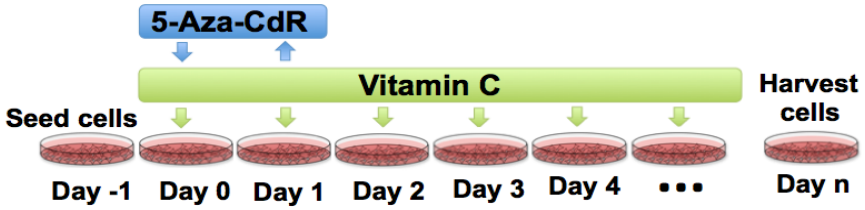
Fig. S10 Screen shots of IGV sequence alignments for 5hmC-seq and RNA-seq data at HERV-Fc1 and LTR12C loci in HCT116 cells after treatment. The 5hmC-seq data were normalized to the "spike-in" control (IC) shown to the left.

Fig. S11 Levels of TETs in A2780 and TET2 KO cells. (A) Expression of TET1, TET2 and TET3 transcripts in A2780 and TET2 KO cells at day 5 after untreated (PBS) or treated with vitamin C, 5-aza-CdR and combination relative to the TATA-binding protein (TBP) levels by qRT-PCR. Values are mean ± SD of three independent experiments. (B)

Western blot analysis on the levels of TET1 and TET2 proteins in A2780 and TET2 KO cells. Loading controls of β-Actin are also shown.

Figure S1

A



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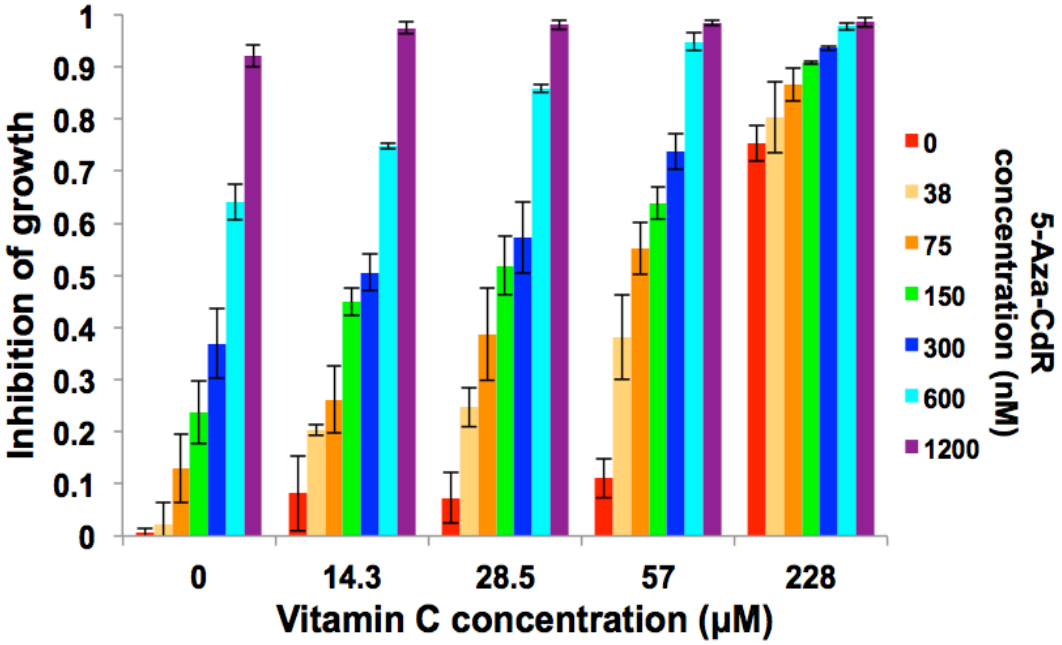

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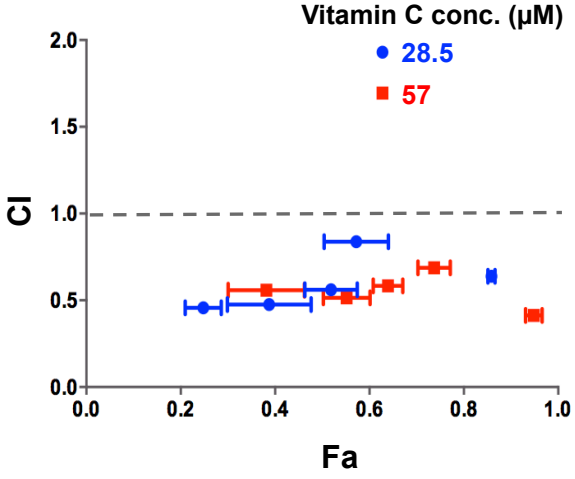

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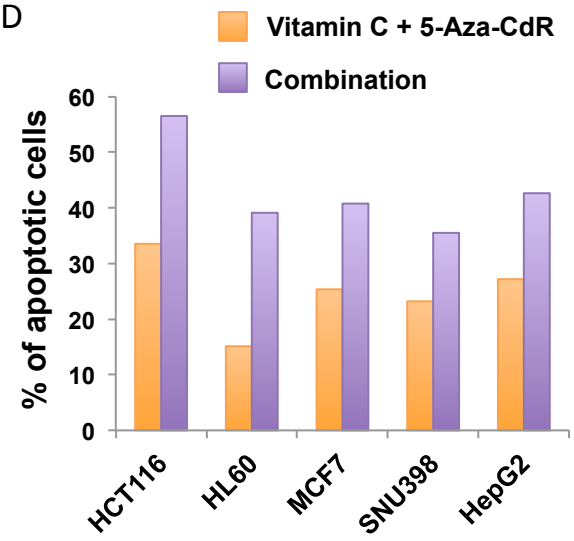
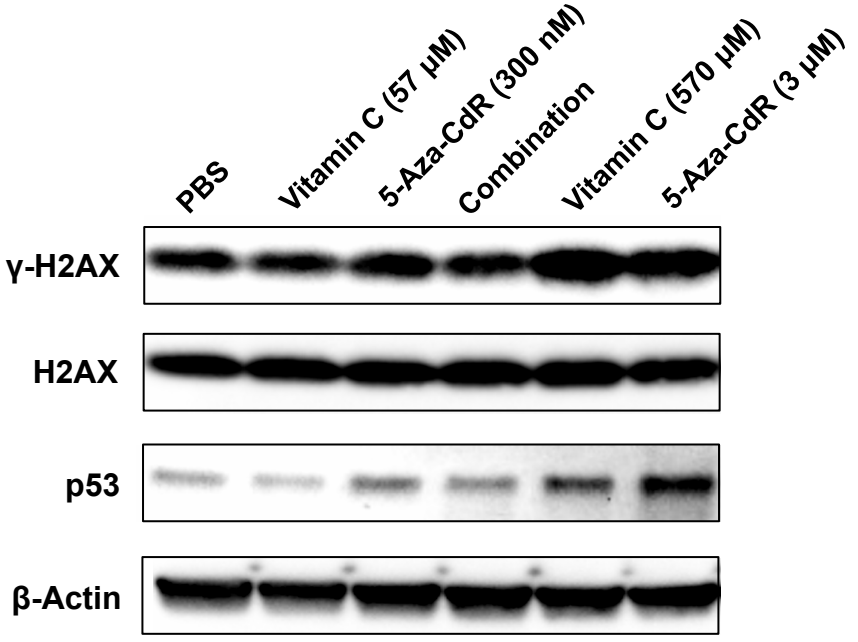


Figure S2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5-Aza-CdR (300 nM)
Combination
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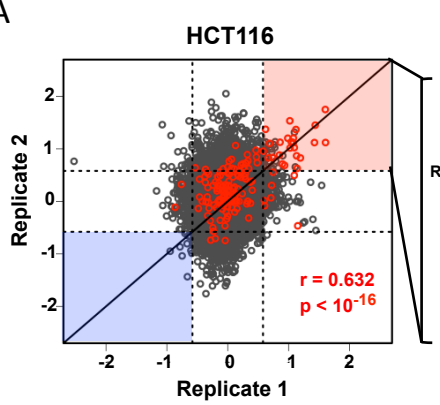
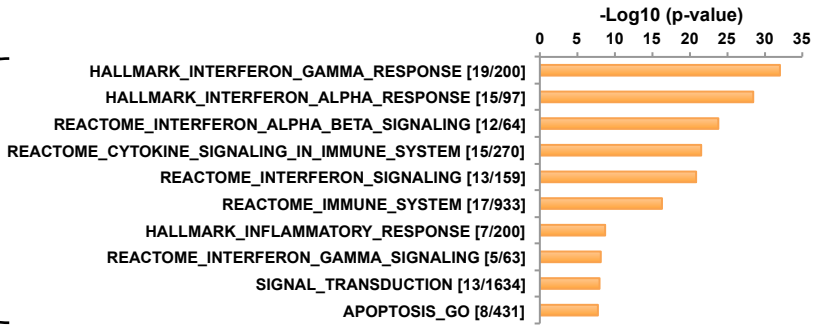
5-Aza-CdR (3 μM)
γ-H2AX
H2AX
p53
β-Actin

Figure S3

A





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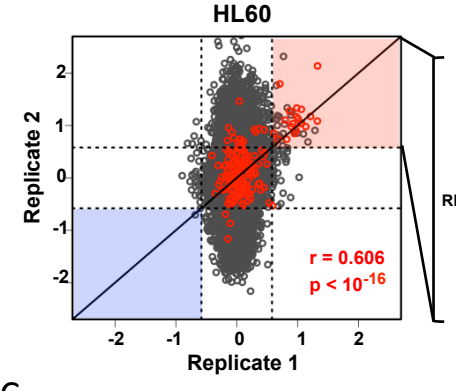

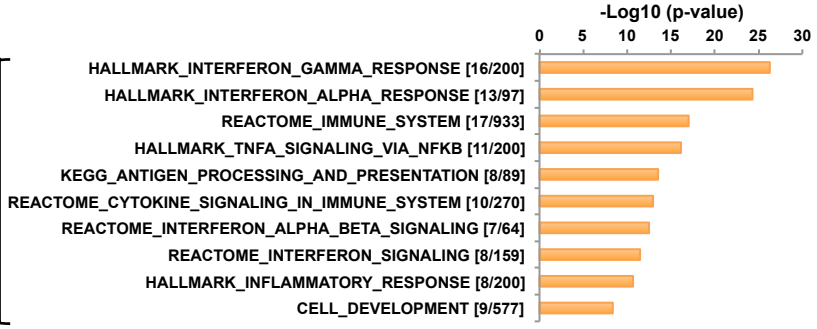

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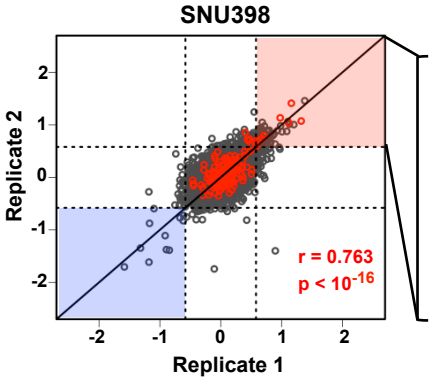


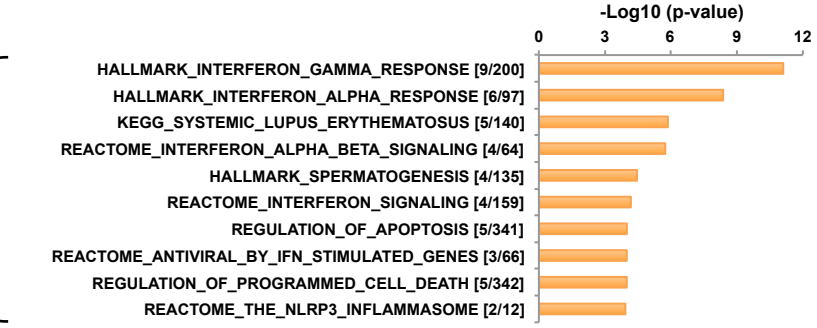


Figure S4

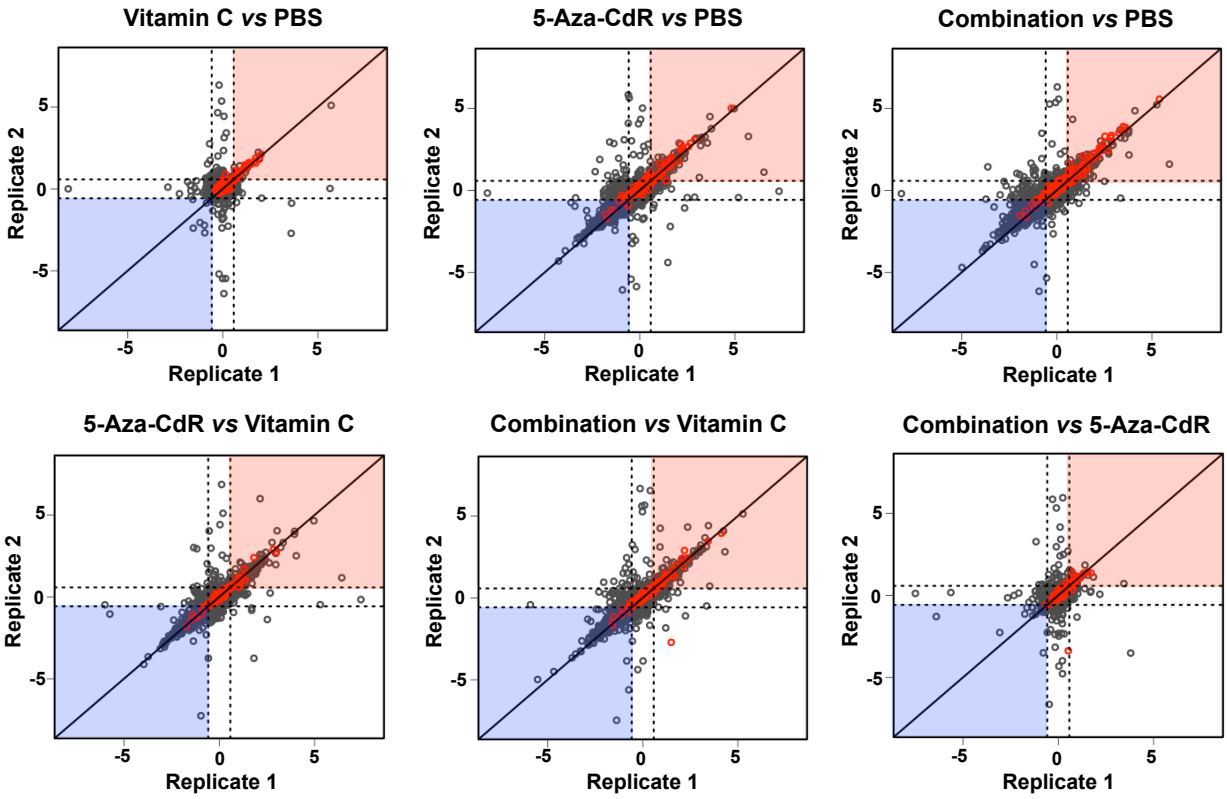

Vitamin C vs PBS
5-Aza-CdR vs PBS
Combination vs PBS
5-Aza-CdR vs Vitamin C
Combination vs Vitamin C
Combination vs 5-Aza-CdR
Replicate 1
Replicate 2
5
0
-5

Figure S5

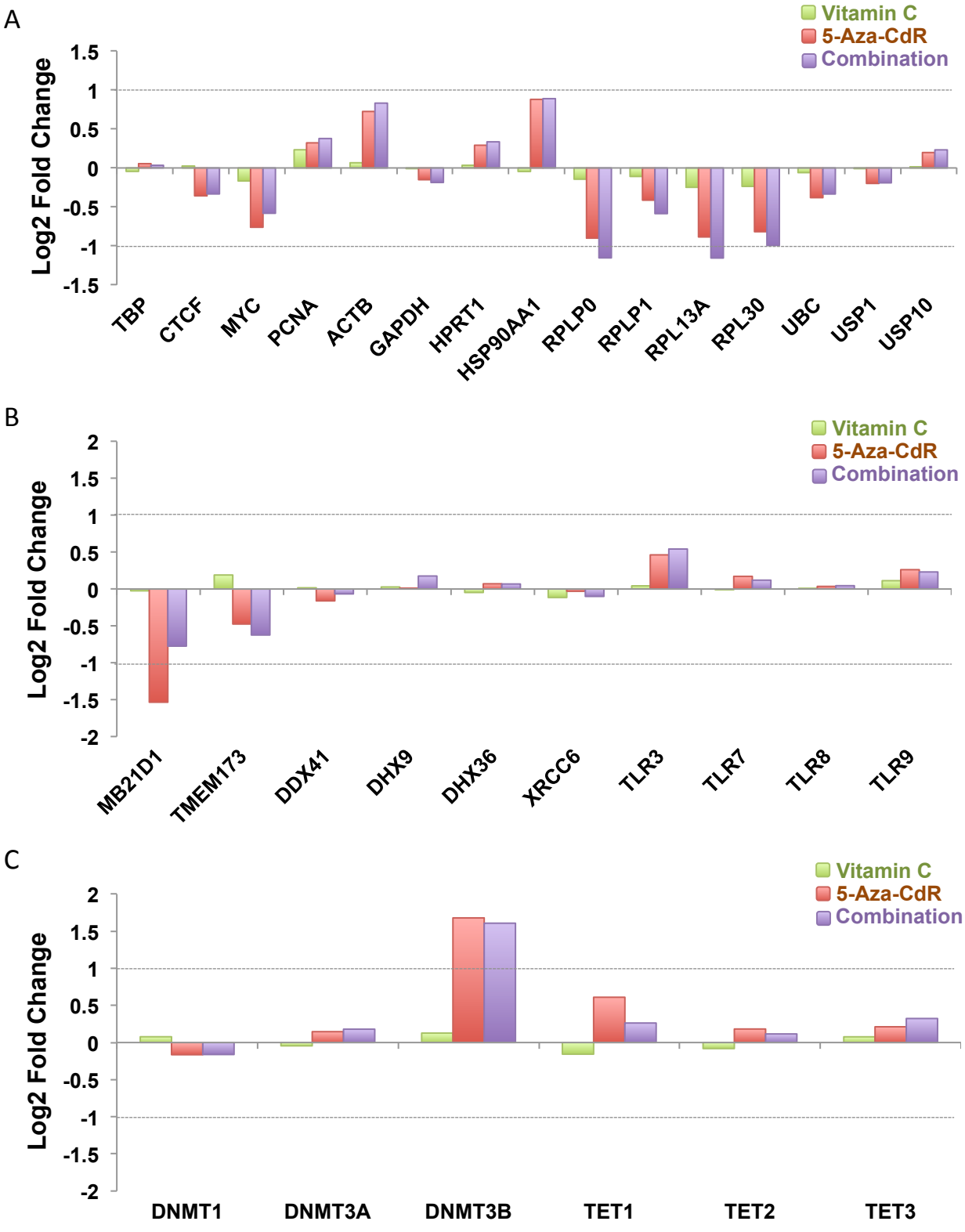

A
Vitamin C
5-Aza-CdR
Combination
Log2 Fold Change
1.5
1
0.5
0
-0.5
-1
-1.5
TBP
CTCF
MYC
PCNA
ACTB
GAPDH
HPRT1
HSP90AA1
RPLP0
RPLP1
RPL13A
RPL30
UBC
USP1
USP10
B
Vitamin C
5-Aza-CdR
Combination
Log2 Fold Change
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
MB21D1
TMEM173
DDX41
DHX9
DHX36
XRCC6
TLR3
TLR7
TLR8
TLR9
C
Vitamin C
5-Aza-CdR
Combination
Log2 Fold Change
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
DNMT1
DNMT3A
DNMT3B
TET1
TET2
TET3

Figure S6

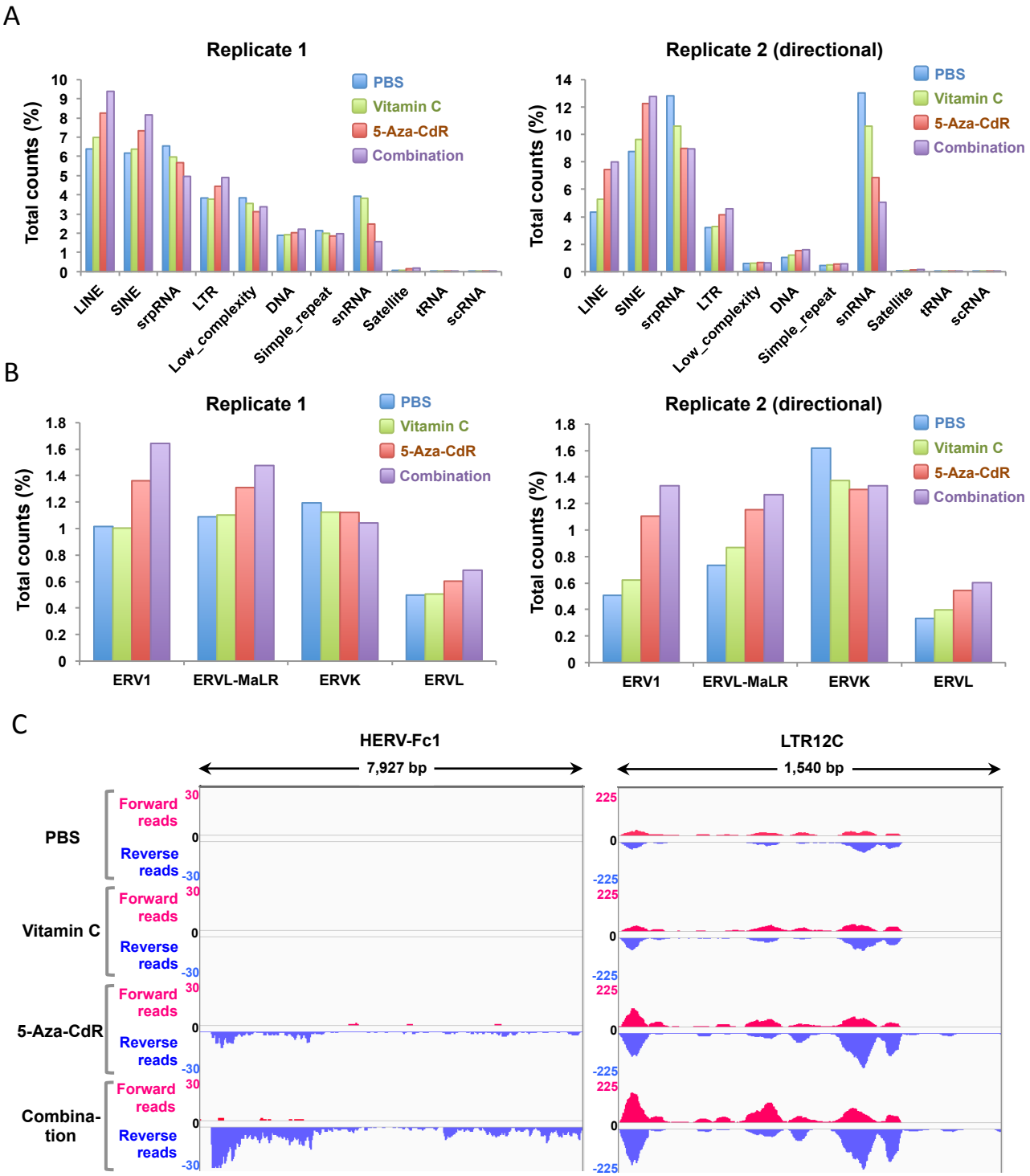

A
Replicate 1
Total counts (%)
PBS
Vitamin C
5-Aza-CdR
Combination
LINE
SINE
srpRNA
LTR
Low_complexity
DNA
Simple_repeat
snRNA
Satellite
tRNA
scRNA
Replicate 2 (directional)
B
ERV1
ERVL-MaLR
ERVK
ERVL
C
HERV-Fc1
7,927 bp
LTR12C
1,540 bp
Forward reads
Reverse reads

Figure S7

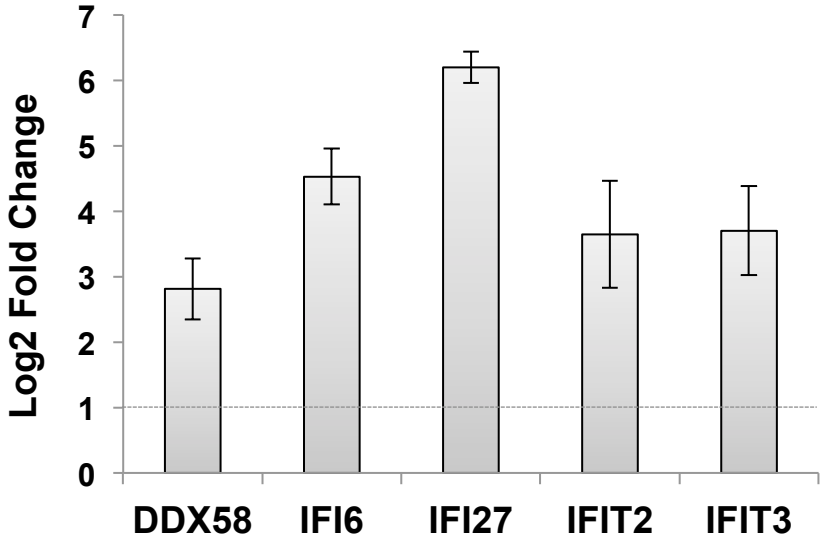


Figure S8

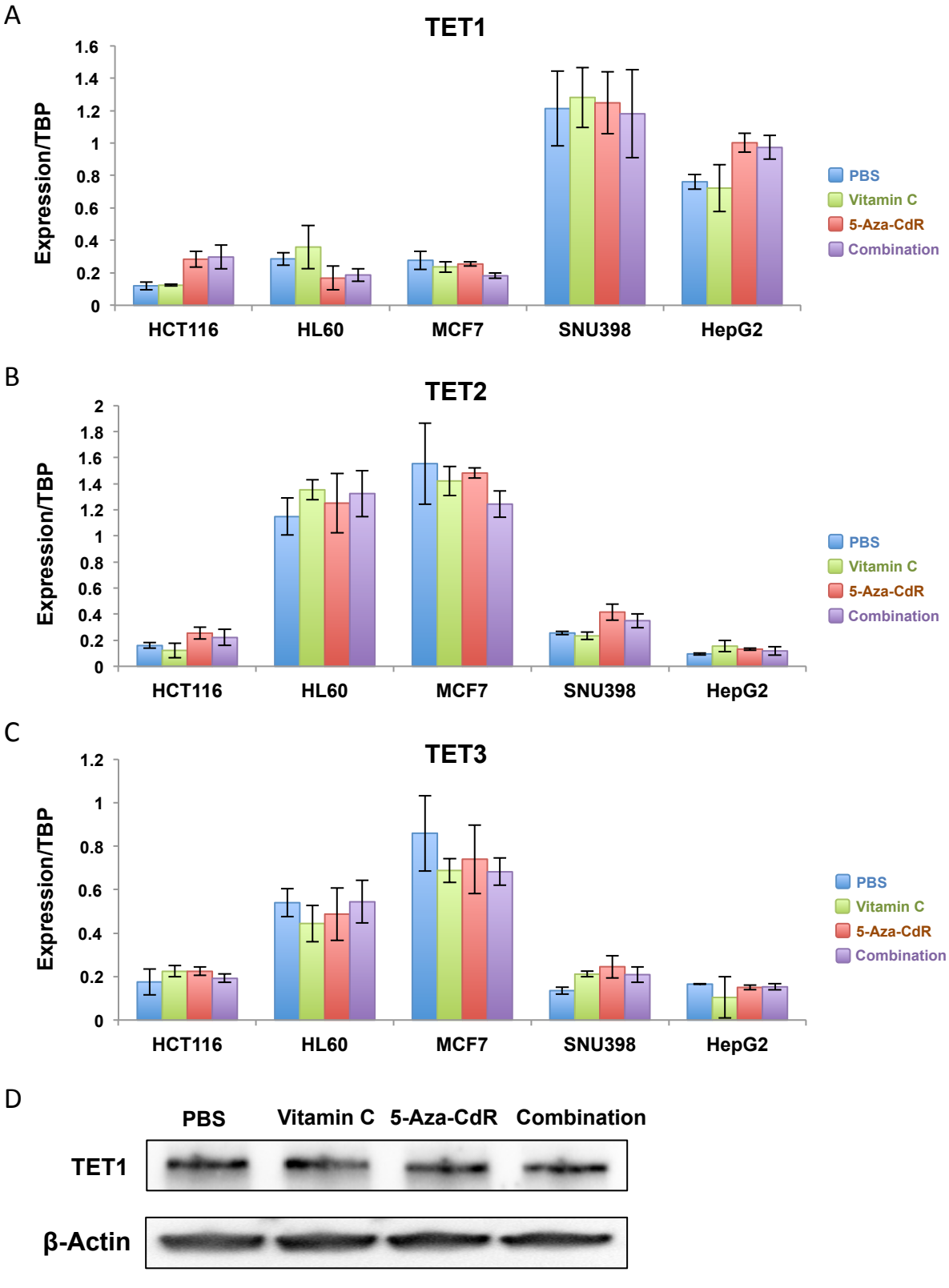
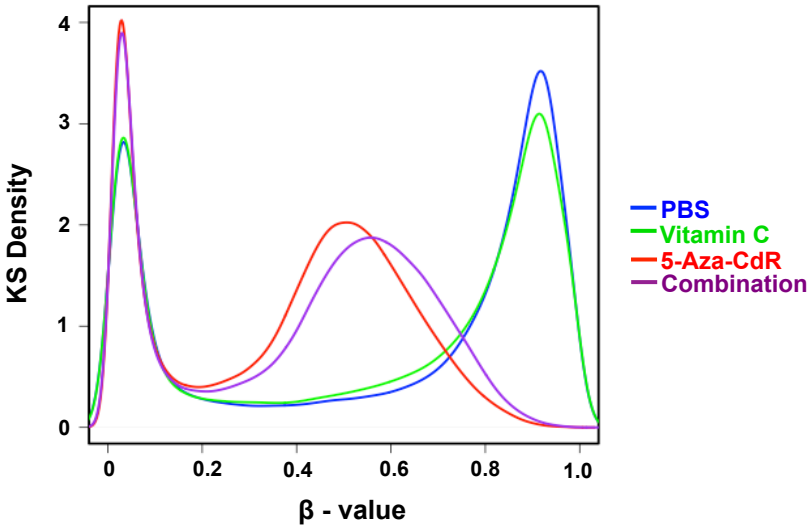

A
TET1
Expression/TBP
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
HCT116
HL60
MCF7
SNU398
HepG2
PBS
Vitamin C
5-Aza-CdR
Combination
B
TET2
Expression/TBP
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
HCT116
HL60
MCF7
SNU398
HepG2
PBS
Vitamin C
5-Aza-CdR
Combination
C
TET3
Expression/TBP
1.2
1
0.8
0.6
0.4
0.2
0
HCT116
HL60
MCF7
SNU398
HepG2
PBS
Vitamin C
5-Aza-CdR
Combination
D
PBS
Vitamin C
5-Aza-CdR
Combination
TET1
β-Actin

Figure S9

A


KS Density
β - value
PBS
Vitamin C
5-Aza-CdR
Combination

B

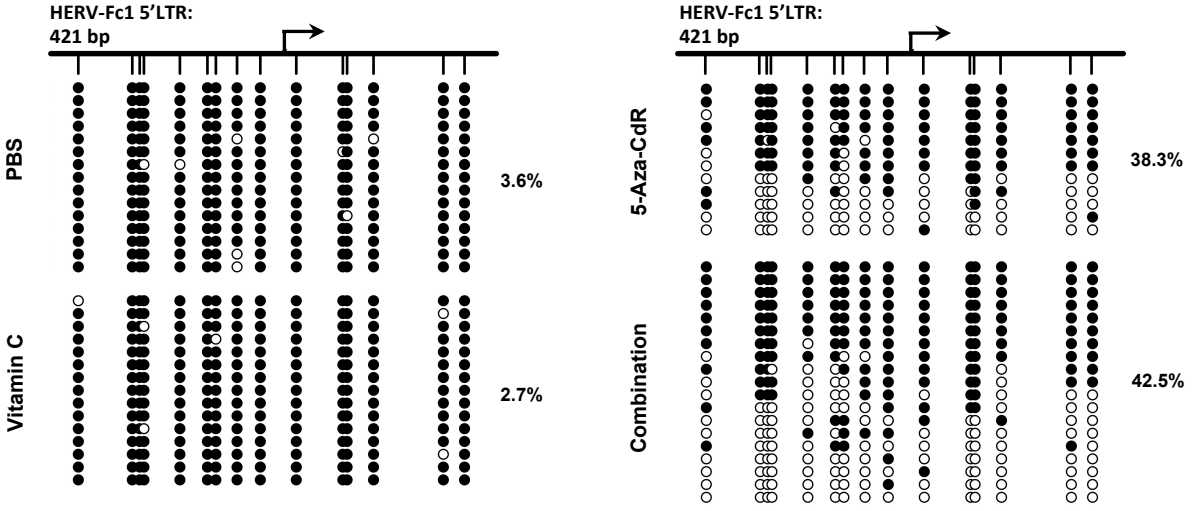

HERV-Fc1 5'LTR:
421 bp
PBS
3.6%
Vitamin C
2.7%
HERV-Fc1 5'LTR:
421 bp
5-Aza-CdR
38.3%
Combination
42.5%

Figure S10

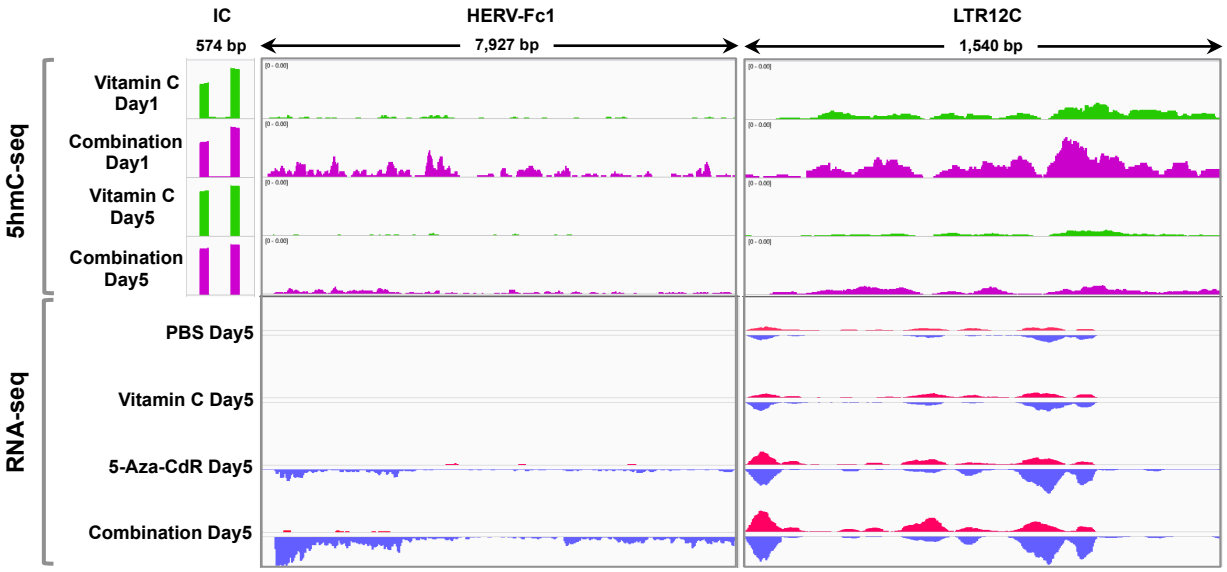

IC
574 bp
HERV-Fc1
7,927 bp
LTR12C
1,540 bp
5hmC-seq
Vitamin C Day1
Combination Day1
Vitamin C Day5
Combination Day5
RNA-seq
PBS Day5
Vitamin C Day5
5-Aza-CdR Day5
Combination Day5

Figure S11

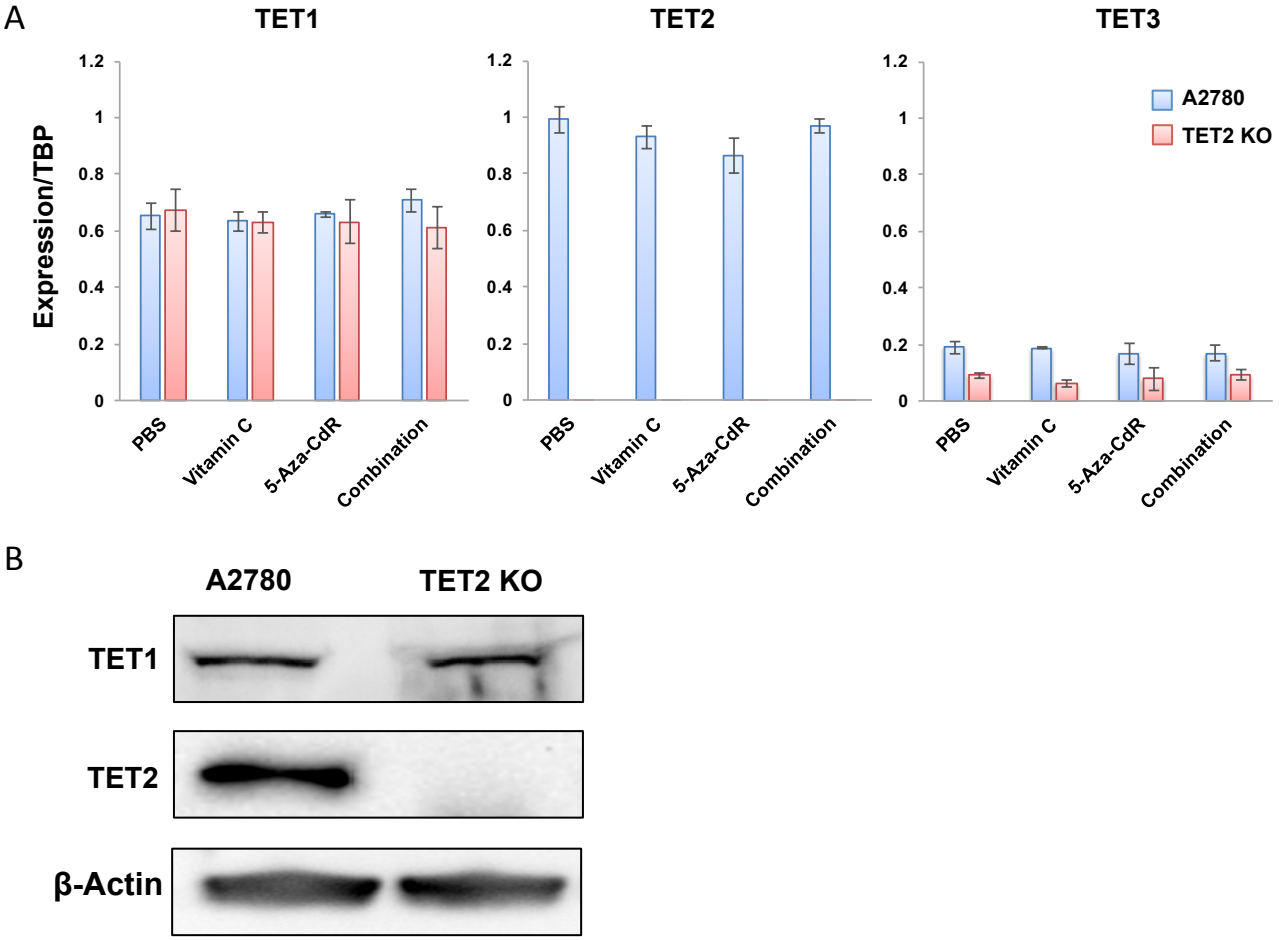

A
TET1
TET2
TET3
Expression/TBP
1.2
1
0.8
0.6
0.4
0.2
0
PBS
Vitamin C
5-Aza-CdR
Combination
A2780
TET2 KO
B
A2780
TET2 KO
TET1
TET2
β-Actin

Table S1. Plasma vitamin C concentrations in patients with hematopoietic malignancies.

Patient number	Gender	Age (years)	Hematological disease	Treatment	Disease status	Plasma vitamin C level (µmol/L)
1	M	63	MCL	Y	First line	4.4
2	M	88	CLL	Y	First line	5.1
3	M	64	DLBCL	Y	Relapse treatment	<3.0
4	M	69	Primary CNS lymphoma	Y	First line	4.0
5	M	67	AML	Y	Refractory AML with neutropenic fever	<3.0
6	M	70	DLBCL	Y	Relapse treatment	<3.0
7	M	45	AML	N	Newly diagnosed before treatment	8.7
8	M	21	ALL	Y	First line, ALL in CR. Consolidation	9.5
9	F	80	MDS	N	RCMD, untreated	9.1
10	M	71	Hairy cell leukemia	N	CR 3 years after treatment	13.3
11	M	52	AML	Y	Relapse treatment	12.5
12	M	76	AML	Y	Secondary AML,cytarabin after 5-aza-CR	<3.0
13	F	47	ALL	Y	First line	35.8
14	F	41	ALL	N	Newly diagnosed before treatment	4.6
15	M	70	MM	Y	Relapse treatment	13.8
16	M	31	ALL	Y	MRD after first line	13.5
17	M	65	MM	Y	Relapse treatment	21.3
18	M	59	DLBCL	Y	First line	9.0
19	F	73	MDS	Y	5-Aza-CR, day 5 in cycle 18	17.7
20	M	69	MDS	Y	5-Aza-CR, day 5 in cycle 8	7.6
21	M	53	MDS (sec. to AML)	Y	5-Aza-CR, day 5 in cycle 3	25.2
22	F	61	AML	Y	5-Aza-CR, relapse after ALLO-HCT	37.6
23	M	79	MDS	Y	5-Aza-CR, day 5 in cycle 4	12.7
24	F	75	MDS	Y	5-Aza-CR, day 5 in cycle 19	3.1
25	*M*	*84*	*MDS*	*N*	*RCMD*	*47.5*
26	*M*	*60*	*AML*	*N*	*5-aza-CR, relapse after ALLO-HCT*	*45.6*

Abbreviations: ALL=acute lymphoblastic leukemia, AML=acute myeloid leukemia, CLL=chronic lymphocytic leukemia, DLBCL=diffuse large B-cell lymphoma, MCL=mantle cell lymphoma, MDS=myelodysplastic syndrome, MM=multiple myeloma. CR=complete remission, RCMD=refractory cytopenia with multi lineage dysplasia, MRD=minimal residual disease, Allo-HCT: allogeneic hematopoietic stem cell support, sec. = secondary. Patients 1-24 take no vitamin C supplement while patients 25 and 26 takes oral vitamin C daily (physiological dose).

Table S2 Primers used in this study.

Primers for qRT-PCR:	
TBP-Fwd	5’-GCCCGAAACGCCGAATAT-3’
TBP-Rev	5’-CCGTGGTTCGTGGCTCTCT-3’
DDX58-Fwd	5’-CCTTCAGACATGGGACGAAG-3’
DDX58-Rev	5’-TGGCTTGGGATGTGGTCTAC-3’
IFI6-Fwd	5’-CTCGGAGAGCTCGGACAG-3’
IFI6-Rev	5’-TACCTATGACGACGCTGCTG-3’
IFI27-Fwd	5’-TCTGGCTCTGCCGTAGTTTT-3’
IFI27-Rev	5’-GAACTTGGTCAATCCGGAGA-3’
IFIT2-Fwd	5’-GTGCAAGGACTTGGGAAATG-3’
IFIT2-Rev	5’-CCAGAGTGTGGCTGATGCT-3’
IFIT3-Fwd	5’-CTGCAAGCAGCCAAATGTTA-3’
IFIT3-Rev	5’-ATCGTCCTACCCGTCACAAC-3’
TET1-Fwd	5’-CCGACCGGCTATACACAGAG-3’
TET1-Rev	5’-TCTCTGGATCAATTCCTTGACA-3’
TET2-Fwd	5’-GGACATGATCCAGGAAGAGC-3’
TET2-Rev	5’-CCCTCAACATGGTTGGTTCT-3’
TET3-Fwd	5’-GGCCTCAACGATGACCGGA-3’
TET3-Rev	5’-AACTTGCGAGGTGTCTTGCT-3’
HERV-Fc1-Fwd	5’-TTTCCCACCGCTGGTAATAG-3’
HERV-Fc1-Rev	5’-AGGCTAAGGATTCGGCTGAG-3’
HERVW1-Fwd	5’-AAGAATCCCTAAGCCTAGCTGG-3’
HERVW1-Rev	5’-GCCTAATTAGCATTTTAGTGAGCTC-3’
LTR12C-Fwd	5’-AGCTAGACATAAAGGTCCTCCACG-3’
LTR12C-Rev	5’-TGGGGCCTTGGAGAACTTTTATG-3’
Primers for bisulfite sequencing:	
HERV-Fc1-Bi-Fwd	5’-TTGTTAGGTAGGTTATTTAAGATGG-3’
HERV-Fc1-Bi-Rev	5’-CTCCCCGACCCTAAATCAAAAA-3’
Primers for 5hmC pulldown-qPCR:	
ACTB-p-Fwd	5’-GAGGGGAGAGGGGGTAAAA-3’
ACTB-p-Rev	5’-TCGAGCCATAAAAGGCAACT-3’
HERV-Fc1-LTRa-Fwd	5’-CCTATCCCAGAAAACCGAAAC-3’
HERV-Fc1-LTRa-Rev	5’-GCGAGGTTCACTGGTCCTAA-3’
HERV-Fc1-LTRb-Fwd	5’-TTAGGCAGGTCACCCAAGAT-3’
HERV-Fc1-LTRb-Fwd	5’-CAGGAAAATCTGGTGGGAGA-3’
HERVW1-LTR-Fwd	5’-TGCAACTGCACTCTTCTGGT-3’
HERVW1-LTR-Rev	5’-GATCCAGAGGGATGGGAGTC-3’