SUPPLEMENTARY MATERIAL

Supplementary material includes 6 supplemental figures and 3 supplementary tables
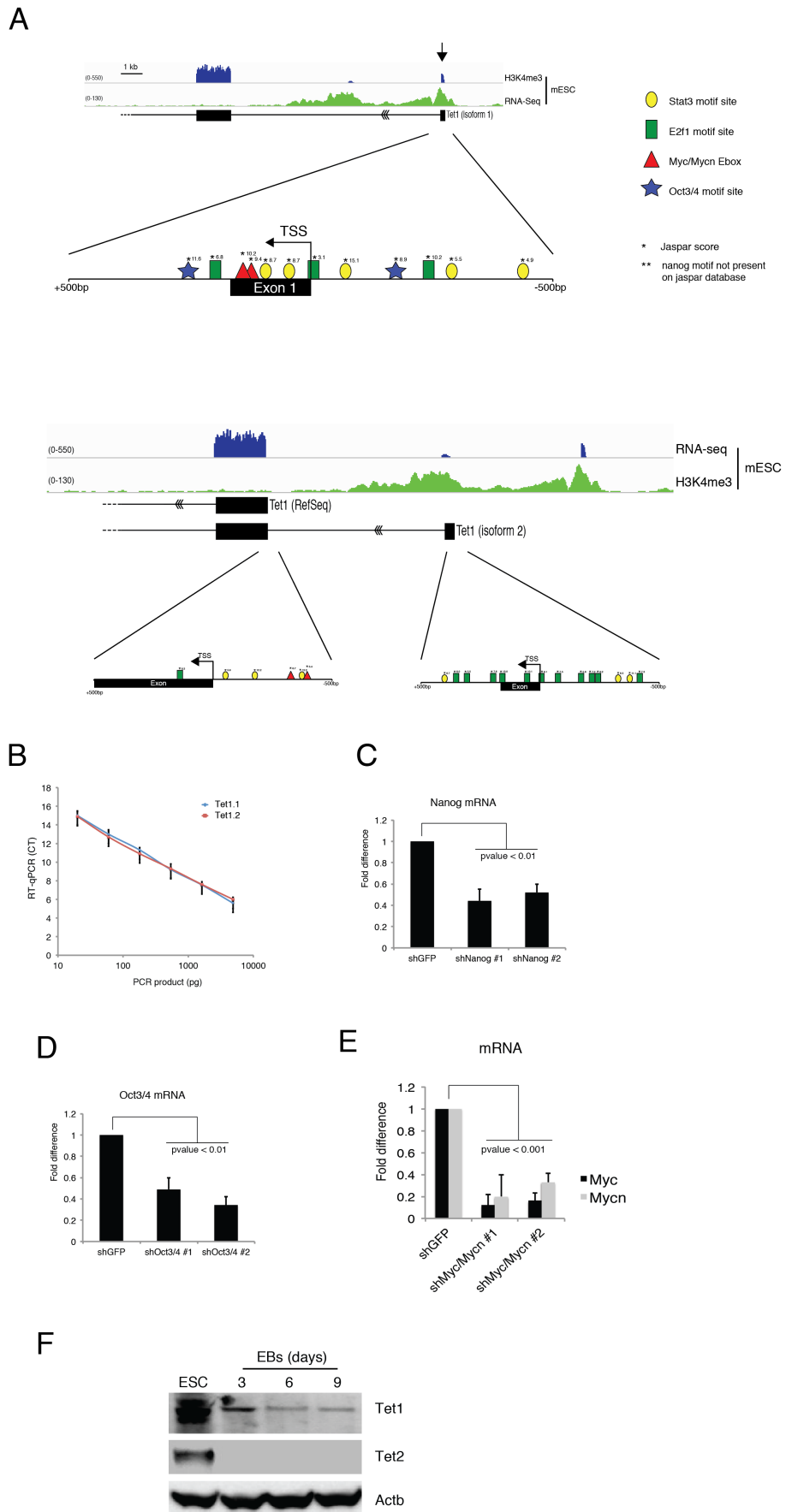
A

Stat3 motif site
E2f1 motif site
Myc/Mycn Ebox
Oct3/4 motif site

* Jaspar score

** nanog motif not present on jaspar database

B

C

Nanog mRNA

pvalue < 0.01

D

Oct3/4 mRNA

pvalue < 0.01

E

mRNA

pvalue < 0.001

Myc
Mycn

F

EBs (days)

ESC 3 6 9

Tet1
Tet2
Actb

Figure S1.

Tet1 is regulated by stemness factors in mESCs. (A) Transcription factor motif sites scanning of 1kb around Tet1.1, Tet1.2 and annotated transcriptional start site was performed using Jaspar software (http://jaspar.binf.ku.dk/). (B) Isoform-specific Tet1 primers amplification coefficient analysis (C-E) RT-qPCR of Nanog, Oct3/4, cMyc, Mycn and Tet1.2 mRNA levels in the indicated knockdown mESCs. Error bars represent the standard deviation of three independent experiments. P-value was calculated by using T-test. (F) Western blot analysis of Tet1 and Tet2 in mESC and differentiating EBs at the indicated passages. Actb was used as a loading control.
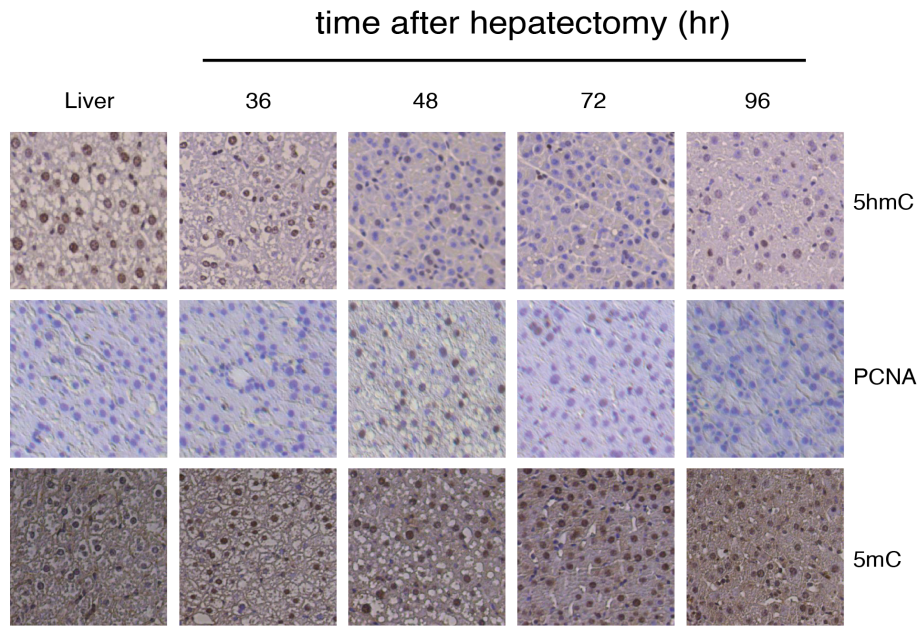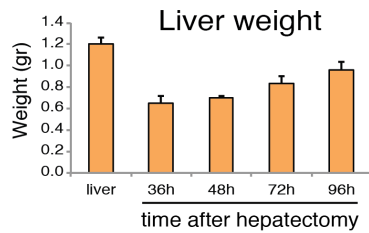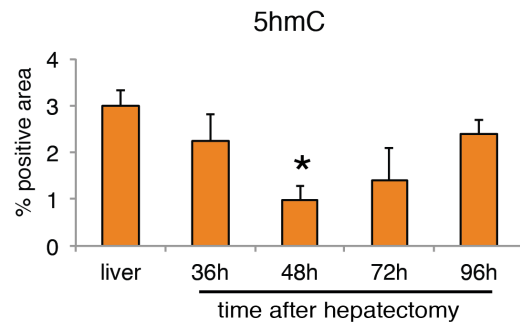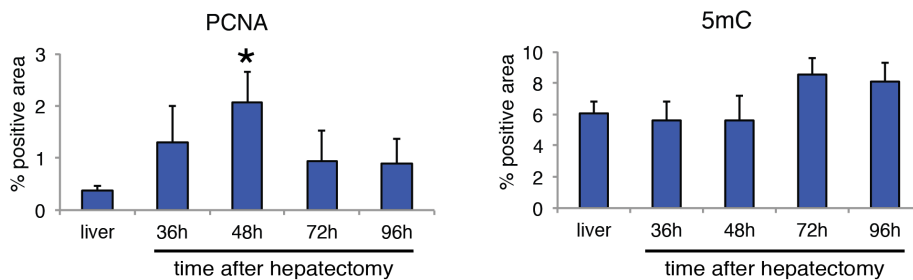
Figure S2.
5hmC decrease in proliferating liver cells *in vivo*. (A) Immunohistochemical staining of 5hmC, 5mC and Pcna in regenerating liver after partial hepatectomy. (B) Weight in grams of a normal liver or at the indicated hours after hepatectomy. (C-D) Quantification of immunohistochemical staining in (A). Error bars represent the standard deviation of three independent experiments. P-value was calculated by using T-test. * P-value < 0.01.
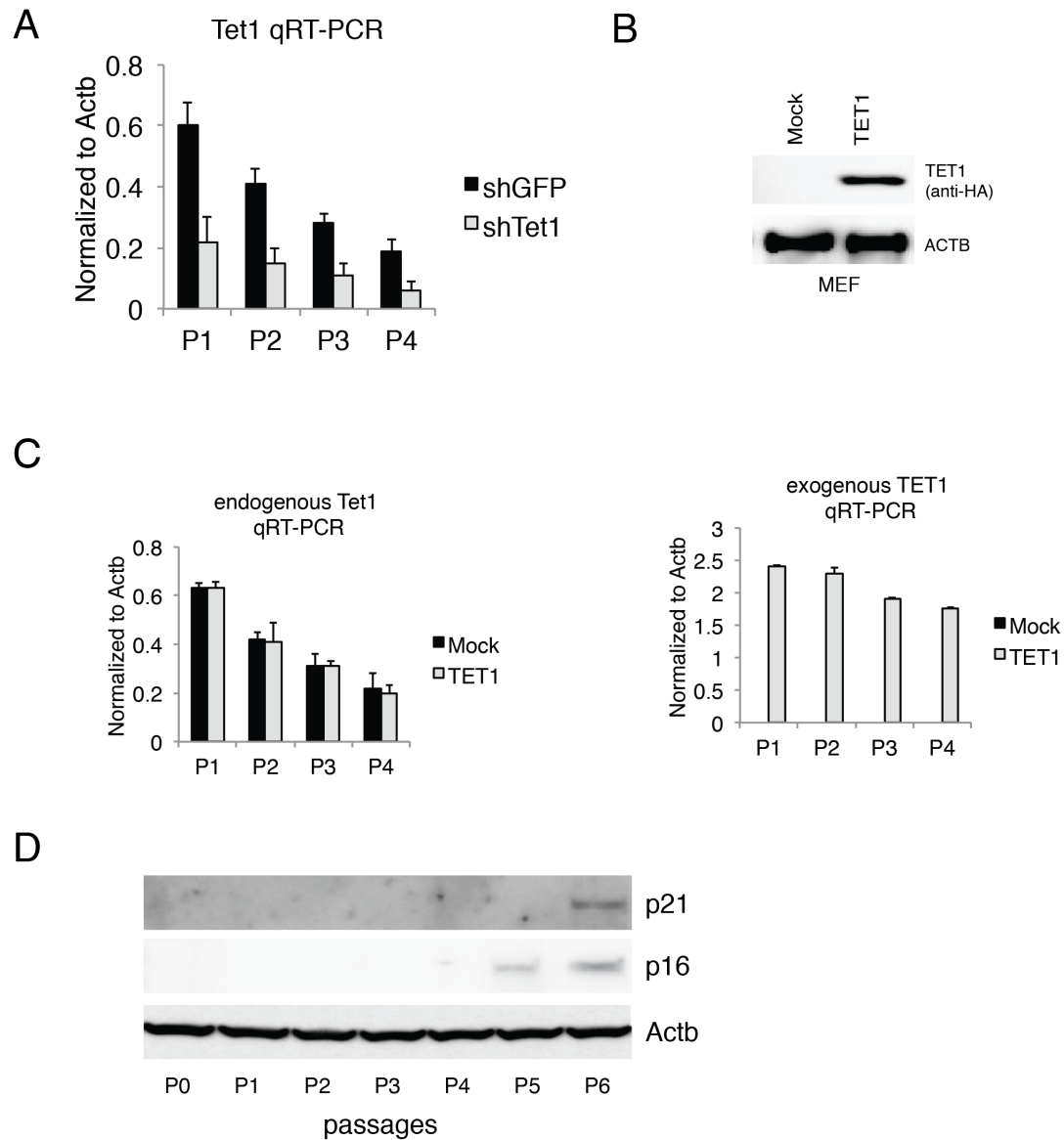
Figure S3.
Tet1 regulates proliferation in primary cells. (A) RT-qPCR of *Tet1* mRNA in shGFP or shTet1 MEFs at the indicated passages. (B) Western blot analysis of TET1 ectopic expression in MEFs transduced with empty vector or Tet1. Actb was used as a loading control. (C) RT-qPCR of the endogenous and exogenous *Tet1* mRNA in MEFs transduced with empty vector or Tet1 at the indicated passages. Error bars represent the standard deviation of three independent experiments. (D) Western blot analysis of senescence markers at the indicated passages. Actb was used as a loading control.
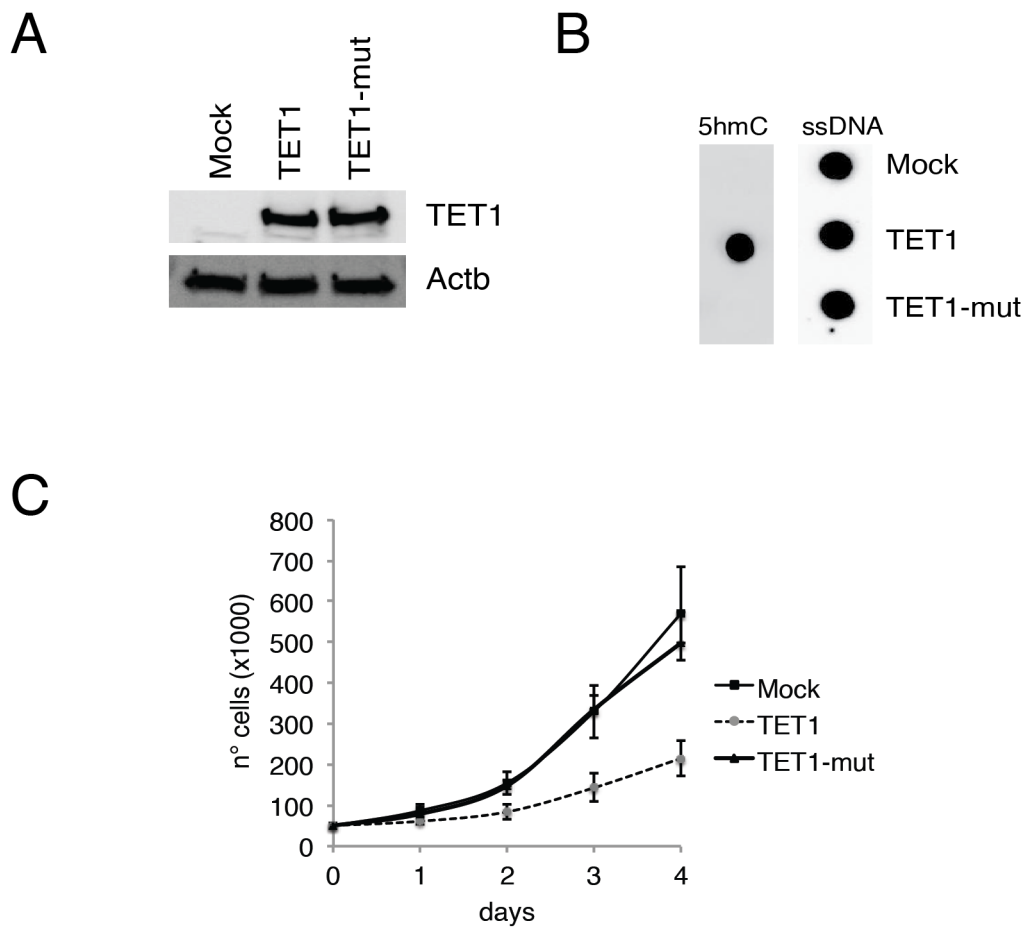
Figure S4.
Tet1 inhibition of proliferation in fibroblast requires its enzymatic activity.
(A) Western blot analysis of TET1 expression in fibroblasts transfected with empty vector, TET1 or catalytically inactive TET1. Actb was used as a loading control. (B) Dot-blot analysis of 5hmC level in fibroblasts treated as in (A). ssDNA was used as a loading control. (C) Cell growth assay in fibroblasts treated as in (A). Error bars represent the standard deviation of 3 independent experiments.
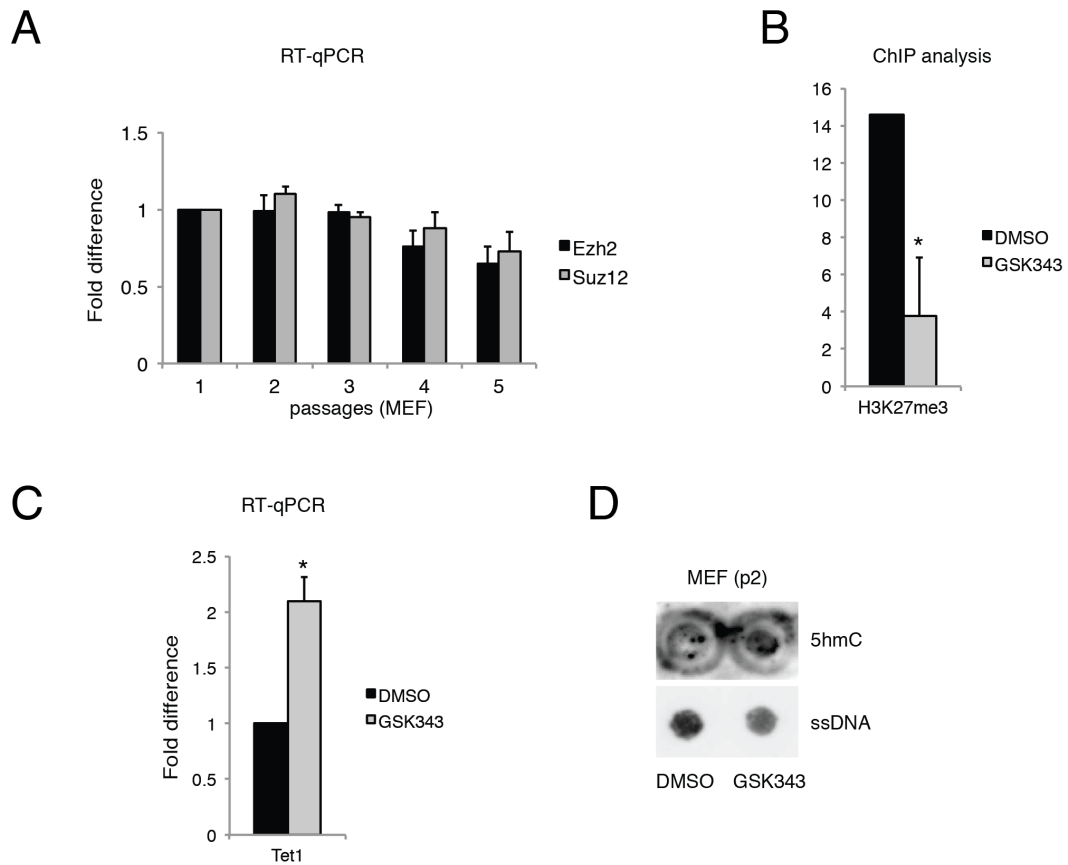
Figure S5.
Tet1 is downregulated by PRC2 in adult cells.
(A) RT-qPCR of *Suz12* and *Ezh2* mRNA in MEFs at the indicated passages. (B) qPCR of ChIP analysis for H3K27me3 in MEFs treated with DMSO (vehicle) or GSK343. (C) RT-qPCR of Tet1 mRNAs in MEFs treated with DMSO (vehicle) or GSK343. (D) Dot-blot analysis of 5hmC level in MEFs treated with DMSO (vehicle) or GSK343. SsDNA was used as a loading control. Error bars represent the standard deviation of 3 independent experiments.
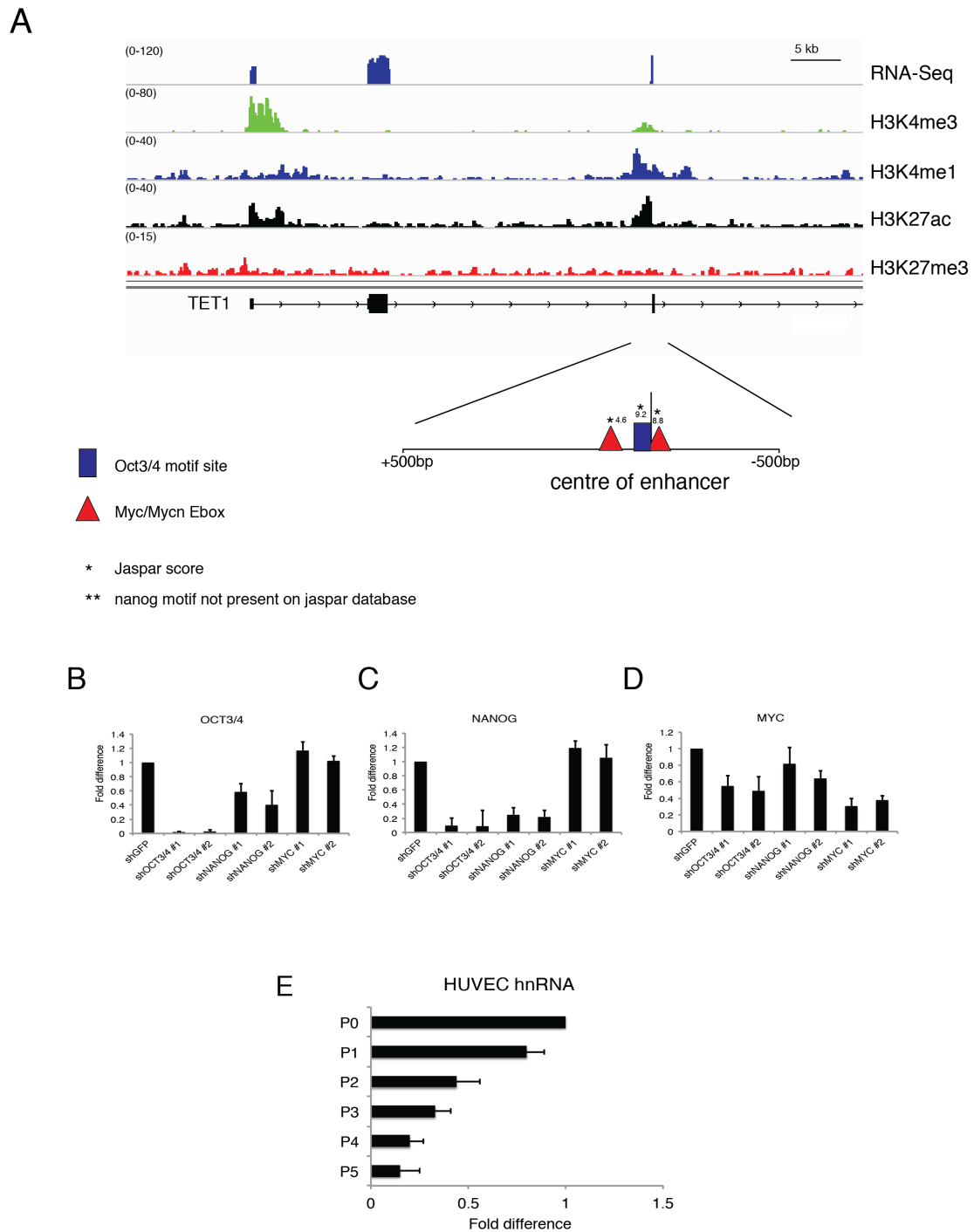
Figure S6.
Regulation of TET1 in human cells. (A) Transcription factor motif sites scanning of 1kb around human TET1 intronic enhancers site was performed using Jaspar software (http://jaspar.binf.ku.dk/). (B-C-D) RT-qPCR of OCT3/4, NANOG and MYC mRNA in hESCs silenced for the indicated transcriptional factors. (E) RT-qPCR of TET1 hnRNA in HUVECs at the indicated passages. Error bars represent the standard deviation of 3 independent experiments.

target	sequence	strand
sh mTet1	CCGGTTTCAACTCCGACGTAAATATCTCGAGATATTTACGTCGGAGTTGAAATTTTTG	fw
sh mTet1	AATTCAAAAATTTCAACTCCGACGTAAATATCTCGAGATATTTACGTCGGAGTTGAAA	rev
sh #1 hOCT3/4	CCGGTCATTCACTAAGGAAGGAATTCTCGAGAATTCCTTCCTTAGTGAATGATTTTTG	fw
sh #1 hOCT3/4	AATTCAAAAATCATTCACTAAGGAAGGAATTCTCGAGAATTCCTTCCTTAGTGAATGA	rev
sh #2 hOCT3/4	CCGGCCCTCACTTCACTGCACTGTACTCGAGTACAGTGCAGTGAAGTGAGGGTTTTTG	fw
sh #2 hOCT3/4	AATTCAAAAACCCTCACTTCACTGCACTGTACTCGAGTACAGTGCAGTGAAGTGAGGG	rev
sh #1 hNANOG	CCGGGCTTTGAAGCATCCGACTGTACTCGAGTACAGTCGGATGCTTCAAAGCTTTTTG	fw
sh #1 hNANOG	AATTCAAAAAGCTTTGAAGCATCCGACTGTACTCGAGTACAGTCGGATGCTTCAAAGC	rev
sh #2 hNANOG	CCGGGAGTATGGTTGGAGCCTAATCCTCGAGGATTAGGCTCCAACCATACTCTTTTTG	fw
sh #2 hNANOG	AATTCAAAAAGAGTATGGTTGGAGCCTAATCCTCGAGGATTAGGCTCCAACCATACTC	rev
sh #1 mOct3/4	CCGGACTGGGACACACAGTAGATAGCTCGAGCTATCTACTGTGTGTCCCAGTTTTTTG	fw
sh #1 mOct3/4	AATTCAAAAAACTGGGACACACAGTAGATAGCTCGAGCTATCTACTGTGTGTCCCAGT	rev
sh #2 mOct3/4	CCGGGGGCTCTCCCATGCATTCAAACCTCGAGGTTTGAATGCATGGGAGAGCCTTTTTG	fw
sh #2 mOct3/4	AATTCAAAAAGGCTCTCCCATGCATTCAAACCTCGAGGTTTGAATGCATGGGAGAGCC	rev

Table S1.
Oligonucleotides used for shRNAs vectors cloning.

target	sequence
mTet1	tgccagcagaaggccaact
mTet1	tcttttccctctggggcct
mTet1 isof. 1	atctgtcctggctgagtgtc
mTet1 isof. 2	ggaacattcgcggagcagc
mTet1 isof. 1-2	tgatttggaaggctttgcgg
mPcna	ttggaatcccagaacaggag
mPcna	cagtggagtggcttttgtga
mc-Myc	TGACCTAACTCGAGGAGGAGCTGGAATC
mc-Myc	AAGTTTGAGGCAGTTAAAATTATGGCTGAAGC
mMycn	ATCCATCAGCAGCACAACTAT
mMycn	TTAGCAAGTCCGAGCGTGTTCG
mTet1 hnRNA	TGCTCAAAAAGAAGCCAGAG
mTet1 hnRNA	CCAATCTACCACAAGATGCC
hTET1	AAGGGAGCCAACAAAAATGT
hTET1	AGGACTCTGGGTTCTGAAAA
hTET1 hnRNA	AACAGAAAGAACAGCCATCA
hTET1 hnRNA	CCTTGACTGTTTGCTTACCT
hc-MYC	ttcgggtagtggaaaaccag
hc-MYC	cagcagctcgaatttcttcc
hOCT3/4	GGGAGCCCTCACTTCACTGC
hOCT3/4	TGCCCCCACCCTTTGTGTTC
hNANOG	TGGACTGAGCTGGTTGCCTC
hNANOG	GGCAAGCTTTGGGGACAAGC
mActb	TCTTTGCAGCTCCTTCGTTG
mActb	ACGATGGAGGGGAATACAGC
mNanog	aagtacctcagcctccagca
mNanog	gtgctgagcccttctgaatc
mOct3/4	GGGAGCCCTCACTTCACTGC
mOct3/4	TGCCCCCACCCTTTGTGTTC
hActb	agaaaatctggcaccacacc
hActb	ggggtgttgaaggtctcaaa
mSuz12	AGGCTGCCTCCATTTGAGA
mSuz12	TGGTTTCTCCTGTCCATCG
mEzh2	TGCCTATAATGTACTCTTGGTCG
mEzh2	GCCATCCTGATCCAGAACTTCA

Table S2.
Oligonucleotides used for RT-qPCR of mRNAs.

target	sequence	strand
mTet1 (a)	ACGTCCCTAGCTGTTCTGGA	fw
mTet1 (a)	AGCTTCAGGTCCCCGATTTG	rev
mTet1 (b)	CAGCTACACTCCTAGAGGTC	fw
mTet1 (b)	TGTACAAACCAGTTGCAGAG	rev
mTet1 (c)	TCTTGCATCATCATGCTCTG	fw
mTet1 (c)	AAGGCAGTATAGGAGTTCCC	rev
hTET1 (d)	TCTTGACACCTCTCTACGTC	fw
hTET1 (d)	GGATTTACCCAAACTGGAGC	rev
hTET1 (e)	GTTGAAGCCTCCTGTGATTT	fw
hTET1 (e)	GCTTAGCAACTCCAAAGTGA	rev

Table S3.
Oligonucleotides used for qPCR of ChIP experiments.