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Supplemental information

HDAC inhibitors improve CRISPR-Cas9 mediated prime editing and base editing

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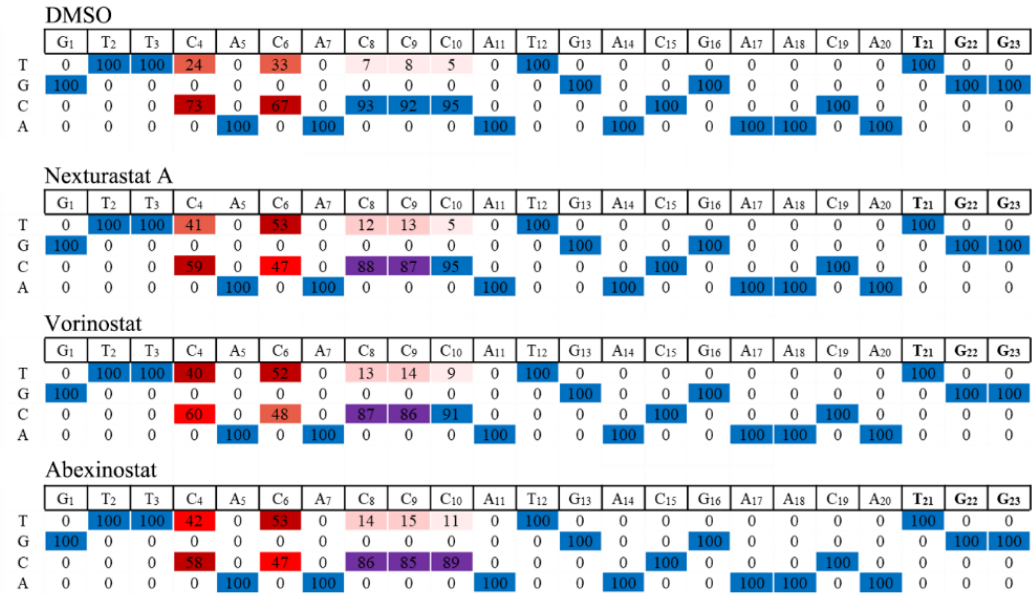

Figure S1. Representative Sanger sequencing results and EditR analysis of site 29 in figure 3a.

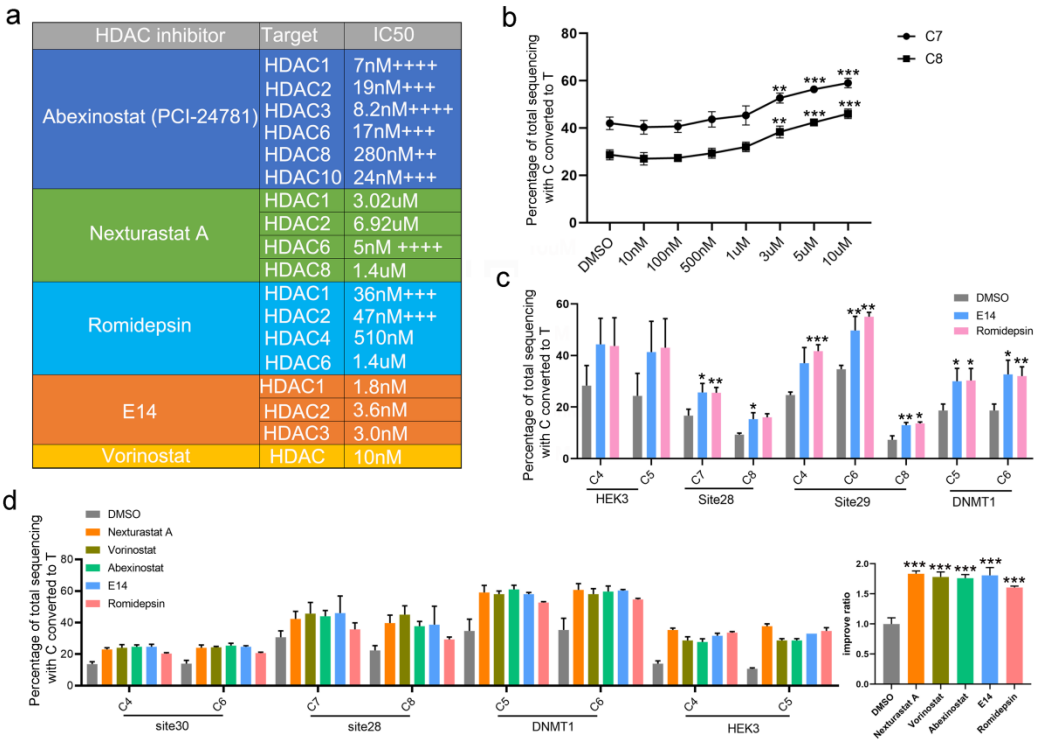

Figure S2. Detailed analysis of the effect of HDAC inhibitors on cytosine base editing. a. Summarization of the IC50 HDAC profile of inhibitors used in this study. The value of each IC50 was obtained from cell-free assay. **b.** Dose dependent effects of Nexturastat A on cytosine base editing. Nexturastat A can only improve the base editing of BE3 when its concentration exceeded 3μM. **c.** Effects of Romidepsin and E14 on cytosine base editing at 4 endogenous sites. Note that Romidepsin and E14 shared two common targets, HDAC1 and HDAC2. **d.** The comparison of improvement of 5 HDAC inhibitors on cytosine base editing at 4 endogenous sites. Data are represented as mean ± SD; Asterisks indicate statistically significant differences between DMSO-treated cells and HDAC inhibitors-treated cells. (*p < 0.05, **p < 0.01, ***p < 0.001).

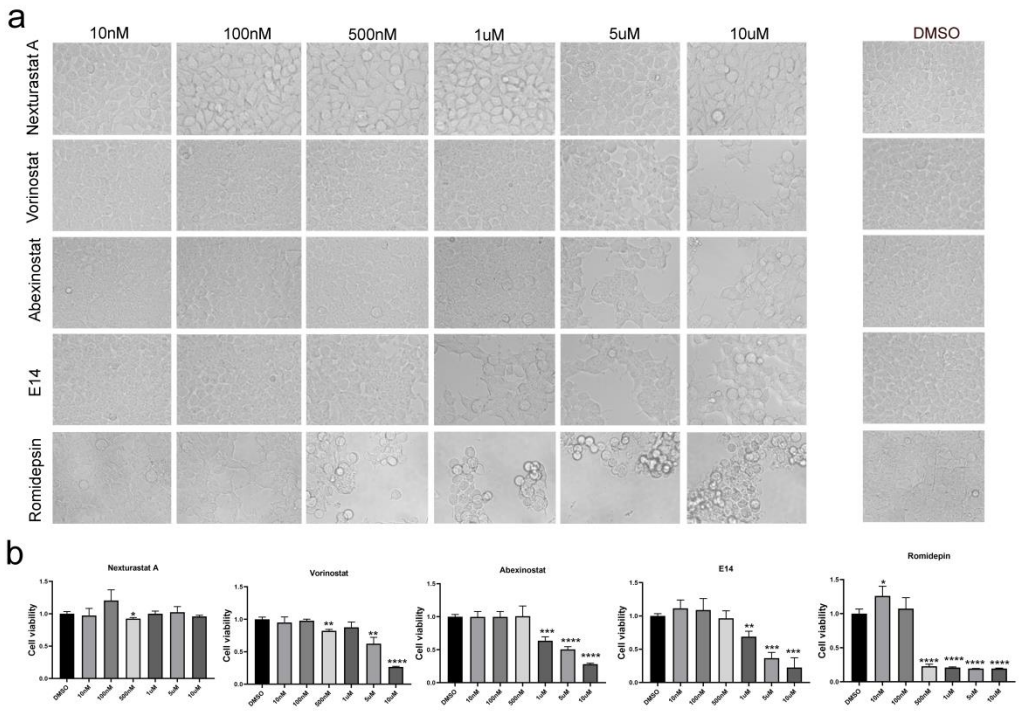

Figure S3. The cell viability assay of HDAC inhibitors on HEK293T cell line. a. The images of phenotypic observation of HEK293T cell treated with different HDAC inhibitor. **b.** Cell viability of HEK293T treated with HDAC inhibitors. Promega #G3582 assay was used to determine cell viability. Data are represented as mean ± SD; Asterisks indicate statistically significant differences between DMSO-treated cells and HDAC inhibitors-treated cells. (*p < 0.05, **p < 0.01, ***p < 0.001).

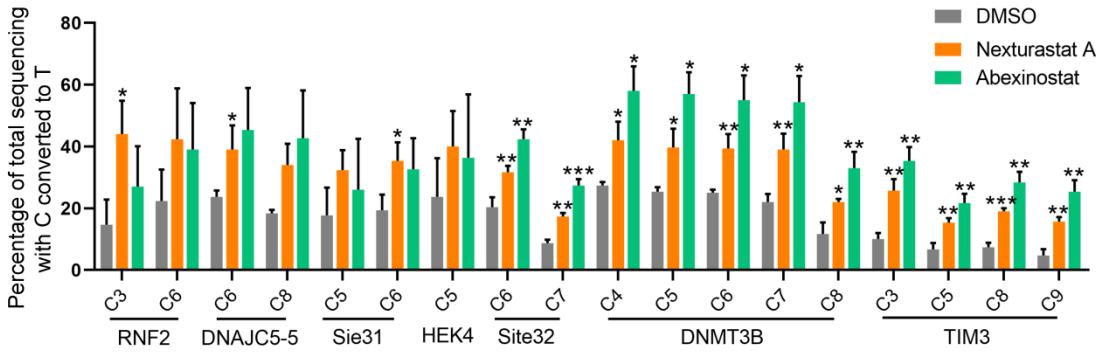

Figure S4. Effects of Nexturastat A and Abexinostat on cytosine base editing of 7 additional endogenous sites. The Nexturastat A and Abexinostat improved the C to T base editing efficiency of BE3 across all 7 endogenous sites. Each experiment was repeated at least three times. Data are represented as mean ± SD; Asterisks indicate statistically significant differences between DMSO-treated cells and HDAC inhibitors-treated cells. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

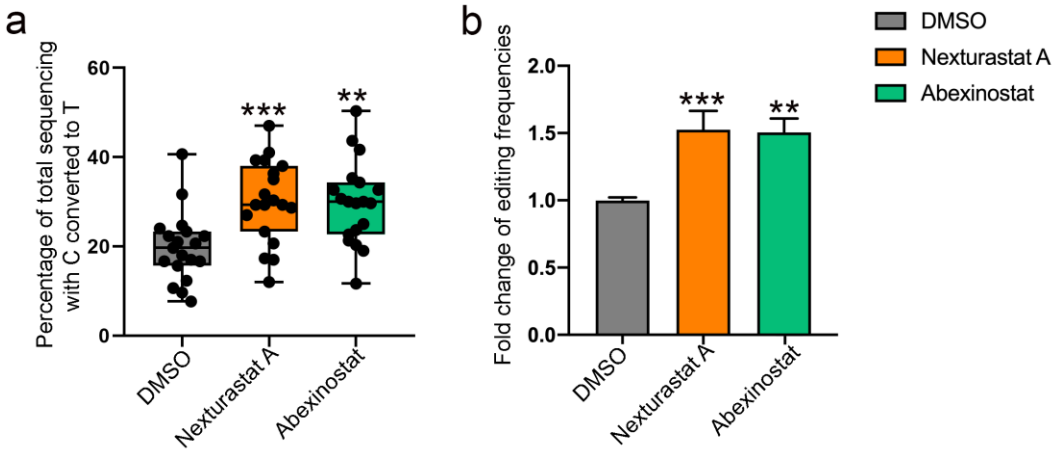

Figure S5. The total cytosine editing efficiency and the editing ratio of SaCas9 derived CBE across tested 5 endogenous sites. Each experiment was repeated at least three times. Data are represented as mean ± SD; Asterisks indicate statistically significant differences between DMSO-treated cells and HDAC inhibitors-treated cells. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

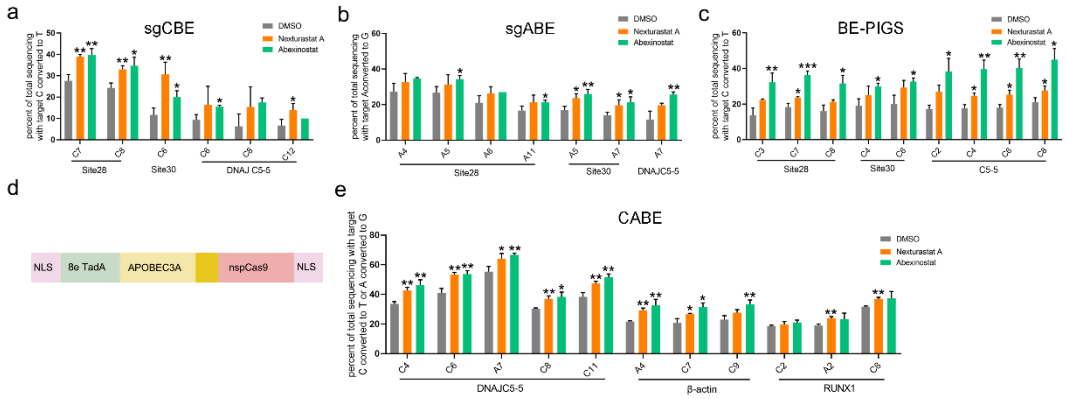

Figure S6. HDAC inhibitors generally improved the action of a wide range of base editing systems. Effect of Nexturastat A and Abexinostat on sgCBE (a), sgABE (b), BE-PIGS (c) and SpCas9-CABE (e). d. Schematic diagram of the structure of CABE base editor. 8e TadA and A3A were fused to the N-terminal of nSpCas9. Each experiment was repeated at least three times. Data are represented as mean ± SD; Asterisks indicate statistically significant differences between DMSO-treated cells and HDAC inhibitors-treated cells. (*p < 0.05, **p < 0.01, ***p < 0.001).

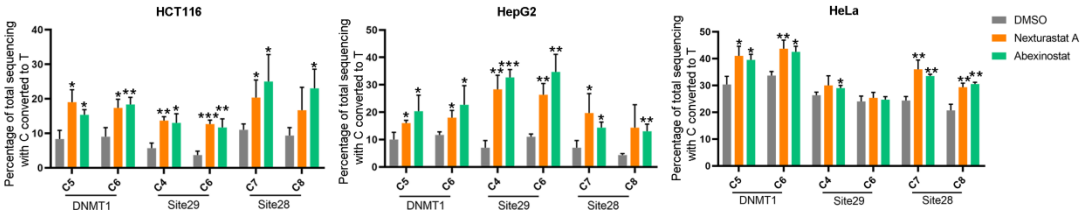

Figure S7. HDAC inhibitors improved the base editing efficiency in different cell lines. The effects of Nexturastat A and Abexinostat on the editing of BE3 in HCT116, HepG2 and HeLa cell line. Each experiment was repeated at least three times. Data are represented as mean ± SD; Asterisks indicate statistically significant differences between DMSO-treated cells and HDAC inhibitors-treated cells. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

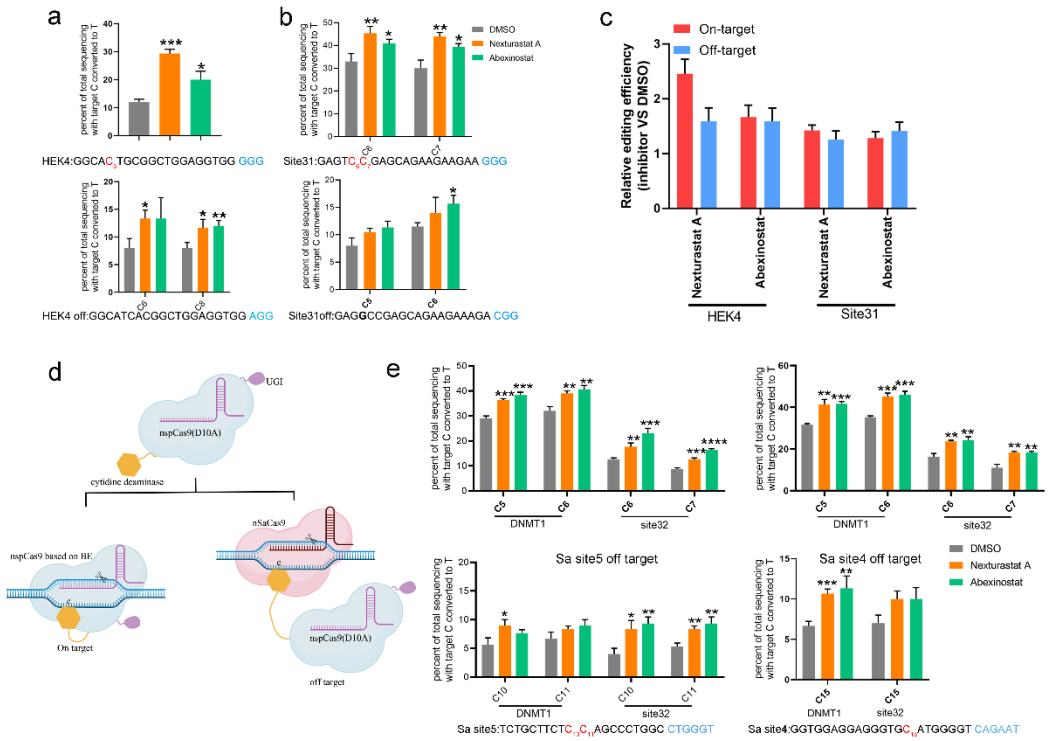

Figure S8. The effect of HDAC inhibitors on DNA off-target editing. a, b. Sequence-dependent off-target editing were investigated with two previously known off-target sites of HEK4 and site31. Editable Cs in each site were shown in red and mismatched bases within off-target sites were shown in bold (1). **c.** Relative improvement of HDAC inhibitor over DMSO in on-target base editing and off-target base editing. d. A diagram showing the mechanism of detecting sequence-independent editing with orthogonal R-loop assay and sequence-independent off-target editing. Two artificial R-loop were generated by transfection of nSaCas9 (D10A) and corresponding sgRNAs. The sequence-independent base editing effects were determined by co-transfection of indicated R-loop constructs and base editors with or without sgRNA. e. On target efficiency of DNMT1 and Site32 and their corresponding independent off-target efficiency on Sa site4 and Sa site5 genomic site. Each experiment was repeated at least three times. Data are represented as mean ± SD; Asterisks indicate statistically significant differences between DMSO-treated cells and HDAC inhibitors-treated cells. (*p < 0.05, **p < 0.01, ***p < 0.001).

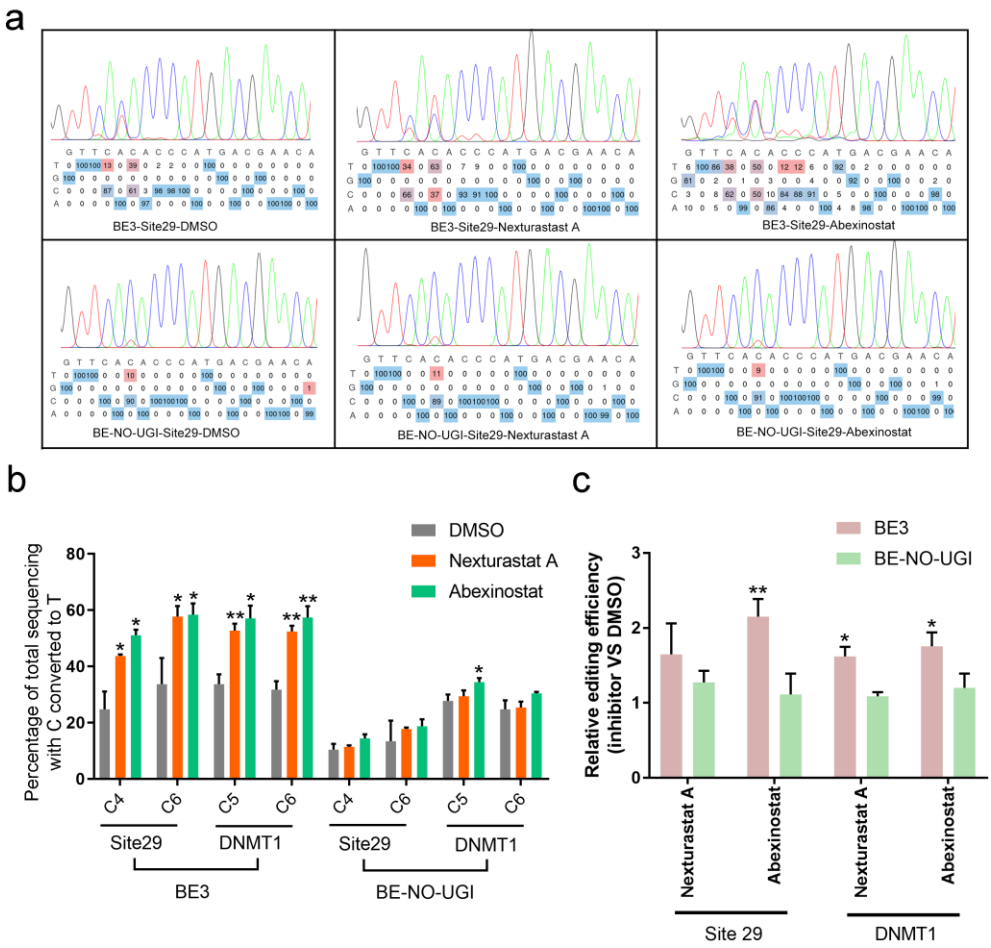

Figure S9. The effect of HDAC inhibitors on the editing of cytosine base editors with (BE3) or without UGI (BE-NO-UGI). a and **b.** The effect of Nexturastat A and Abexinostat on the editing of BE3 and BE-NO-UGI. a. Representative Sanger sequencing results and EditR analysis of site 29. c. Relative improvement of HDAC inhibitor over DMSO in base editing of BE3 and BE-NO-UGI. Each experiment was repeated at least three times. Data are represented as mean ± SD; Asterisks indicate statistically significant differences between DMSO-treated cells and HDAC inhibitors-treated cells. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Table S1. List of the 54 drugs used in the screening.

	Drug	Target	Relative editing ratio
D01-a-2	I-CBP112 hydrochloride	Epigenetic Reader Domain inhibitor	1.10
D01-a-3	Tenovin-1	Epigenetic Reader Domain inhibitor	1.17
D01-a-4	NSC 42834	JAK inhibitor	0.77
D01-a-5	Doxorubicin hydrochloride	Topoisomerase inhibitor	0.71
D01-a-7	Docetaxel	Bcr-Abl inhibitor; Microtubule Associated inhibitor; Others	0.64
D01-a-8	Theophylline	AChR antagonist; HDAC activator; PDE inhibitor	1.10
D01-a-11	Curcumin	Epigenetic Reader Domain inhibitor; Others	1.09
D01-b-2	Zebularine	DNA Methyltransferase inhibitor	0.67
D01-b-4	Entacapone	Transferase inhibitor	1.00
D01-b-5	Resveratrol	COX inhibitor; DNA/RNA Synthesis inhibitor; IκB/IKK inhibitor; Lipoxygenase inhibitor; NADPH inhibitor; Sirtuin inhibitor	0.63
D01-b-7	Bortezomib (PS-341)	Proteasome inhibitor	0.55
D01-b-8	BAY 87-2243	HIF inhibitor	0.53
D01-b-9	Vorinostat (SAHA, MK0683)	HDAC inhibitor	1.57
D01-b-10	Aminophylline	PDE inhibitor	0.67
D01-b-11	Daptomycin	DNA/RNA Synthesis inhibitor	0.87
D01-c-3	BET bromodomain inhibitor	Epigenetic Reader Domain inhibitor	1.05
D01-c-4	AMI-1	Histone Methyltransferase inhibitor	0.80
D01-c-5	EPZ6438	Histone Methyltransferase inhibitor	1.33
D01-c-6	CUDC-907	HDAC inhibitor	0.86
D01-c-7	Baricitinib (Phosphate)	JAK inhibitor; Tyrosine Kinases inhibitor	1.01
D01-c-8	SP2509	Histone Demethylase inhibitor	1.00

D01-c-9	SC-514	Aurora Kinase inhibitor; CDK inhibitor; IκB/IKK inhibitor; p38 MAPK inhibitor; Serine Protease inhibitor	1.00
D01-c-10	PFI-3	Epigenetic Reader Domain inhibitor	0.84
D01-c-11	TCS PIM-1 1	Pim inhibitor	1.03
D01-d-2	PJ34 hydrochloride	PARP inhibitor	1.08
D01-d-4	PCI-24781 (Abexinostat)	HDAC inhibitor	1.60
D01-d-5	Decernotinib(VX-509)	JAK inhibitor; Tyrosine Kinases inhibitor	1.19
D01-d-6	J147	Epigenetic Reader Domain	1.08
D01-d-7	SMI-4a	Pim inhibitor	0.87
D01-d-8	Daphnetin	Others inhibitor	1.13
D01-d-9	(-)-Epigallocatechin Gallate	EGFR inhibitor; PKA inhibitor; PKC inhibitor	1.52
D01-d-10	Curcumol	JAK inhibitor	1.30
D01-d-11	Sodium Aescinate	HIF inhibitor	0.98
D01-e-3	Imatinib (STI571)	Bcr-Abl inhibitor; c-Kit inhibitor; PDGFR inhibitor	1.01
D01-e-4	GSK J4 hydrochloride	Histone Demethylase inhibitor	0.76
D01-e-5	Procarbazine hydrochloride	DNA/RNA Synthesis inhibitor	1.01
D01-e-6	Methotrexate	Dehydrogenase inhibitor	1.21
D01-e-7	(R)-Ruxolitinib (INCB018424)	JAK inhibitor	1.52
D01-e-8	Belinostat (PXD101)	HDAC inhibitor	1.11
D01-e-9	Momelotinib (CYT387)	JAK inhibitor	1.33
D01-e-10	Picropodophyllin (PPP)	Adenosine Receptor antagonist; Aurora Kinase inhibitor	1.27
D01-e-11	IOX 2	HIF/HIF Prolyl-Hydroxylase inhibitor	1.05
D01-f-2	Nexturastat A	HDAC inhibitor	2.06
D01-f-4	JW55	PPAR inhibitor	1.35
D01-f-5	WHI-P258	EGFR inhibitor; JAK inhibitor	1.13
	SKLB-681	SETDB1 inhibitor	1.17

cis-24b	SETDB1 inhibitor	1.08
(*R*,*R*)-32b	SETDB1 inhibitor	1.24
(*R*,*R*)-59	SETDB1 inhibitor	1.12
(*S*,*S*)-45	SETDB1 inhibitor	1.53
SKLB-A011	ATM inhibitor	0.94
XAJ-A017	ATM inhibitor	0.98
AZD1390	ATM inhibitor	1.58
AZD0156	ATM inhibitor	1.38

Table S2. List of Sequences of pegRNAs.

pegRNA	spacer sequence	3'extension	PBS length (nt)	RT template length(nt)
β-actin +2 C to A	GGCTATTCTCGCAGCTCACCA	TCGACGACGAGCGCGGCGATATCATCATCCATAGTGAGCTGCGAGAA	13	34
β-actin +1 KI36	GGCTATTCTCGCAGCTCACCA	TCGACGACGAGCGCGGCGATATCATCATCCATGGAGCCGCAGCACCCTGGACAGCAAGGCATGGAAGCTTTGAGCTGCGAGAA	13	40
β-actin del1-40	GGCTATTCTCGCAGCTCACCA	CGTCGCCCGCGAAGCCGGCCTTGCACATGCCGGATGAGCTGCGAGAA	13	41
DNMT 1+1 KI27	GATTCCTGGTGCCAGAAACA	AGGAGGAGGAAGCTGCTAAGGACTAGTTCTGCCCTCCCGTCACCCCTGTCTTGTAATCCATGGATCCGAGCTCGGT	11	76
DNMT 1 del1-24	GATTCCTGGTGCCAGAAACA	TAAAATAAAGGAGGAGGAAGCTGCTAAGGACTATTCTGGCACCAGG	11	33
DNMT 1+6 G to C	GATTCCTGGTGCCAGAAACA	GTCACGCCTGTTTCTGGCACCAGG	11	13
VEGFA +1 KI40	GATGTCTGCAGGCCAGATGA	AGGGGCCACAGTGTGTCCCTCTGACAATGTGCCATCTGGAGCCCTCACTCGCTGCTCCCTGGGGCTAGCAGCGAGACAGGGGATCCTCTGGCCTGCAGA	13	87
VEGFA	GATGTCTGCAG	AGTTGCTTCATGTACAG	13	33

del1-50	GCCAGATGA	AGAGCCCAGGGCTGGG TCTGGCCTGCAGA		
VEGFA +5 G to C	GATGTCTGCAG GCCAGATGA	AATGTGCCATCTGGAGC ACTCATCTGGCCTGCAG A	13	22
HEK3 +1 KI40	GGCCCAGACT GAGCACGTGA	TGGAGGAAGCAGGGCT TCCTTTCCTCTGCCATCA ATAACTTCGTATAATGTA TGCTATACGAAGTTATAA CAATCGTGCTCAGTCTG	13	74
HEK3del1-40	GGCCCAGACT GAGCACGTGA	AGGAGCTGCACATACTA GCCCCTGTCTAGGAAAA GCTGTCCTGCGACCGTG CTCAGTCTG	13	47
HEK3+3 A to C	GGCCCAGACT GAGCACGTGA	TGGAGGAAGCAGGGCT TCCTTTCCTCTGCCACC ACGTGCTCAGTCTG	13	34

Table S3. List of sgRNAs and Oligos sequence

sgRNA	target sequence	Oligo F	Oligo R	Reference
Site31	GAGTCCGAGC AGAAGAAGAA **GGG**	CACCGAGTCC GAGCAGAAGA AGAA	AAACTTCTTC TTCTGCTCGG ACTC	(2)
RNF2	GTCATCTTAGT CATTACCTG **AGG**	CACCGTCATCT TAGTCATTACC TG	AAACCAGGT AATGACTAA GATGAC	(2)
DNAJC5-5	GCGCTCACTGT CTACCTCTG **GGG**	CACCGCGCTC ACTGTCTACCT CTG	AAACCAGAG GTAGACAGT GAGCGC	(3)
β-actin	GCTATTCTCGC AGCTCACCA **TGG**	CACCGCTATTC TCGCAGCTCA CCA	AAACTGGTG AGCTGCGAG AATAGC	This study
SiteE	CACACACACAC TTAGAATCTG **TGG**	CACCGCACAC ACACACTTAG AATCTG	AAACCAGAT TCTAAGTGTG TGTGTG	(4)
HEK4	GGCACTGCGGC TGGAGGTGG **GGG**	CACCGGCACT GCGGCTGGAG GTGG	AAACCCACC TCCAGCCGC AGTGCC	(5)
Site30	GAACACAAAG CATAGACTGC **GGG**	CACCGAACAC AAAGCATAGA CTGC	AAACGCAGT CTATGCTTTG TGTTC	(2)
Site28	GACAAACCAG AAGCCGCTCC	CACCGACAAA CCAGAAGCCG	AAACGGAGC GGCTTCTGGT	(6)

	TGG	CTCC	TTGTC	
Sa	GATGTTCCAAT	CACCGATGTTC	AAACTGCGT	(7)
Site6	CAGTACGCA	CAATCAGTACG	ACTGATTGG	
	GAGAGT	CA	AACATC	
HEK3	GGCCCAGACTG	CACCGGCCCA	AAACTCACG	(8)
	AGCACGTGA	GACTGAGCAC	TGCTCAGTCT	
	TGG	GTGA	GGGCC	
Site29	GTTCACACCCA	CACCGTTCAC	AAACTGTTC	This study
	TGACGAACA	ACCCATGACG	GTCATGGGT	
	TGG	AACA	GTGAAC	
DNMT	GATTCCTGGTG	CACCGATTCCT	AAACTGTTTC	(8)
1	CCAGAAACA	GGTGCCAGAA	TGGCACCAG	
	GGG	ACA	GAATC	
VEGF	ATGTACAGAGA	CACCGATGTAC	AAACGCCCT	This study
A3	GCCCAGGGC	AGAGAGCCCA	GGGCTCTCT	
	TGG	GGGC	GTACAT C	
VEGF	GATGTCTGCAG	CACCGATGTCT	AAACTCATCT	(8)
A1	GCCAGATGA	GCAGGCCAGA	GGCCTGCAG	
	GGG	TGA	ACATC	
Site32	GAAGACCAAG	CACCGAAGAC	AAACGCAGT	(6)
	GATAGACTGC	CAAGGATAGA	CTATCCTTGG	
	TGG	CTGC	TCTTC	
RUNX	GCATTTTCAGG	CACCGCATTTT	TCGCTTCCTC	(8)
1	AGGAAGCGA	CAGGAGGAAG	CTGAAAATG	
	TGG	CGA	C	
HEK2	GTTAAGAACAC	CACCGTTAAG	AAACCCTTTA	This study
site2	GTTTAAAGG	AACACGTTTAA	AACGTGTTCT	
	GGG	AGG	TAAC	
VEGF	GCTCCATTCAC	CACCGCTCCAT	AAACGGGAA	This study
A11	CCAGCTTCCC	TCACCCAGCTT	GCTGGGTGA	
	TGTGGT	CCC	ATGGAGC	
EXM1-	CCTCCCTCCCT	CACCGCCTCCC	AAACACCTG	(9)
1	GGCCCAGGT	TCCCTGGCCCA	GGCCAGGGA	
	GAAGGT	GGT	GGGAGGC	
RUNX	GTACTCACCTC	CACCGTACTCA	AAACAGTGC	(9)
14	TCATGAAGCAC	CCTCTCATGAA	TTCATGAGA	
	T **GTGGGT**	GCACT	GGTGAGTAC	
HEK3-	GTACTCACCTC	CACCGTACTCA	AAACAGTGC	(2)
2	TCATGAAGCAC	CCTCTCATGAA	TTCATGAGA	
	T **GTGGGT**	GCACT	GGTGAGTAC	
HEK3-	TCTGCTTCTCC	CACCGTCTGCT	AAACGCCAG	(2)
1	AGCCCTGGC	TCTCCAGCCCT	GGCTGGAGA	
	CTGGGT	GGC	AGCAGAC	
Sa site4	GGTGGAGGAG	CACCGGTGGA	AAACACCCC	(7)

	GGTGCATGGGG	GGAGGGTGCA	ATGCACCCTC	
	T **CAGAAT**	TGGGGT	CTCCACC	
Sa site5	TCTGCTTCTCC	CACCGTCTGCT	AAACGCCAG	(7)
	AGCCCTGGC	TCTCCAGCCCT	GGCTGGAGA	
	CTGGGT	GGC	AGCAGAC	

Table S4. Primers used to amplify each target sites for sanger sequencing

Target site	chromosome	Forward primer	Reverse primer
Site31	Chr2	CAGCTCAGCCTGA GTGTTGA	CTCGTGGGTTTGTGG TTGC
RNF2	Chr1	ACCACTGTTCACC CAGTACC	TCCCTTCCAAATACT AAAATTG
DNAJC5-5	Chr20	TCTGTCTGTGCAC GTGGCAA	AGCTGTGACCAGTTC AACGC
β-actin	Chr7	GACCCGGCGCTGT TTGAA	AAAGCGCCCTTGCCT CC
SiteE	Chr1	TTCGAGGTGGAGC TCAAGAT	TTCTGCAGGCGAGA ACCTG
HEK4	Chr20	CAGCGAGGTCAAA GTCACC	TCCTTTCAACCCGAA CGGAG
Site30	Chr5	ACAGGCTACCCCC TAAGT	TCCCAAGTGAGAAG CCAGTG
Site28	Chr3	GGCACAAAGGATG AAGGCT	GCTCAGTCTTGCATG AAACAC
Sa site6	Chr11	ATGACTGGCATCAT CTCGCA	GGTGCTGACGTAGGT AGTGC
HEK3	Chr9	TGGGTCACAGTGG CAAATGA	ATGCAGGTGCTGAAA GCCAC
Site29	Chr12	CAAAGAAAGAGG GAGCGGGG	GCTGAGTACGTCGTG GAGTC
DNMT1	Chr19	AGTCCCGTGCAAA TCACGAA	CCGTGAACGTTCCCT TAGCA
VEGFA site3	Chr6	GGAACAAGGGCCT CTGTCTG	GCCGTTCCCTCTTTG CTAGG
VEGFA site1	Chr6	GGAACAAGGGCCT CTGTCTG	GCCGTTCCCTCTTTG CTAGG
Site32	Chr22	TTCCAACCTTCCC ACAGG	GGGCATCATAGCGAG AC
RUNX1	Chr21	GTTCTCACGCACC GACTGAA	GAGTCCCAGAGGTAT CCAGC
HEK2 site2	Chr5	ACAGGCTACCCCC TAAGT	TCCCAAGTGAGAAG CCAGTG
VEGFA11	Chr5	GGAACAAGGGCCT CTGTCTG	GCCGTTCCCTCTTTG CTAGG

EXM1-1	Chr2	AATCTACCACCCC AGGCTCT	GCCCCTAACCCTATG TAGCC
RUNX14	Chr21	GTTCTCACGCACC GACTGAA	GAGTCCCAGAGGTAT CCAGC
HEK3-2	Chr9	AGAATGGGTCACA GTGGCAA	TAGGAAAAGCTGTCC TGCGA
HEK3-1	Chr9	AGAATGGGTCACA GTGGCAA	TAGGAAAAGCTGTCC TGCGA
Sa site4	Chr1	AGGAACAACCTGT CCGCAAG	AGGCATACACTCCTG GCATC
Sa site5	Chr9	AGAATGGGTCACA GTGGCA	TAGGAAAAGCTGTCC TGCGA

Table S5. HTS Primers used to amplify each target sites.

sample name	Primer Name	Sequence
DNMT1+1 KI 27-DMSO	HTS-1-for	GTGAACA accacacatgtgaacggaca
	HTS-1-rev	CGTGTTCCCCAGAGTGACTT
DNMT1+1 KI 27-Nexturastat A	HTS-2-for	GTGACTC accacacatgtgaacggaca
	HTS-2-rev	CGTGTTCCCCAGAGTGACTT
DNMT1+1 KI 27-Vorinostat	HTS-3-for	GTGTCAA accacacatgtgaacggaca
	HTS-3-rev	CGTGTTCCCCAGAGTGACTT
DNMT1+1 KI 27-Abexinostat	HTS-4-for	AACTGTC accacacatgtgaacggaca
	HTS-4-rev	CGTGTTCCCCAGAGTGACTT
DNMT1 del 1-24-DMSO	HTS-5-for	AAGTCAG accacacatgtgaacggaca
	HTS-5-rev	CGTGTTCCCCAGAGTGACTT
DNMT1 del 1-24-Nexturastat A	HTS-6-for	ATCAGTG accacacatgtgaacggaca
	HTS-6-rev	CGTGTTCCCCAGAGTGACTT
DNMT1 del 1-24-Vorinostat	HTS-7-for	ATCTGCT accacacatgtgaacggaca
	HTS-7-rev	CGTGTTCCCCAGAGTGACTT
DNMT1 del 1-24-Abexinostat	HTS-8-for	ATGTGAC accacacatgtgaacggaca
	HTS-8-rev	CGTGTTCCCCAGAGTGACTT
β-actin +1 KI36-DMS	HTS-9-for	CTGATGT ccccctggcggccta
	HTS-9-rev	CACGATGGAGGGGAAGACG
β-actin +1 KI36-Nexturastat A	HTS-10-for	CTGTAGA ccccctggcggccta
	HTS-10-rev	CACGATGGAGGGGAAGACG
β-actin +1 KI36-Vorinostat	HTS-11-for	GAACACT ccccctggcggccta
	HTS-11-rev	CACGATGGAGGGGAAGACG
β-actin +1 KI36-Abexinostat	HTS-12-for	GATCTCA ccccctggcggccta
	HTS-12-rev	CACGATGGAGGGGAAGACG
β-actin del 1-40-DMSO	HTS-13-for	GACTACA ccccctggcggccta
	HTS-13-rev	CACGATGGAGGGGAAGACG
β-actin del 1-40-Nexturastat A	HTS-14-for	GACTCAC ccccctggcggccta
	HTS-14-rev	CACGATGGAGGGGAAGACG
β-actin del 1-40-Vorinostat	HTS-15-for	GAGTCGT ccccctggcggccta

	HTS-15-rev	CACGATGGAGGGGAAGACG
β-actin del 1-40-Abexinostat	HTS-16-for	GTACAGA ccccctggcggccta
	HTS-16-rev	CACGATGGAGGGGAAGACG
VEGFA+1 KI40nt-DMSO	HTS-17-for	CACACTGccaaaggaccccagtcactc
	HTS-17-rev	TGGGACTGGAGTTGCTTCAT
VEGFA+1 KI40-Nexturastat	HTS-18-for	CACTTGAccaaaggaccccagtcactc
A	HTS-18-rev	TGGGACTGGAGTTGCTTCAT
VEGFA+1 KI40-Vorinostat	HTS-19-for	CACTGATccaaaggaccccagtcactc
	HTS-19-rev	TGGGACTGGAGTTGCTTCAT
VEGFA+1 KI40-Abexinostat	HTS-20-for	CAGATCAccaaaggaccccagtcactc
	HTS-20-rev	TGGGACTGGAGTTGCTTCAT
VEGFA del1-50-DMSO	HTS-21-for	CAGTACTccaaaggaccccagtcactc
	HTS-21-rev	TGGGACTGGAGTTGCTTCAT
VEGFA del1-50-Nexturastat	HTS-22-for	CAGTCTAccaaaggaccccagtcactc
A	HTS-22-rev	TGGGACTGGAGTTGCTTCAT
VEGFA del1-50-Vorinostat	HTS-23-for	CTAGACAccaaaggaccccagtcactc
	HTS-23-rev	TGGGACTGGAGTTGCTTCAT
VEGFA del1-50-Abexinostat	HTS-24-for	CTTGAGTccaaaggaccccagtcactc
	HTS-24-rev	TGGGACTGGAGTTGCTTCAT
HEK3 del 1-40-DMSO	HTS-41-for	TCAGAGAtgcatttgtaggcttgatgc
	HTS-41-rev	GTCAACCAGTATCCCGGTGC
HEK3 del 1-40-Nexturastat	HTS-42-for	TGACTGAtgcatttgtaggcttgatgc
A	HTS-42-rev	GTCAACCAGTATCCCGGTGC
HEK3 del 1-40-Vorinostat	HTS-43-for	TGAGACTtgcatttgtaggcttgatgc
	HTS-43-rev	GTCAACCAGTATCCCGGTGC
HEK3 del 1-40-Abexinostat	HTS-44-for	TGTCAGTtgcatttgtaggcttgatgc
	HTS-44-rev	GTCAACCAGTATCCCGGTGC

Table S6. List of off-target sites

Target site	sequence
HEK4 off Target	GGCATCACGGCTGGAGGTGG
Site31 off Target	GAGGCCGAGCAGAAGAAAGA

Table S7. primers used for amplifying off-target sites

Sample name	Forward primer	Reverse primer
HEK4 off Target	CAGGTGTTCAGCTTTGCCA	AGTAGAGACAGGCCCAGAG
Site31 off Target	TGCAGGAGCTAGACTCCGA	TCCTCGTCCTGCTCTCACTT

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