Supplemental Figure legends:

Supplemental Figure 1. RT-qPCR results and locus-specific bisulfite sequencing data showed 1 h and 3 h TNF-α treatment can activate *IL-32* transcription in a DNA demethylation-independent manner in THP-1 (A) and HAP1 (B) cells.

Supplemental Figure 2. In HAP1 cells, *IL-32* basal transcription is upregulated after long-term TNF-α treatment and is accompanied by sustained hypomethylation at the promoter and CGI. *A*, RT-qPCR results showed that *IL-32* transcription can be efficiently activated by 12 d TNF-α treatment in HAP1 cells. Averages from two independent experiments are shown, and error bars represent standard deviation in the RT-qPCR results. *B*, *IL-32* basal expression level is upregulated after 12 d TNF-α treatment. Averages from two independent experiments are shown, and error bars represent standard deviation in the RT-qPCR results. *C*, Bisulfite sequencing data revealed that *IL-32* regulatory regions are demethylated by 12 d TNF-α treatment and the low methylation status can be sustained after TNF-α withdrawal in HAP1 cells.

Supplemental Figure 3. The sequence of mutant alleles in *TET1* KO, *TET2* KO and *TET3* KO HEK293 cells determined by Sanger sequencing. The exons encoding the catalytic cysteine-rich and DSBH domains are indicated. The red stars show the exons targeted by the CRISPR-Cas9 system to abrogate the activity of *TET* catalytic domain.

Supplemental Figure 4. *TET* TKO HEK293 cells. *A*, The sequences of mutant alleles in *TET* TKO HEK293 cells determined by Sanger sequencing. The exons encoding the catalytic cysteine-rich and DSBH domains are indicated. Red stars indicate the exons that are targeted by the CRISPR-Cas9 system to abrogate the activity of the *TET* catalytic domain. *B*, UHPLC-MRM MS/MS quantitation of 5mC and 5hmC in the genomic DNA extracted from HEK293 WT and *TET* TKO cells. 5hmdC indicates 5-hydroxymethyl-2'-deoxycytidine and dC indicates 2'-deoxycytidine. SD represents standard deviation. *C*, FPKM value of *DNMT* genes in RNA-seq experiments are shown in WT and *TET* TKO cells.

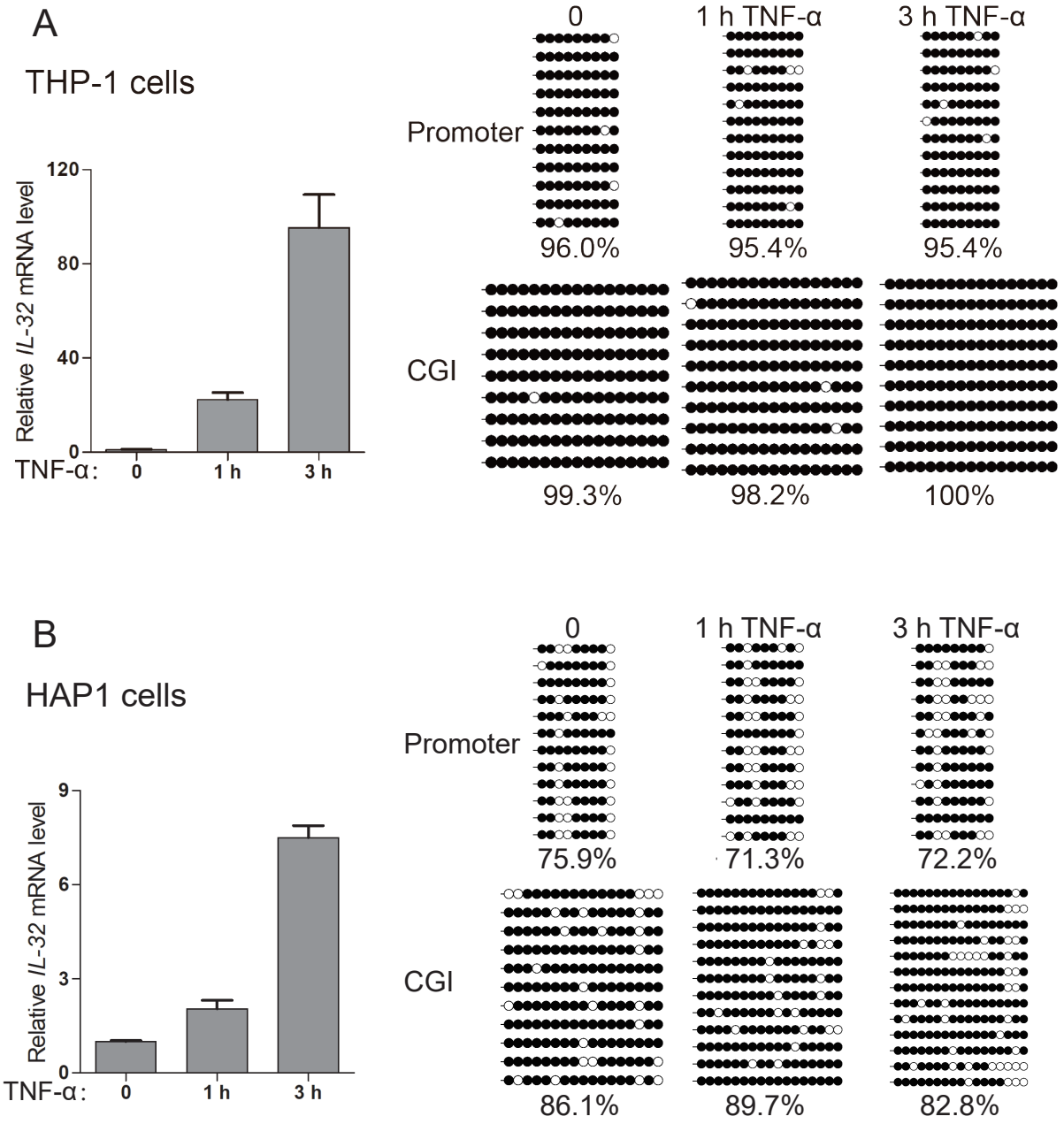
Supplemental Figure 5. RT-qPCR data revealed that short-term and long-term TNF-α treatment can activate *IL-32* transcription in *TET1* KO, *TET2* KO, *TET3* KO and *TET* TKO cells. Averages from three independent experiments are shown and error bars represent standard deviation.

Supplemental Figure 6. *RELA* KO cells. *A*, Strategy for CRISPR-Cas9 system to knockout human *RELA* gene. *B*, Validation of *RELA* KO cells by PCR (primer sequence shown in Supplemental Table 4). *C*, Validation of *RELA* KO cells by Sanger sequencing. *D*, FAIRE assay results show increased chromatin accessibility at the *IL-32* promoter of 12 h TNF-α treated cells. The promoter of *ACTB* was used as a control to determine relative DNA recovery ratios. Error bars represent standard deviations from two biological replicates. qPCR primer sequences are shown in Supplemental Table 5.

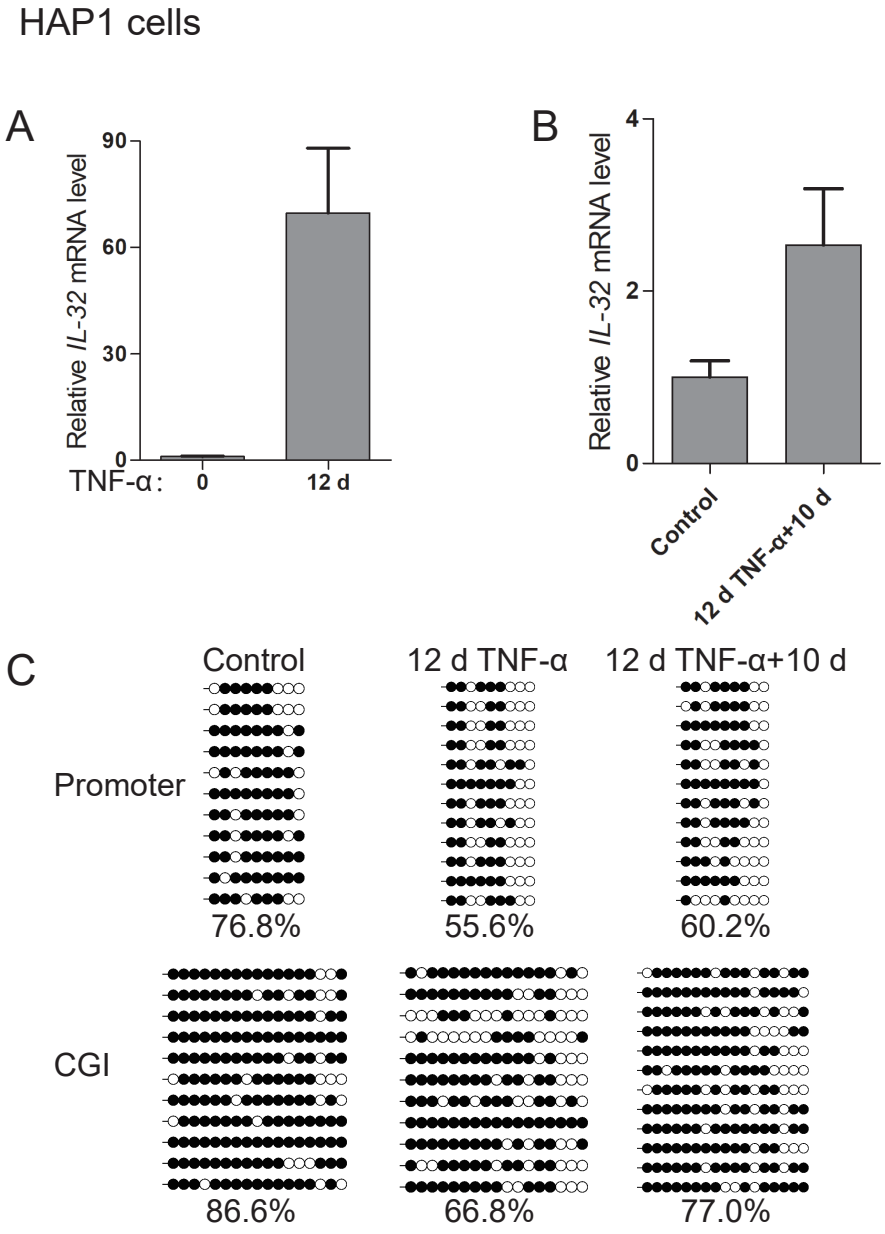
Supplemental Figure 7. The upregulation of *IL-32* transcription after long-term TNF-α stimulation can be sustained independent of CREB and *IL-32* promoter CRE in HEK293 cells. *A*, Schematic representation of CRE (TGACGTCA) in *IL-32* promoter. *B*, The sequence of mutant alleles in *CREB1* KO HEK293 cells. The exons encoding the bZIP domain are indicated. The red star shows the exon targeted by the CRISPR-Cas9 system to abrogate DNA binding and dimerization. *C*, RT-qPCR data showed that *IL-32* transcription can still be induced in *CREB1* KO cells. *D*, RT-qPCR data revealed the upregulation of *IL-32* transcription through long-term TNF-α treatment in *CREB1* KO cells. *E*, Sanger sequencing of genomic DNA PCR product confirmed the *IL-32* promoter CRE mutated cells. *F*, RT-qPCR data showed the upregulation of *IL-32* transcription through long-term TNF-α treatment in *IL-32* promoter CRE mutated cells.

Supplemental Figure 8. HPLC-MRM MS/MS experiments at various time points following TNF-α treatment showed a subtle decline of global 5mC level.

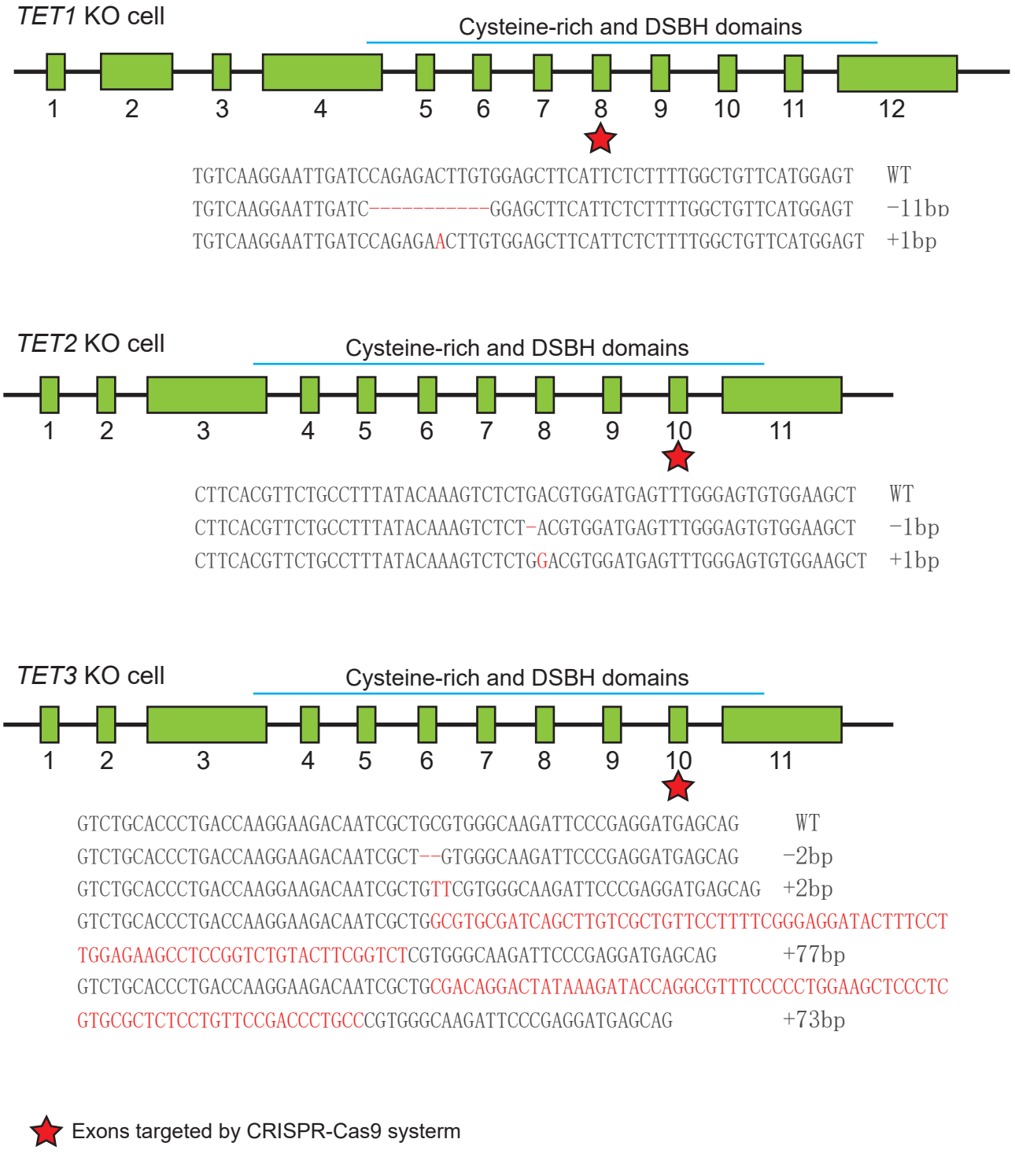
Supplemental Figure 1



Supplemental Figure 2

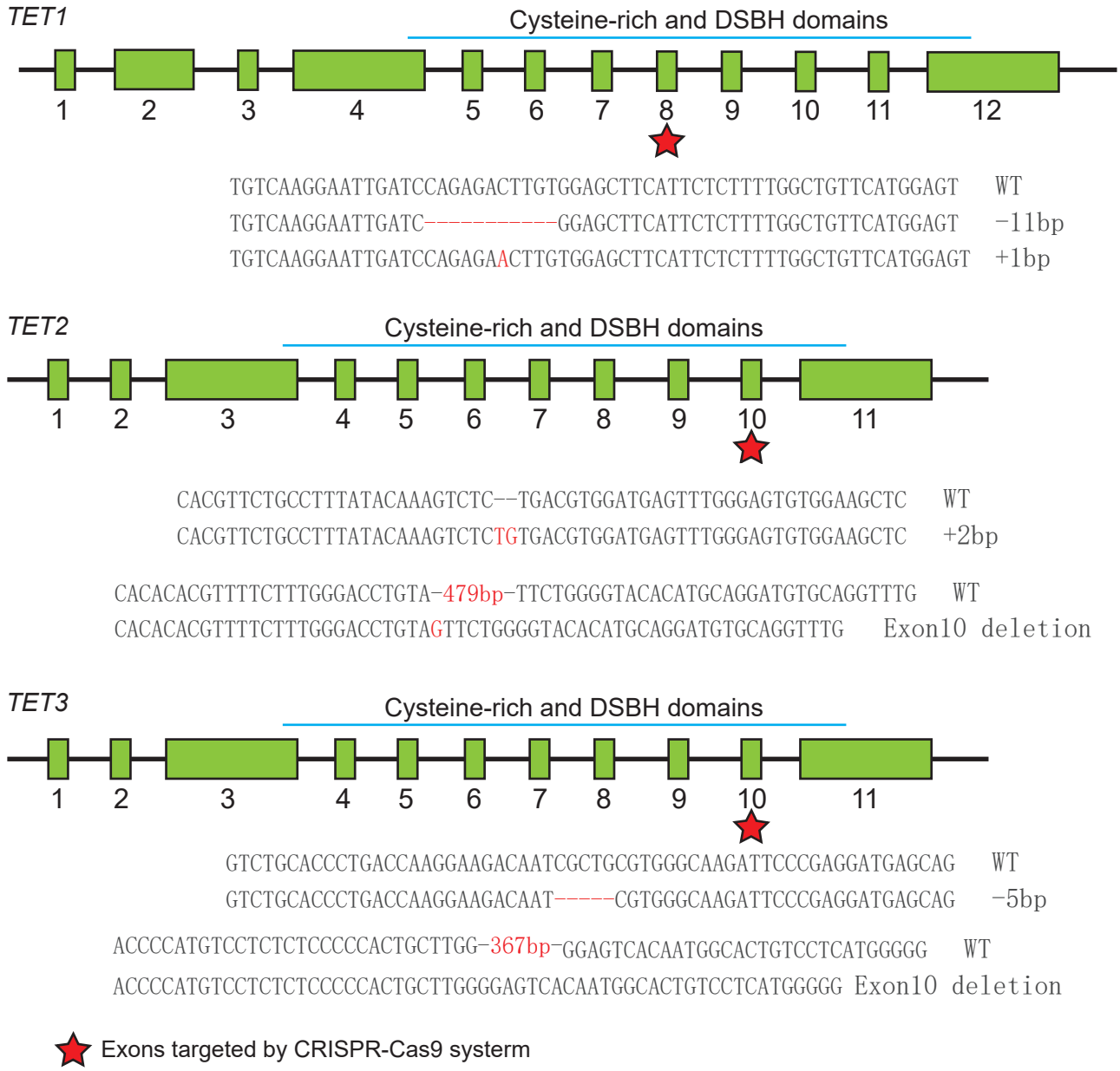


Supplemental Figure 3



Supplemental Figure 4

A *TET* TKO cell



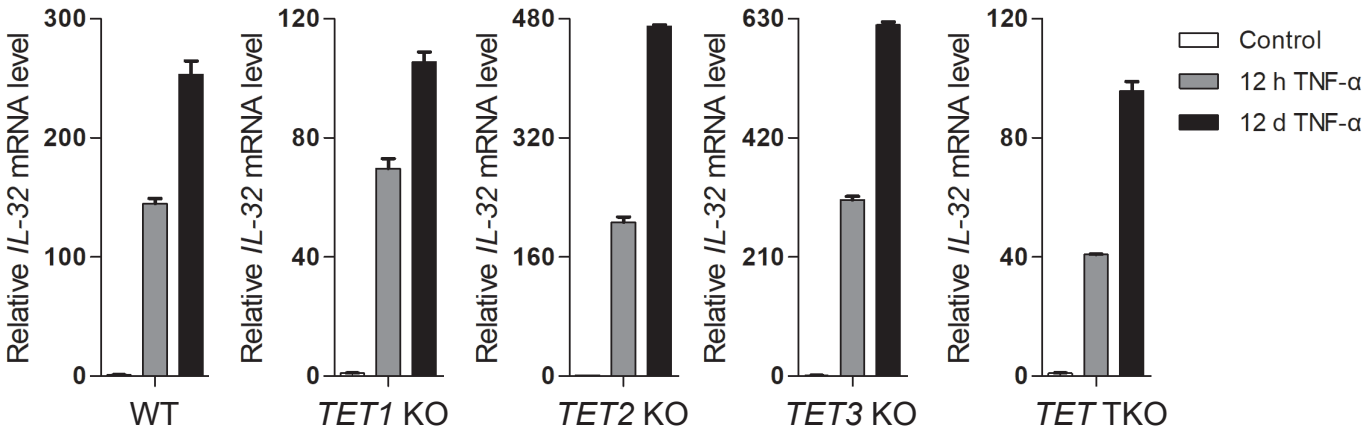
B

Sample	5mdC/10^2 dC	SD	5hmdC/10^6 dC	SD
WT	2.35	0.02	22.23	0.23
TET TKO	2.60	0.03	Not Detected	

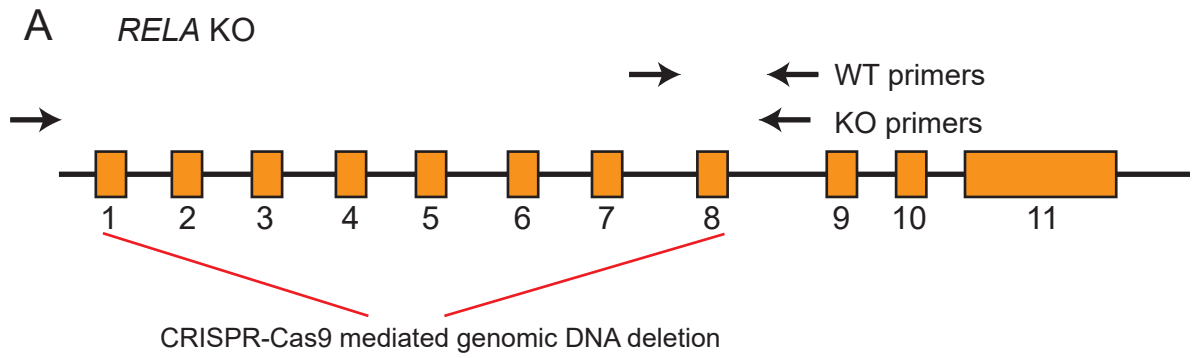
C

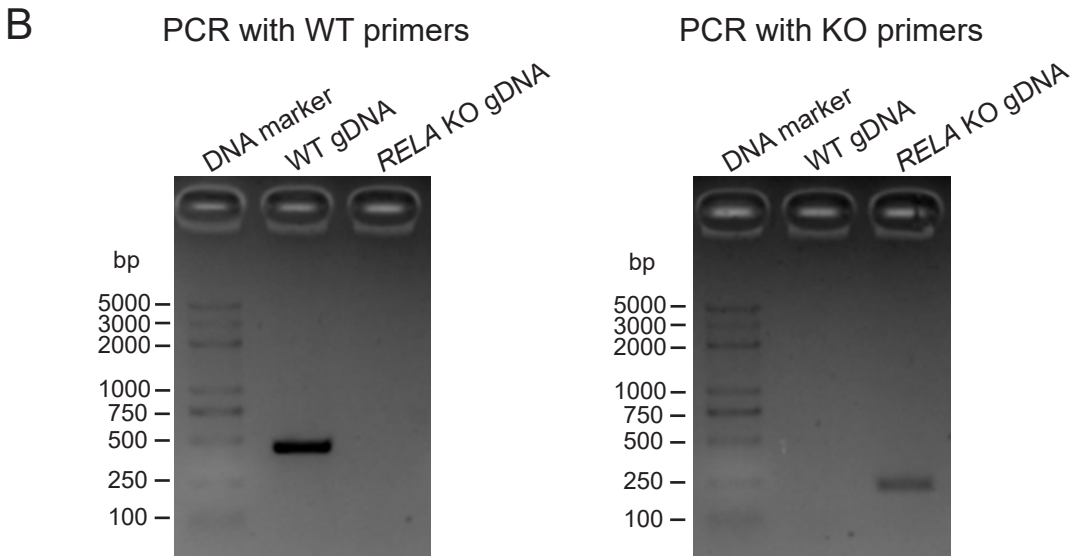
	WT (repeat 1)	WT (repeat 2)	*TET* TKO (repeat 1)	*TET* TKO (repeat 2)
DNMT1	96.6	82.8	94.3	98.1
DNMT3A	4.11	3.63	4.53	5.08
DNMT3B	7.39	5.85	3.89	4.16

Supplemental Figure 5



Supplemental Figure 6

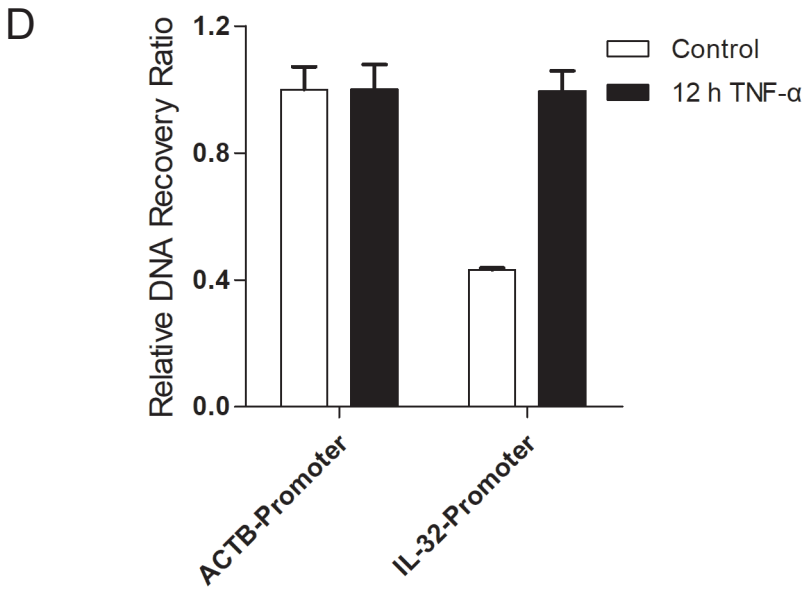




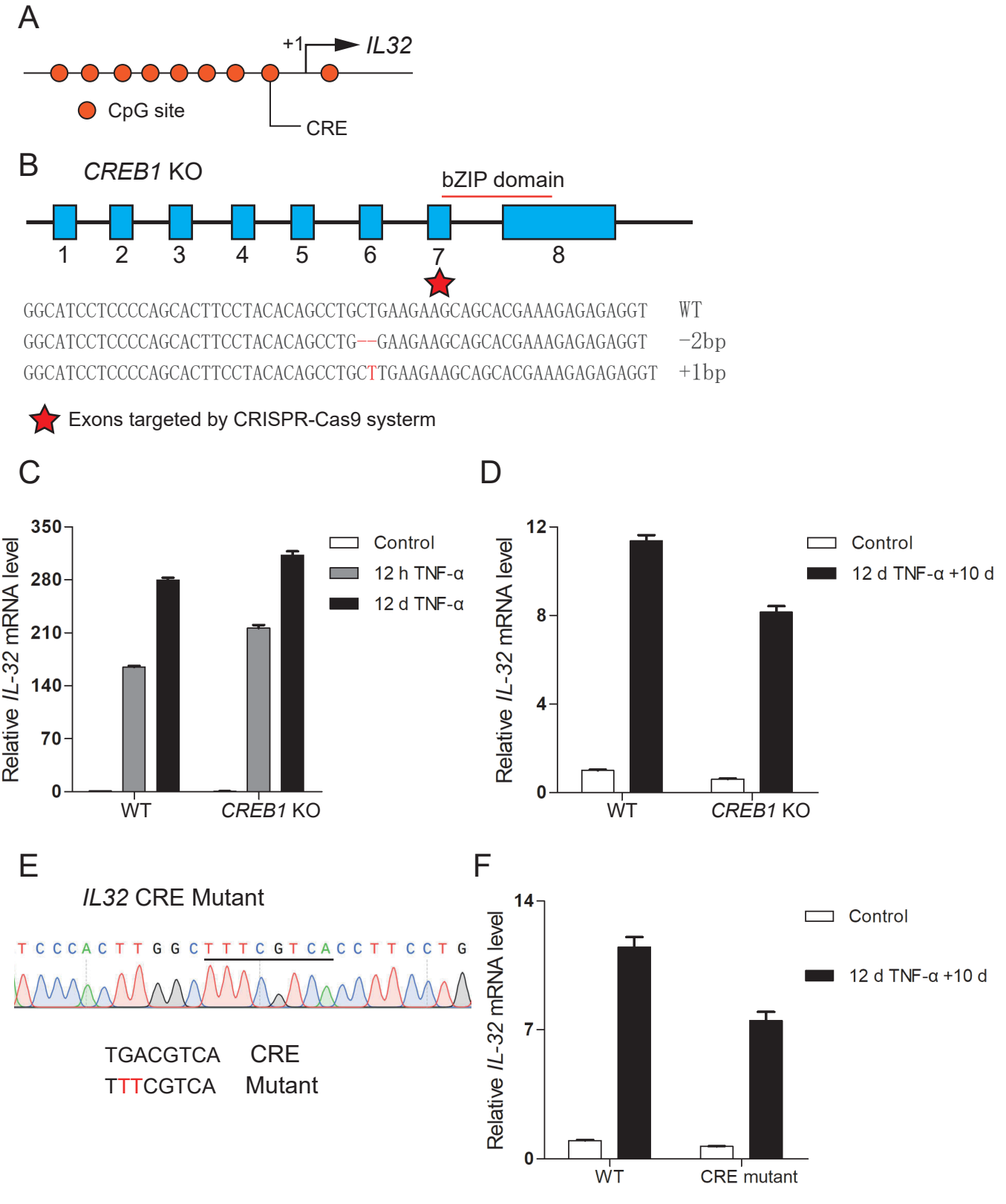
C

GGCCGGATTCCGGGCAGTGACGCGA-4640bp-AGATACAGGTACACAGCTGGGGTGG WT

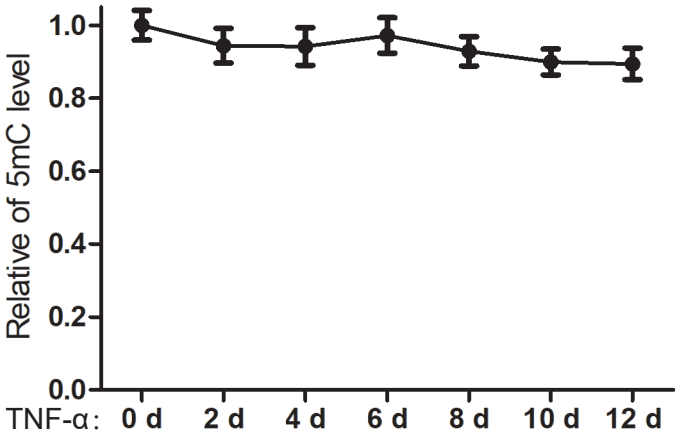
GGCCGGATTCCGGGCAGTGACGCGAAGATACAGGTACACAGCTGGGGTGG *RELA* KO



Supplemental Figure 7



Supplemental Figure 8



Supplemental Tables

Supplemental Table 1: *IL-32* promoter and CpG island genomic sequence for methylation analysis.

	Sequence (5' to 3')
IL-32 promoter	CGGCCCCAGATGGCCTTGCCTTGGCCCAGAAGGGTCAG AAGGACCTGGTCAGCCAAGCTCAGACAGCCGGCAGGAT GCCTTCCACCCTGCAGAGGGTCCTATCTTGTCCCACAGG TAGATCTACATCACCACTAGCCACCCCTCCAACGTGCAC AGGCCCCTGCCCTCACGGCGCCCCTCTTAGGTCCGGCAG TTCCTGCCTCCTTCTGATCCAGAAGTTTCTCTGGCCTCTG GAGCCGGGGCACACCTCATGCAAGGACAGGGTCCAAAT TCCTTTGTCCTTGGATCCCACTTGGCTGACGTCACCTTCC TGTACTCAGGGAGTTTCCCCAGCCAGCTGTCCCGAGTCT GGACTTTCCCTCTGCCCCTCCCCACTCTCAGGCTGGTGG GGTGGGGAAAGCAGCCCATTCCTGGGCTCAGAGACTCC CACCCCAGCTCAGAGGGAGCAGGGGCCCAGCCAGGGAC GGACCCTCATTCCTCCCAGGGACCCCAGACCTCTGTCTC TCTCG
IL-32 CGI	CGCCAGCAGGCCTTGGCTCCTTGAACTTTTGGCCGCCAT GTGCTTCCCGAAGGTGAGTGAGAGGCTGCGTGTGCTTTT GTGGGCATGTCTGAAAACAGACCGTAAGGGTGCGGGTG CCCTCAGTATTTCCCGAGGTGCCTGTGTGTCAGGGCTCA GTCAGGGGCACCCAGCGGCAGGAGGATAGTGATGGGGT GAGAGTGTCAGTGGAGGCGCTGGAGGTCATATGTGTCGG GGGCGCTGGAGAACGGCAGGGGTGTGGATGAGAGGGA GCACCTGTCCCAGGAGCCCTTCACAGCCCGGAAAGCCC GGGGCAGGGGTGGGGCAGGGCTCTGCTGGAAACGACTC GGAGAATGCTTCTCTCAGAGGCCG

Supplemental Table 2: *IL-32* promoter and CGI nested PCR primers for methylation analysis

Primer name	Sequence
IL-32-promoter-first PCR-F	TTAGTTAGGTTGGAGGGTTAGAATT
IL-32-promoter-first PCR-R	TCAAAACAAAAAACCTTACTAAATA
IL-32-promoter-second PCR-F	GGGGAGTTTTAAGATTGTTGAGATT
IL-32-promoter-second PCR-R	AAACAAACAAAAATAAAAACTTACC
IL-32-CGI-first PCR-F	GAGGATTTTTTGGGGAGGAGGGTGT
IL-32-CGI-first PCR-R	AACACCAAAACCCACACAAACCTTA
IL-32-CGI-second PCR-F	TGAGATATTTTTTTTTTTTATATT
IL-32-CGI-second PCR-R	TACTCTTAAACCCACCCAACTAAAC

Supplemental Table 3: gRNA sequence used for CRISPR-Cas9 system mediated genome editing

Cell line	gRNA sequence	Target
TET1 KO cells	AATGAAGCTCCACAAGTCTC	Frameshift mutation in *TET1* exon 8
TET2 KO cells	TTTATACAAAGTCTCTGACG	Frameshift mutation in *TET2* exon 10
TET3 KO cells	AAGGAAGACAATCGCTGCGT	Frameshift mutation in *TET3* exon 10
CREB1 KO cells	TTTCGTGCTGCTTCTTCAGC	Frameshift mutation in *CREB1* exon 7
IL-32 promoter CRE mutant	GGAAGGTGACGTCAGCCAAG	Homology-directed repair to edit *IL-32* promoter CRE
RELA KO cells	TCCGGGCAGTGACGCGACGG	Deletion of *RELA* exon 1~8
	GCTGTGTACCTGTATCTGGC	
TET TKO cells	AATGAAGCTCCACAAGTCTC	Frameshift mutation in *TET1* exon 8
	TTTATACAAAGTCTCTGACG	Frameshift mutation in *TET2* exon 10
	GACATTACAGCCTCAACTAC	Deletion of *TET2* exon 10
	CCCCATCAACTTGTAAGTTC	
	AAGGAAGACAATCGCTGCGT	Frameshift mutation in *TET3* exon 10
	TCTCCCCCACTGCTTGGGGC	Deletion of *TET3* exon 10
	AGTGCCATTGTGACTCCAAG	

Supplemental Table 4: PCR primers for *RELA* KO cells validation

Primer name	Sequence
RELA WT Primer-F	CACCGCATCTCCTGCAGAGG
RELA WT Primer-R	CAGTGAAGTGCCTGGAATAG
RELA KO Primer-F	TGTGCGTGCAGCCTCTTCGTC
RELA KO Primer-R	TCTGACTCCAGCTTCTGGCC

Supplemental Table 5: qPCR primers for FAIRE assay

Primer name	Sequence
ACTB-Promoter-F	AAAGGCAACTTTCGGAACGG
ACTB-Promoter-R	TTCCTCAATCTCGCTCTCGC
IL-32-Promoter-F	TGTCCTTGGATCCCACTTGG
IL-32-Promoter-R	CAGAGGGAAAGTCCAGACTC