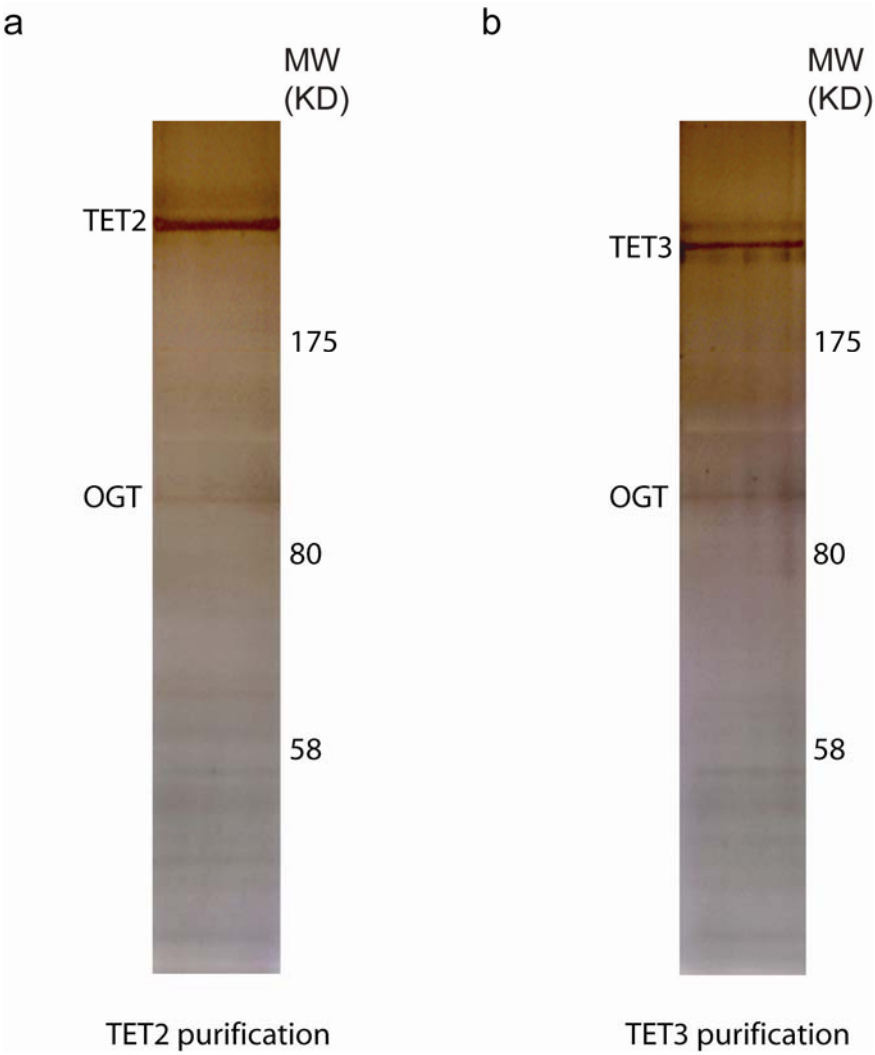
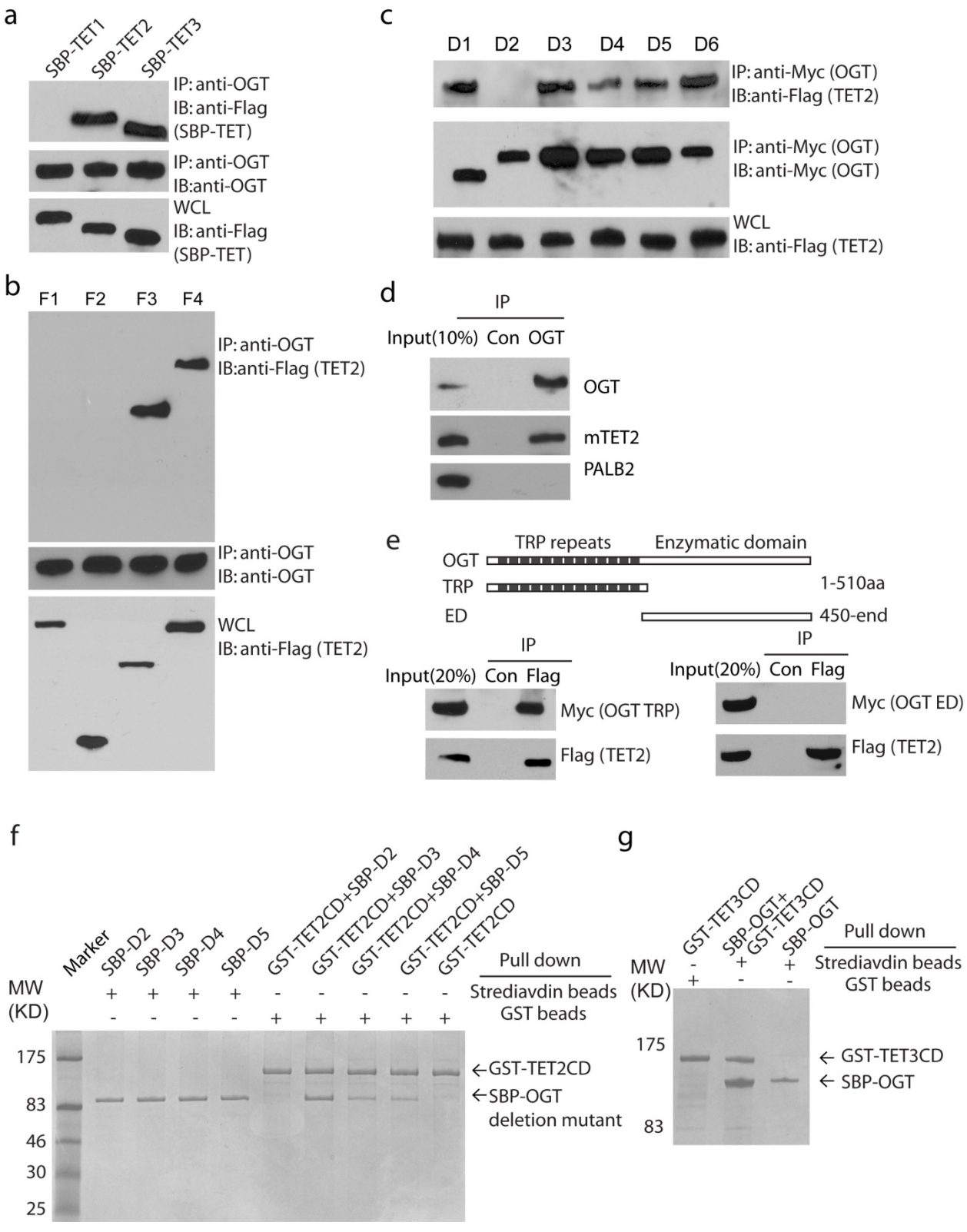
Supplementary Figures



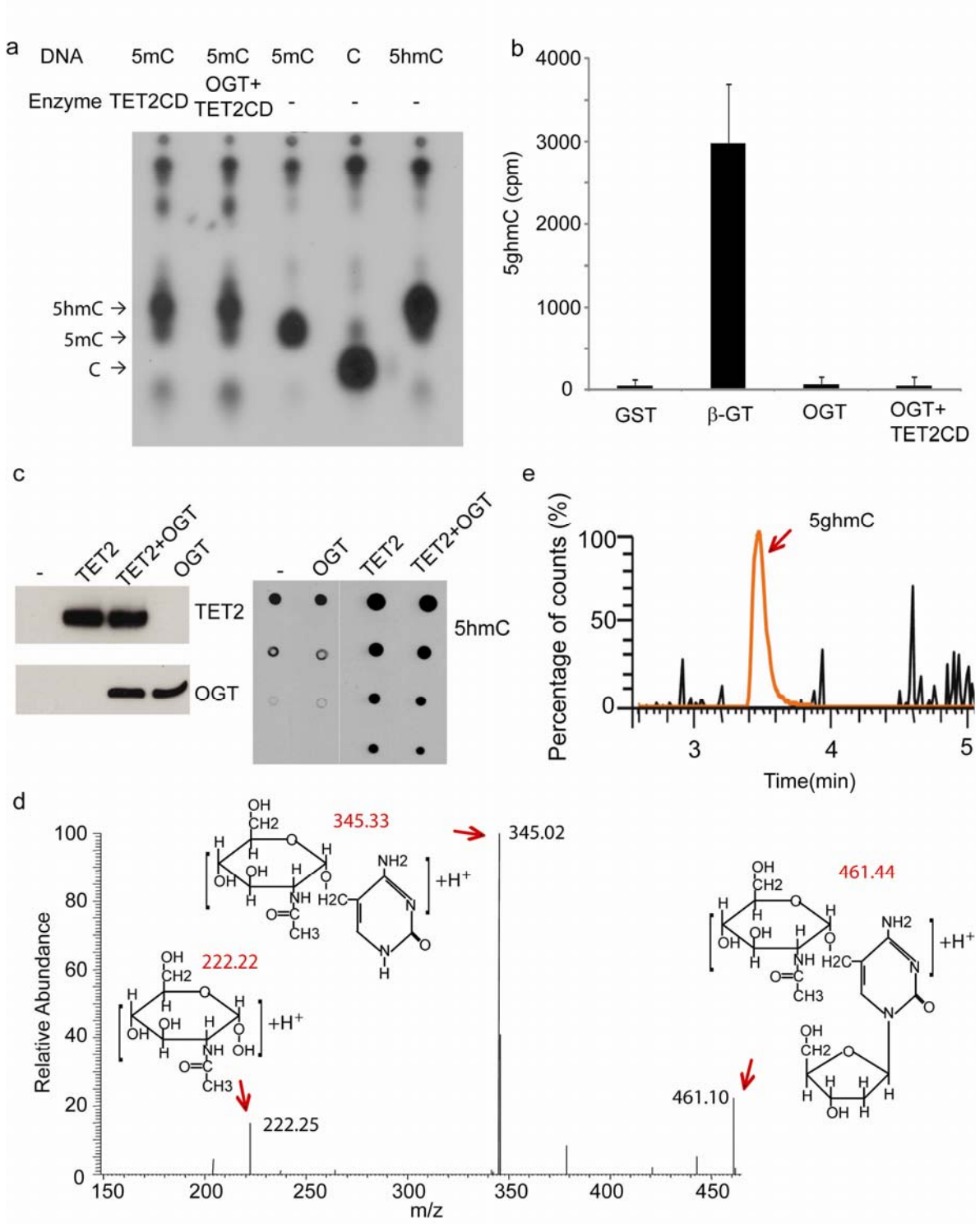
Supplementary Fig. 1. OGT is a partner of TET2 and TET3.

(a, b) Silver staining of SBP-TET2 (**a**) and TET3 (**b**) purifications.


a
SBP-TET1
SBP-TET2
SBP-TET3
IP: anti-OGT
IB: anti-Flag
(SBP-TET)
IP: anti-OGT
IB:anti-OGT
WCL
IB: anti-Flag
(SBP-TET)
b
F1
F2
F3
F4
IP: anti-OGT
IB:anti-Flag (TET2)
IP: anti-OGT
IB: anti-OGT
WCL
IB: anti-Flag (TET2)
c
D1
D2
D3
D4
D5
D6
IP: anti-Myc (OGT)
IB:anti-Flag (TET2)
IP: anti-Myc (OGT)
IB: anti-Myc (OGT)
WCL
IB: anti-Flag (TET2)
d
IP
Input(10%)
Con
OGT
OGT
mTET2
PALB2
e
TRP repeats
Enzymatic domain
OGT
TRP
1-510aa
ED
450-end
IP
Input(20%)
Con
Flag
Myc (OGT TRP)
Flag (TET2)
IP
Input(20%)
Con
Flag
Myc (OGT ED)
Flag (TET2)
f
Marker
SBP-D2
SBP-D3
SBP-D4
SBP-D5
GST-TET2CD+SBP-D2
GST-TET2CD+SBP-D3
GST-TET2CD+SBP-D4
GST-TET2CD+SBP-D5
GST-TET2CD
Pull down
Strediavdin beads
GST beads
MW
(KD)
175
83
46
30
25
←GST-TET2CD
←SBP-OGT
deletion mutant
g
GST-TET3CD
SBP-OGT+
GST-TET3CD
SBP-OGT
Pull down
Strediavdin beads
GST beads
MW
(KD)
175
83
← GST-TET3CD
← SBP-OGT

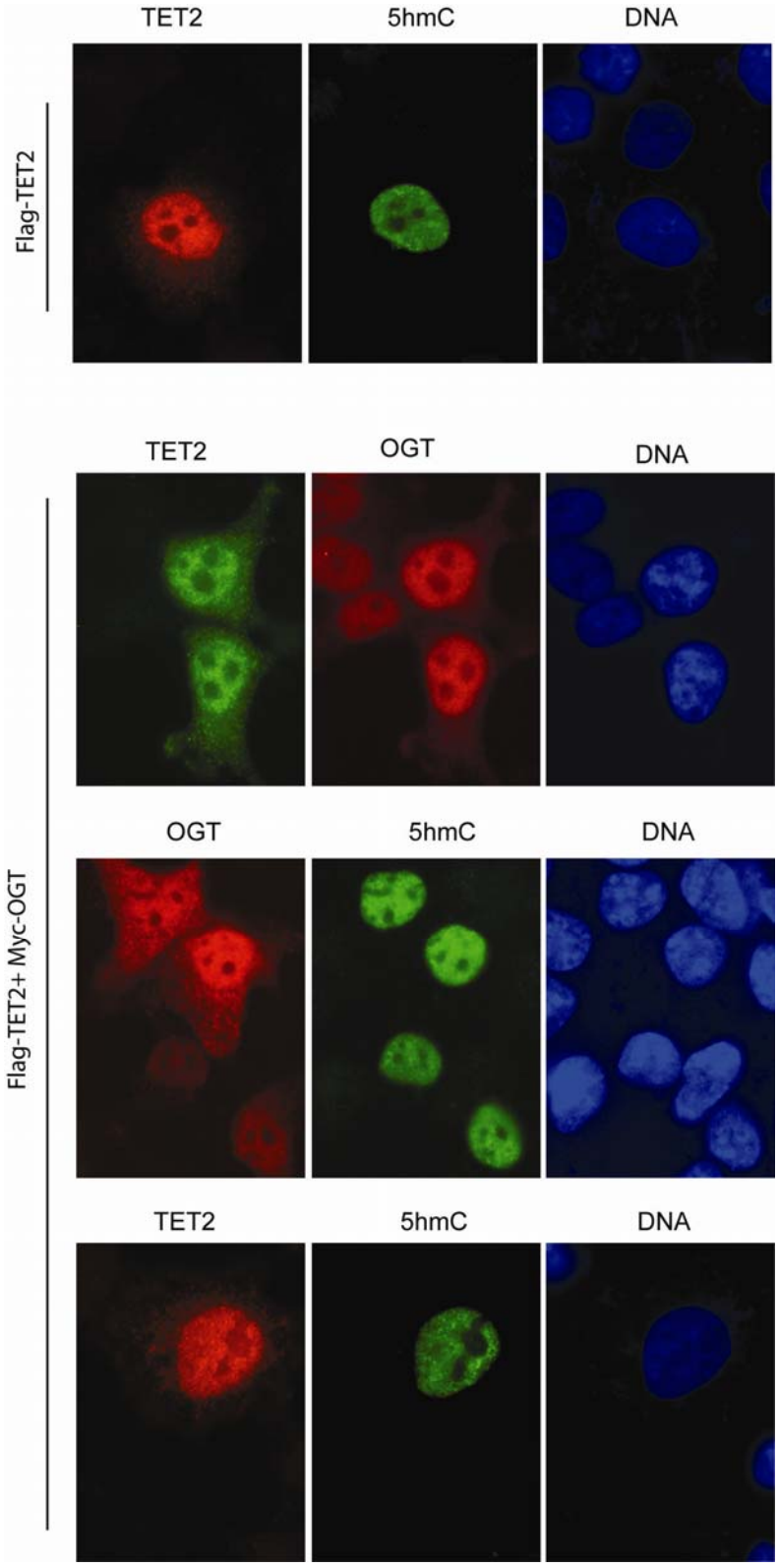
Supplementary Fig. 2. OGT interacts with TET2 and TET3.

(a) OGT interacts with TET2 and TET3 but not TET1. SBP tagged TET1-3 were expressed in 293T cells. Cells lysates were examined by IP and Western blot with indicated antibodies. The whole cell lysates (WCL) was used to examine the input of SBP-TET proteins by Western blot with anti-Flag antibody. **(b)** The DSBH domain of TET2 interacts with OGT. SBP tagged TET2 mutants were expressed in 293T cells. The F3 mutant containing the DSBH domain (catalytic domain of TET2) interacts with OGT. **(c)** TPR5 and 6 of OGT interacts with TET2. The D2 mutant of OGT that is deleted TPR5 and 6 abolished the interaction with TET2. **(d)** TET2 interacts with OGT endogenously in ES cells. ES cell lysates were examined by indicated antibodies. The whole cell lysates were used as the input (10%). Irrelevant IgG was used as the IP control (Con). PALB2 was used as negative control. **(e)** TPR repeats of OGT, but not the enzymatic domain (ED), are required for the interaction with TET2. Myc-OGT, TPR and ED coexpressed with SBP-TET2. IP and Western blot were performed to examine the interaction. **(f)** The D3, D4 and D5 mutants of OGT, but not the D2 mutant, directly bind TET2CD. Sf9 cells were infected with baculoviruses encoding SBP-OGT mutants and GST-TET2CD. The protein complex was purified by Streptavidin beads or GST beads as indicated. **(g)** OGT directly binds TET3CD. Sf9 cells were infected with baculoviruses encoding SBP-OGT and GST-TET3CD. The protein complex was purified by Streptavidin beads or GST beads as indicated.


a
DNA
5mC
5mC
5mC
C
5hmC
Enzyme
TET2CD
OGT+ TET2CD
-
-
-
5hmC
5mC
C
b
5ghmC (cpm)
4000
3000
2000
1000
0
GST
β-GT
OGT
OGT+ TET2CD
c
-
TET2
TET2+OGT
OGT
TET2
OGT
-
TET2+OGT
5hmC
e
Percentage of counts (%)
100
50
0
5ghmC
3
4
5
Time(min)
d
Relative Abundance
100
80
60
40
20
0
345.33
345.02
222.22
222.25
461.44
461.10
+H+
150
200
250
300
350
400
450
m/z

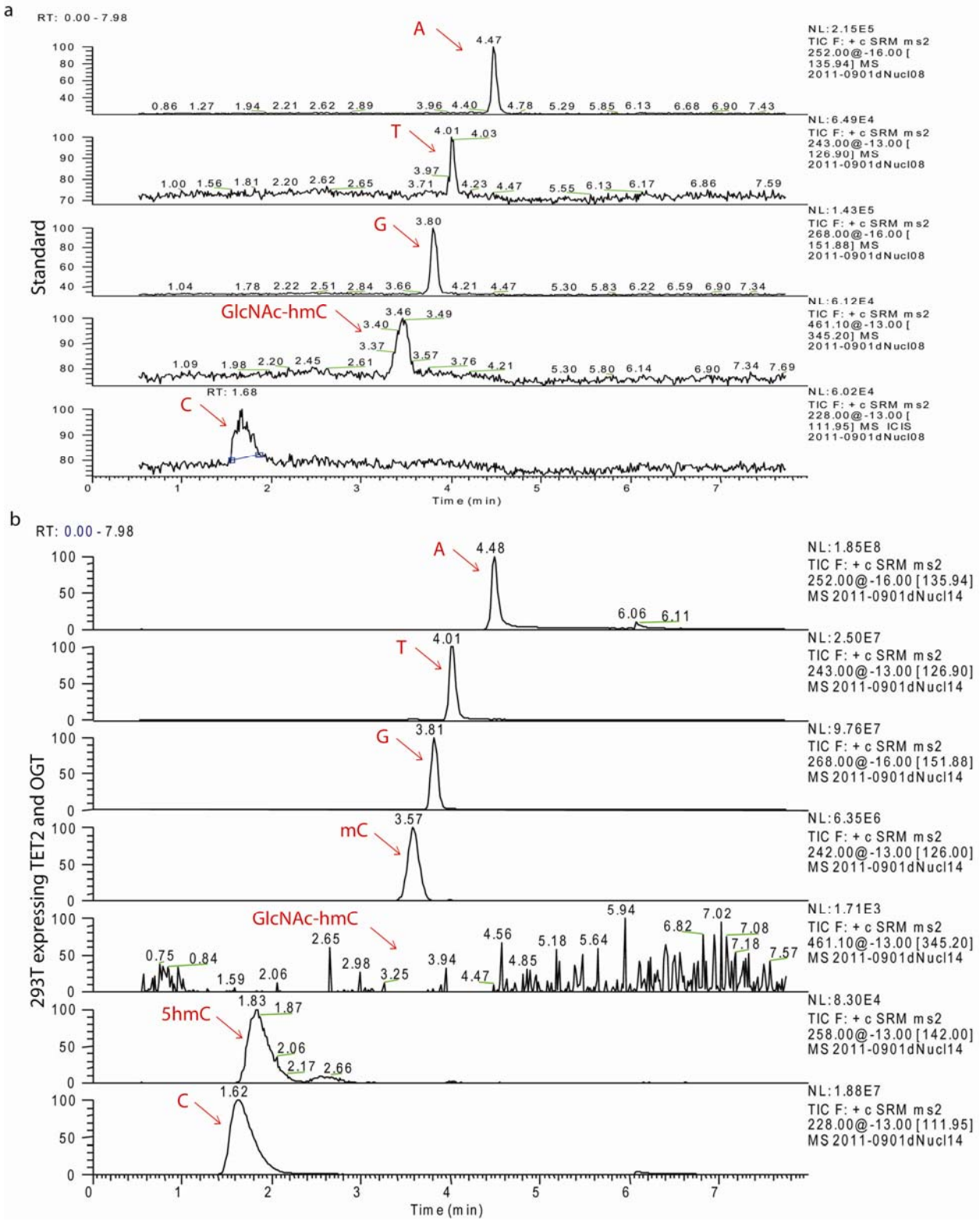
Supplementary Fig. 3. OGT could not glycosylate 5hmC.

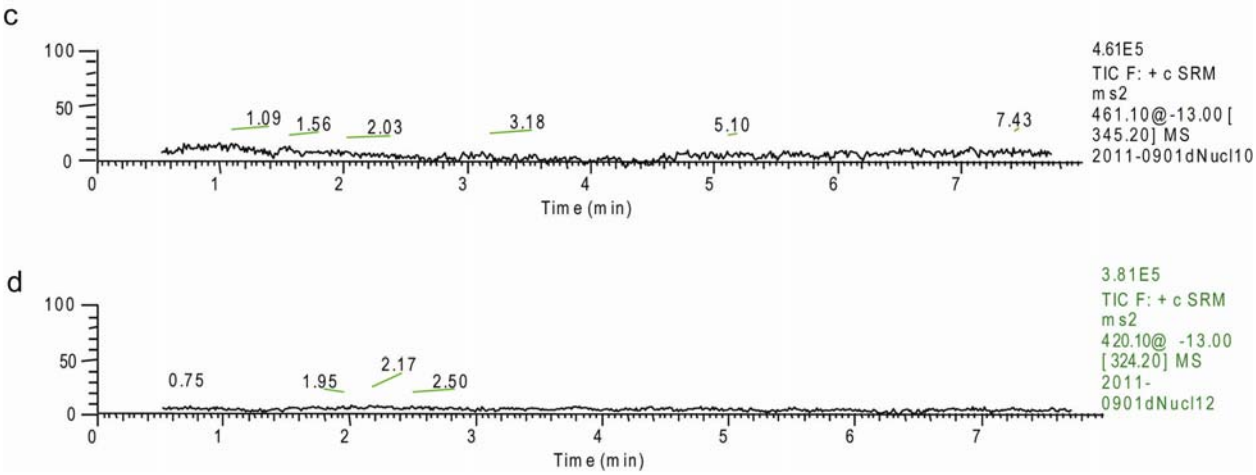
(a) OGT could not O-GlcNAcylate 5hmC *in vitro*. DNA containing 5hmC was incubated with TET2CD and/or OGT. Migration of labeled C and its modified forms were examined by TLC. ^{32}P labeled C, 5mC and 5hmC was used as markers. **(b)** β-GT but not OGT could O-GlcNAcylate 5hmC *in vitro*. Biotin labeled 5hmC oligos incubated with indicted recombinant proteins and ^{3}H-labeled UDP-GlcNAc. Following 1 hour reaction, DNA was purified by streptavidin beads and subjected to scintillation counter. All error bars denote s.d., n=3. **(c)** OGT does not regulate TET2-dependent 5hmC. Genomic DNA from 293T cells expressing TET2CD and/or OGT were examined by dot blot with anti-5hmC antibody. A Western blot shows the expression level of TET2 and OGT. **(d)** Mass spectrometry analysis of 5-glycosyl-hydroxylmethyl C (5ghmC) derived from in *vitro assay* using β-GT. Structural formulas are shown for the major peaks. **(e)** HPLC–tandem mass spectrometry (MS/MS) detection of genomic 5ghmC. MRM elution profiles of negative-ion mass transitions from a precursor to its two product ions are shown. Red line represents DNA hydrolysates of the in vitro synthetic 5ghmC; black line represents genomic DNA hydrolysates from 293T cells expressing TET2 and OGT.


TET2
5hmC
DNA
Flag-TET2
TET2
OGT
DNA
OGT
5hmC
DNA
TET2
5hmC
DNA
Flag-TET2+ Myc-OGT

Supplementary Fig. 4. OGT does not affect TET2-dependent 5hmC *in vivo*.

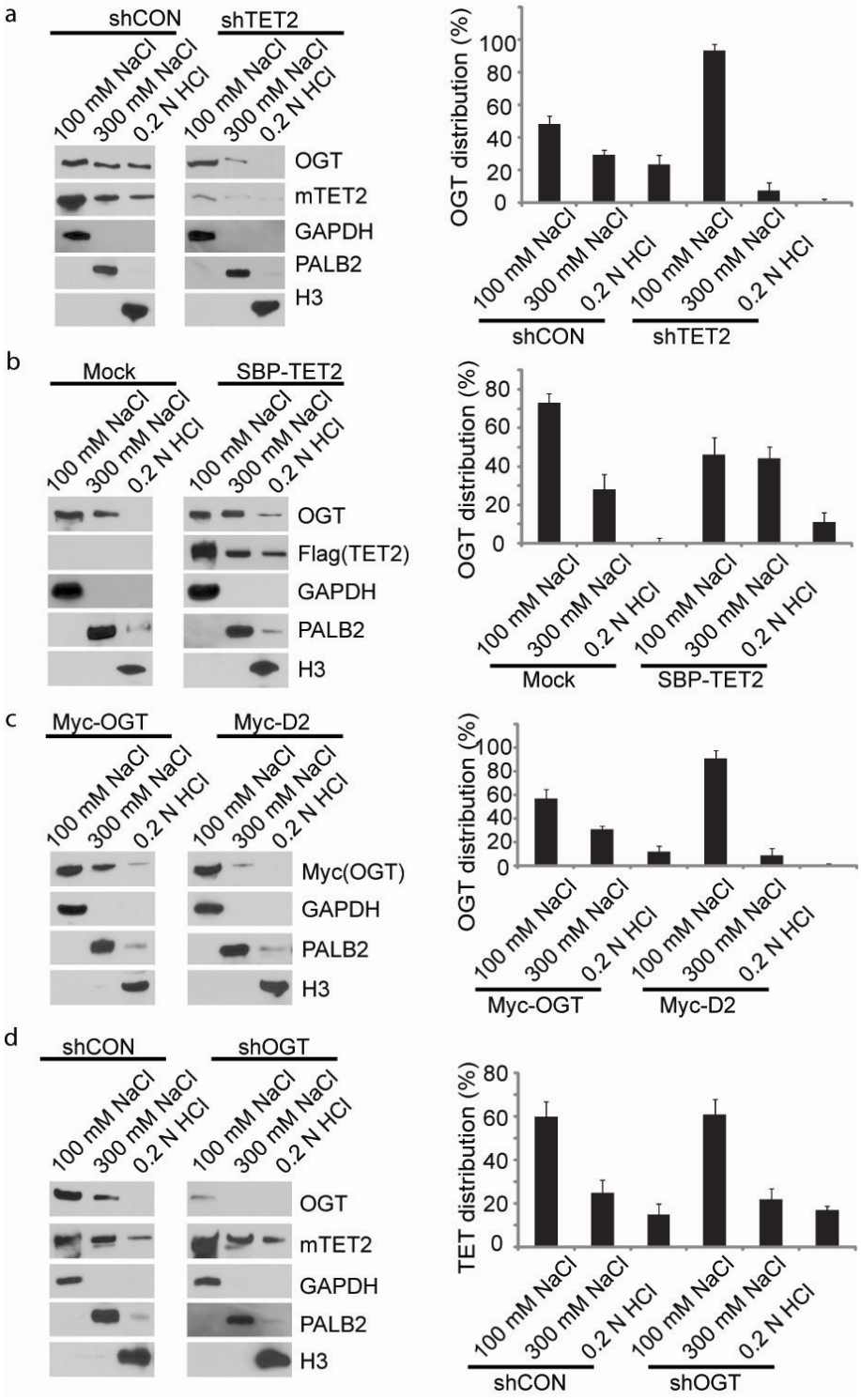
293T cells were co-transfected with plasmids encoding Flag-TET2 and Myc-OGT at a ratio of 1:10 to ensure that TET2 positive expressing cells also expressed OGT. The cells were fixed and denatured with 0.2 N NaOH. Anti-5hmC, Myc and Flag antibodies were used for immumofluorescence staining.


a
RT: 0.00 - 7.98
A
4.47
NL:2.15E5
TIC F: + c SRM ms2
252.00@-16.00 [
135.94] MS
2011-0901dNucl08
T
4.01
4.03
NL:6.49E4
TIC F: + c SRM ms2
243.00@-13.00 [
126.90] MS
2011-0901dNucl08
G
3.80
NL:1.43E5
TIC F: + c SRM ms2
268.00@-16.00 [
151.88] MS
2011-0901dNucl08
Standard
GlcNAc-hmC
3.46
3.49
3.40
3.37
3.57
NL:6.12E4
TIC F: + c SRM ms2
461.10@-13.00 [
345.20] MS
2011-0901dNucl08
RT: 1.68
C
NL:6.02E4
TIC F: + c SRM ms2
228.00@-13.00 [
111.95] MS ICIS
2011-0901dNucl08
Time (min)
b
RT: 0.00 - 7.98
A
4.48
6.06
6.11
NL:1.85E8
TIC F: + c SRM ms2
252.00@-16.00 [135.94]
MS 2011-0901dNucl14
T
4.01
NL:2.50E7
TIC F: + c SRM ms2
243.00@-13.00 [126.90]
MS 2011-0901dNucl14
G
3.81
NL:9.76E7
TIC F: + c SRM ms2
268.00@-16.00 [151.88]
MS 2011-0901dNucl14
mC
3.57
NL:6.35E6
TIC F: + c SRM ms2
242.00@-13.00 [126.00]
MS 2011-0901dNucl14
293T expressing TET2 and OGT
GlcNAc-hmC
2.65
2.98
3.25
3.94
4.47
4.56
4.85
5.18
5.64
5.94
6.82
7.02
7.08
7.18
7.57
NL:1.71E3
TIC F: + c SRM ms2
461.10@-13.00 [345.20]
MS 2011-0901dNucl14
5hmC
1.83
1.87
2.06
2.17
2.66
NL:8.30E4
TIC F: + c SRM ms2
258.00@-13.00 [142.00]
MS 2011-0901dNucl14
C
1.62
NL:1.88E7
TIC F: + c SRM ms2
228.00@-13.00 [111.95]
MS 2011-0901dNucl14
Time (min)

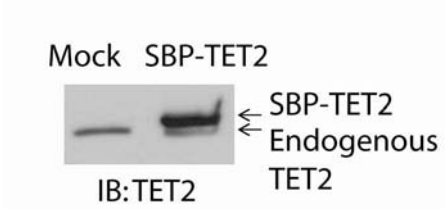


Supplementary Fig. 5. 5ghmC could not be detected by LC-MS/MS.

(a) LC-MS/MS analyses show the standard samples using MRM mode. **(b)** The genomic DNA isolated from 293T overexpressing TET2 and OGT was analyzed by LC-MS/MS using MRM mode. **(c)** The genomic DNA from mouse brain was analyzed by LC-MS/MS using SRM mode to examine the existence of GlcNAc-hmC. **(d)** The genomic DNA from 293T overexpressing TET2 and OGT was analyzed by LC-MS/MS to examine the existence Glucose-hmC.

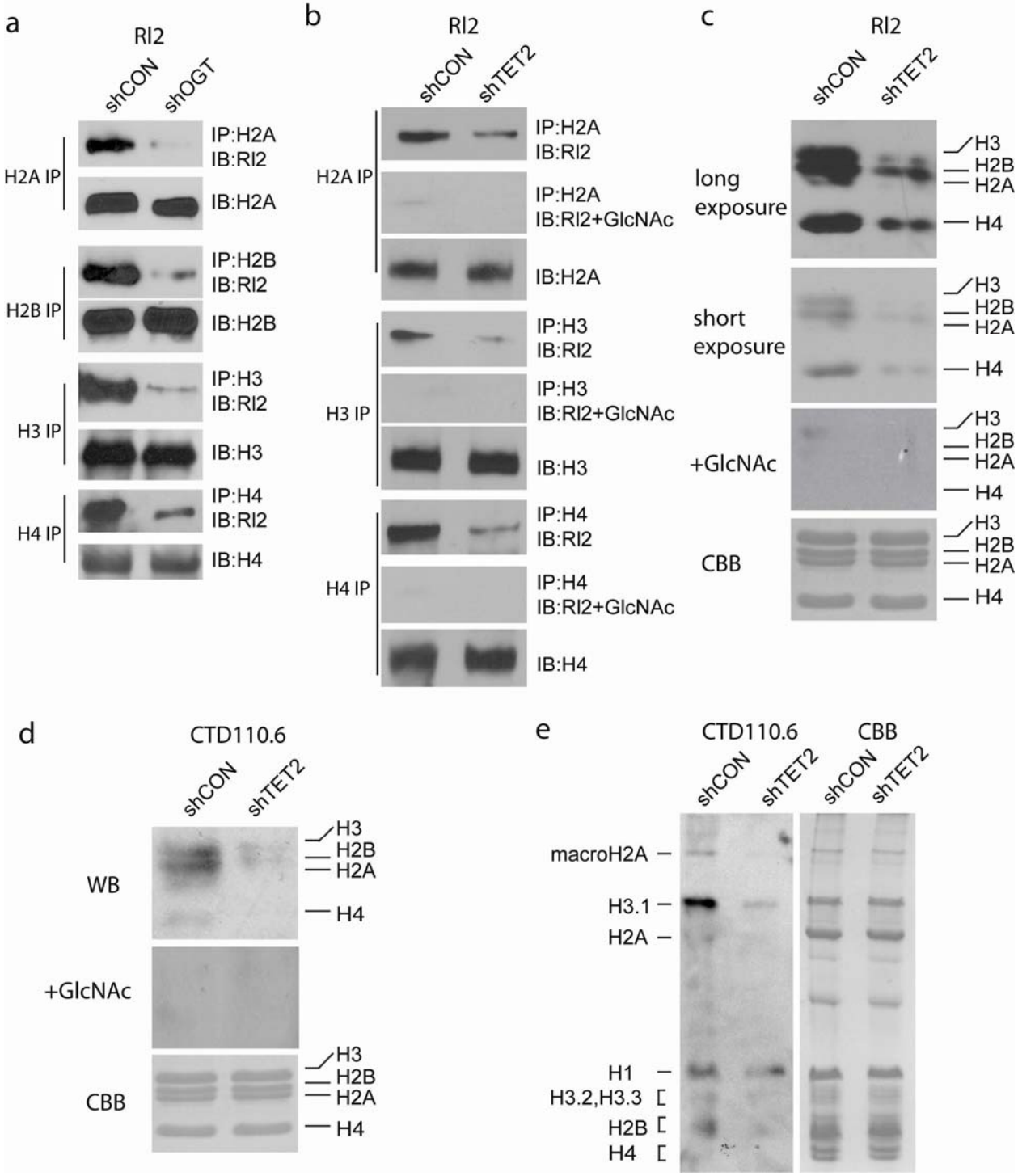

a
shCON
shTET2
100 mM NaCl
300 mM NaCl
0.2 N HCl
OGT
mTET2
GAPDH
PALB2
H3
OGT distribution (%)
b
Mock
SBP-TET2
Flag(TET2)
c
Myc-OGT
Myc-D2
Myc(OGT)
d
shOGT
TET distribution (%)

Supplementary Fig. 6. TET2 facilitates OGT to associate with the chromatin.
(a) Down-regulation of TET2 abrogates the chromatin-bound OGT. ES cells expressing control shRNA (shCON) or TET2 shRNA were lyzed with NETN100 buffer. Chromatin-associated proteins were further eluted with NETN300 buffer and 0.2 N HCl. All three fractions were examined by Western blotting with indicated antibodies. GAPDH, PALB2 and histone H3 were used as the markers in three different fractions. (**b)** Up-regulation of TET2 induces OGT to associate with chromatin. 293T cells were transfected with empty vector or vector encoding SBP-TET2. Exogenous TET2 was examined by anti-Flag antibody. The endogenous and exogenous TET2 was compared in Supplementary Fig. 7. (**c)** The interaction between OGT and TET2 facilitates the chromatin retention of OGT. 293T cells stably expressing SBP-TET2 were transfected with plasmids encoding Myc-OGT or Myc-D2 mutant. (**d)** Down-regulation of OGT does not regulate chromatin-bound TET2. Histogram in each panel shows the percentage of the distribution of OGT in each fraction using Glyco Band-Scan software. All error bars denote s.d., n=3.



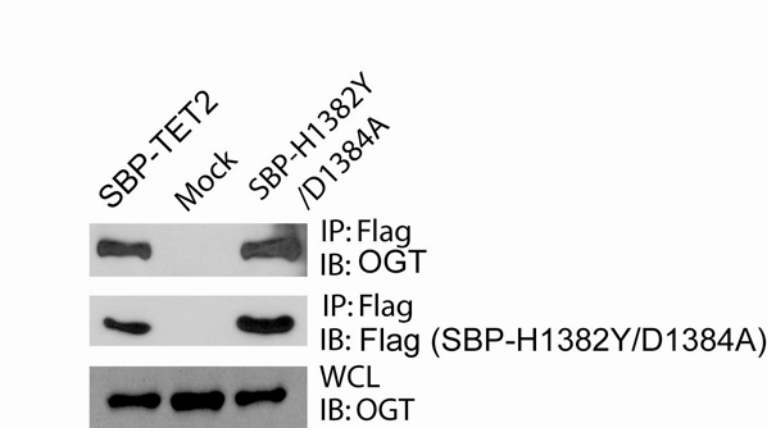
Supplementary Fig. 7. Expression of exogenous TET2 is higher than that of endogenous TET2.

Endogenous and exogenous TET2 in 293T cells were examined by Western blot with anti-TET2 antibody.


a
Rl2
shCON
shOGT
H2A IP
IP:H2A
IB:Rl2
IB:H2A
H2B IP
IP:H2B
IB:Rl2
IB:H2B
H3 IP
IP:H3
IB:Rl2
IB:H3
H4 IP
IP:H4
IB:Rl2
IB:H4
b
Rl2
shCON
shTET2
H2A IP
IP:H2A
IB:Rl2
IP:H2A
IB:Rl2+GlcNAc
IB:H2A
H3 IP
IP:H3
IB:Rl2
IP:H3
IB:Rl2+GlcNAc
IB:H3
H4 IP
IP:H4
IB:Rl2
IP:H4
IB:Rl2+GlcNAc
IB:H4
c
Rl2
shCON
shTET2
long exposure
short exposure
+GlcNAc
CBB
H3
H2B
H2A
H4
d
CTD110.6
shCON
shTET2
WB
+GlcNAc
CBB
H3
H2B
H2A
H4
e
CTD110.6
CBB
shCON
shTET2
shCON
shTET2
macroH2A
H3.1
H2A
H1
H3.2,H3.3
H2B
H4

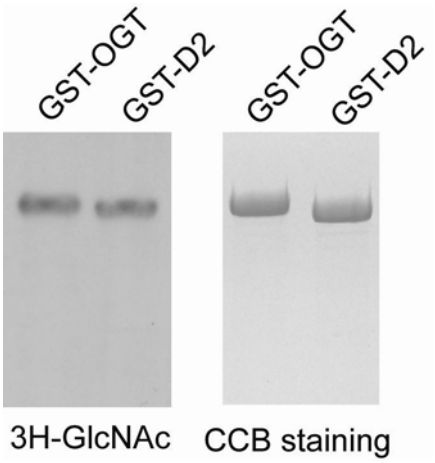
Supplementary Fig. 8. TET2 regulates histone GlcNAcylation.

(a) Down-regulation of OGT impairs H2A, H2B, H3 and H4 GlcNAcylation in ES cells. ES cells were treated with control shRNA or OGT shRNA. Histones GlcNAcylation was examined by IP with indicated antibodies and blot with anti-GlcNAc antibody (RL2). **(b)** TET2 regulates histone GlcNAcylation. TET2 was knocked down by shRNA in ES cell. Histones GlcNAcylation was examined by IP with indicated antibodies and blot with anti-GlcNAc antibody (RL2). 1 M of free GlcNAc was added into the primary antibody blotting solution to compete binding with anti-GlcNAc antibody. **(c, d)** Acid extracted histones from control shRNA or TET2 shRNA treat ES cells were blotted with Rl2 (**c**) or CTD110.6 (**d**) antibodies in the presence or absence of 1 M GlcNAc. The loading of histones were examined by Coomassie blue staining. **(e)** Histone fractions were also examined by 15 % TAU gel and Western blot with CTD110.6. The position of each histone was indicated.

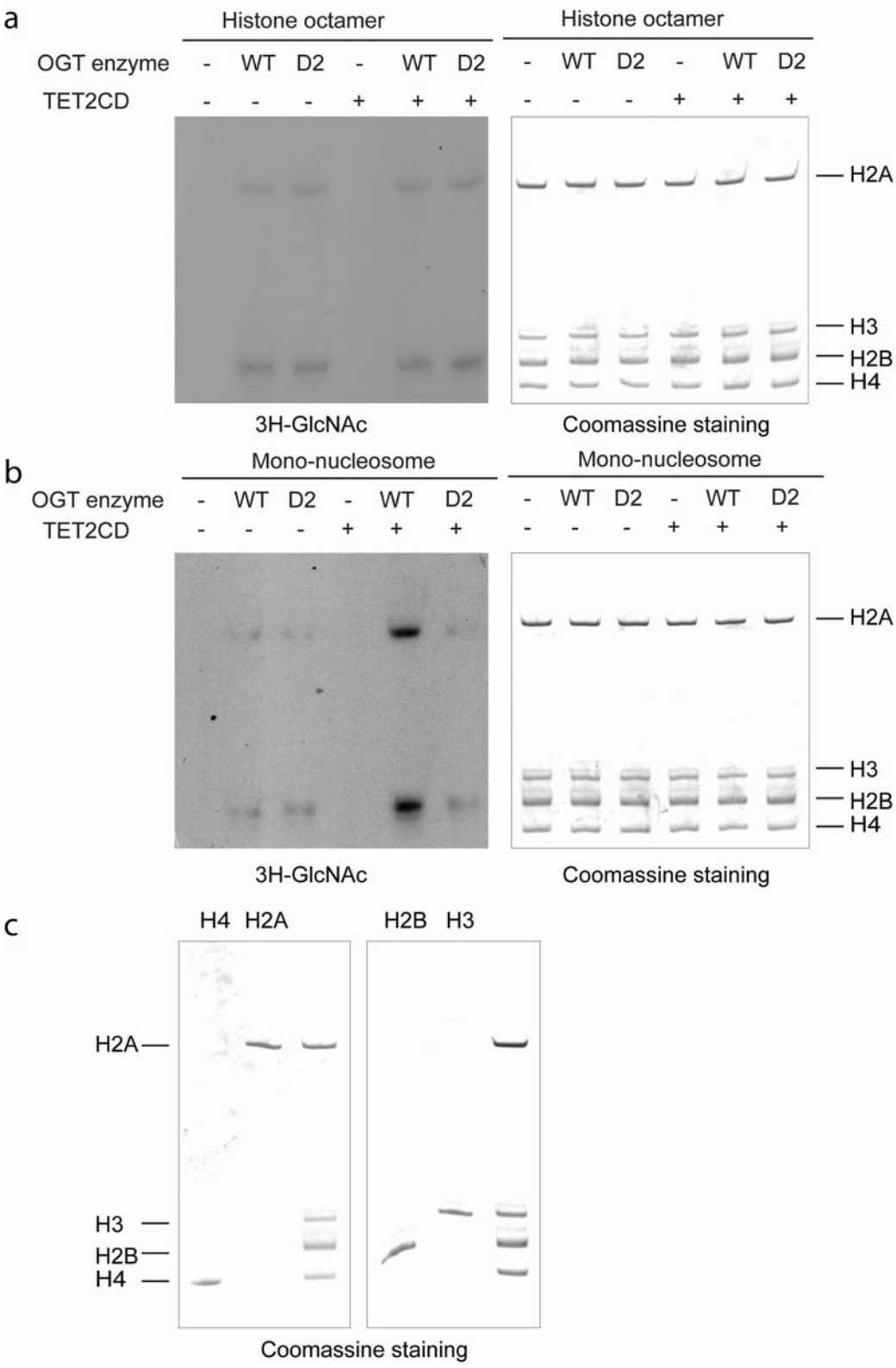


Supplementary Fig. 9. OGT interacts with TET2 (H1382Y/D1384A)

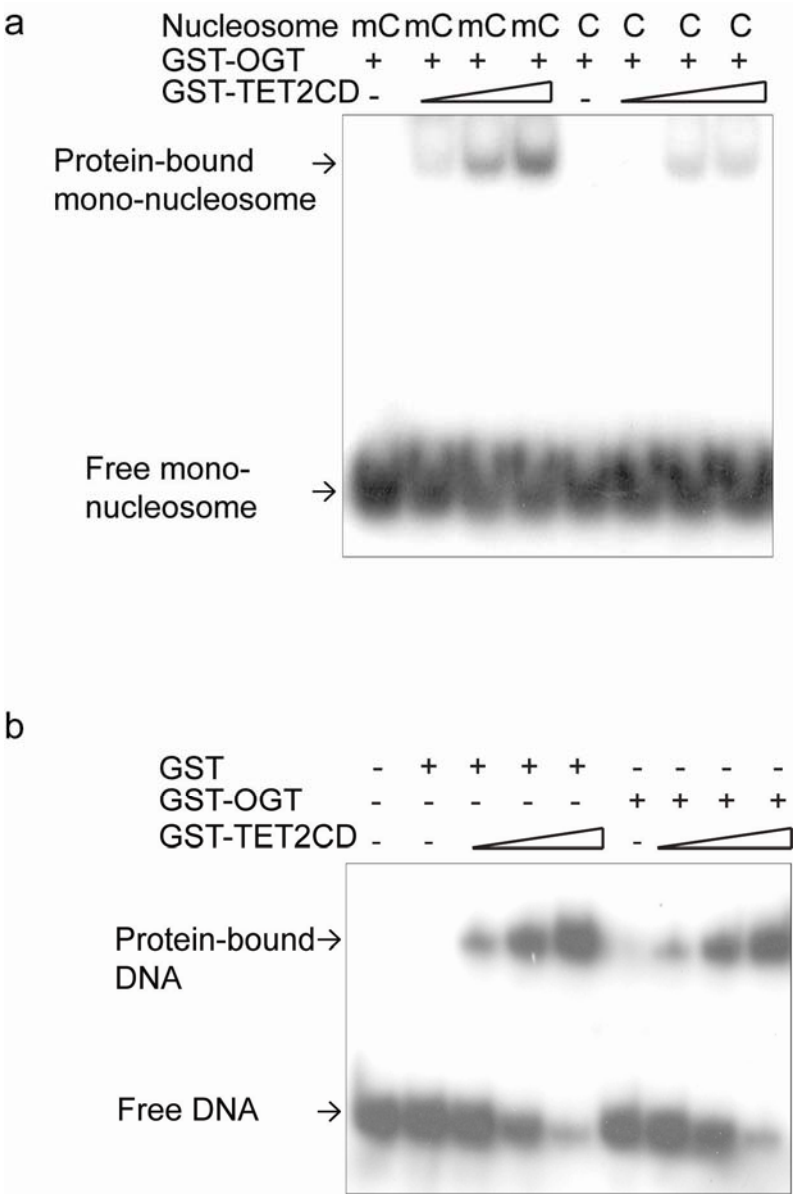
SBP tagged TET2 (H1382Y/D1384A) and wild type TET2 were expressed in 293T cells. Cells lysates were examined by IP and Western blot with indicated antibodies. The whole cell lysates (WCL) was used to examine the input of SBP-TET2 proteins by Western blot with anti-Flag antibody.



Supplementary Fig. 10. The D2 mutant of OGT does not abolish the enzymatic activity. GST recombinant OGT and the D2 mutant were incubated in the *in vitro* GlcNAcylation assay. OGT auto-glycosylation was examined to determine the enzymatic activity (left panel). The loading of the recombinant proteins was examined by Coomassie blue staining (right panel).

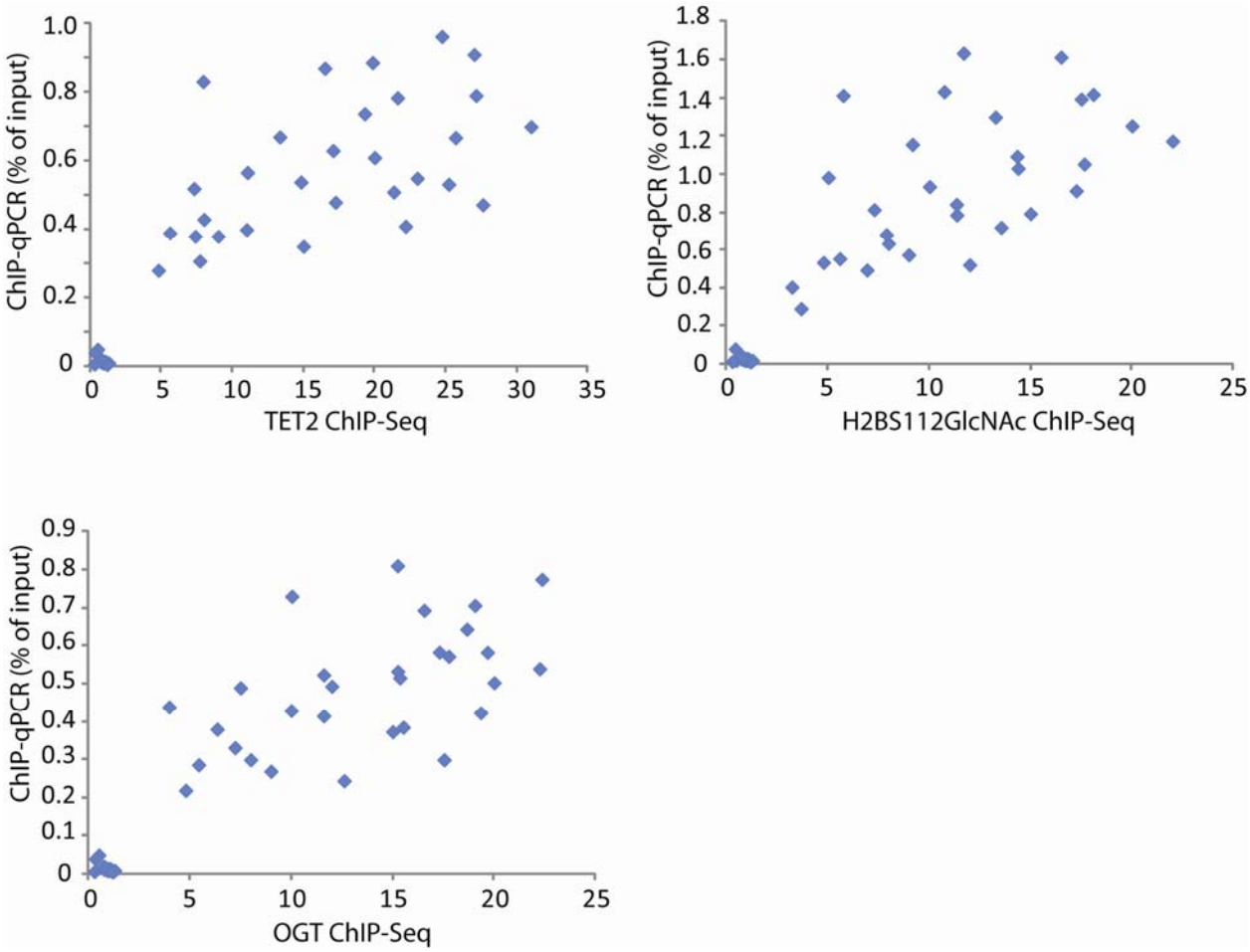

a
Histone octamer
Histone octamer
OGT enzyme
- WT D2 - WT D2 - WT D2 - WT D2
TET2CD
- - - + + + - - - + + +
H2A
H3
H2B
H4
3H-GlcNAc
Coomassine staining
b
Mono-nucleosome
Mono-nucleosome
OGT enzyme
- WT D2 - WT D2 - WT D2 - WT D2
TET2CD
- - - + + + - - - + + +
H2A
H3
H2B
H4
3H-GlcNAc
Coomassine staining
c
H4 H2A
H2B H3
H2A
H3
H2B
H4
Coomassine staining

Supplementary Fig. 11. TET2 facilitates OGT-dependent histone O-GlcNAcylation.
(a, b) TET2 does not affect OGT to glycosylate histone octamers (**a**), but induces OGT-dependent histone glycosylation when mono-nucleosome was used as the substrate (**b**). Core histones or mono-nucleosomes were separated by 15 % TAU gel. Tritium-labeled GlcNAc was incorporated into the histones in the *in vitro* GlcNAcylation assay. **(c)** Recombinant core histones and H2A, H2B, H3, H4 were separated by 15 % TAU gel to mark the position of each histone.



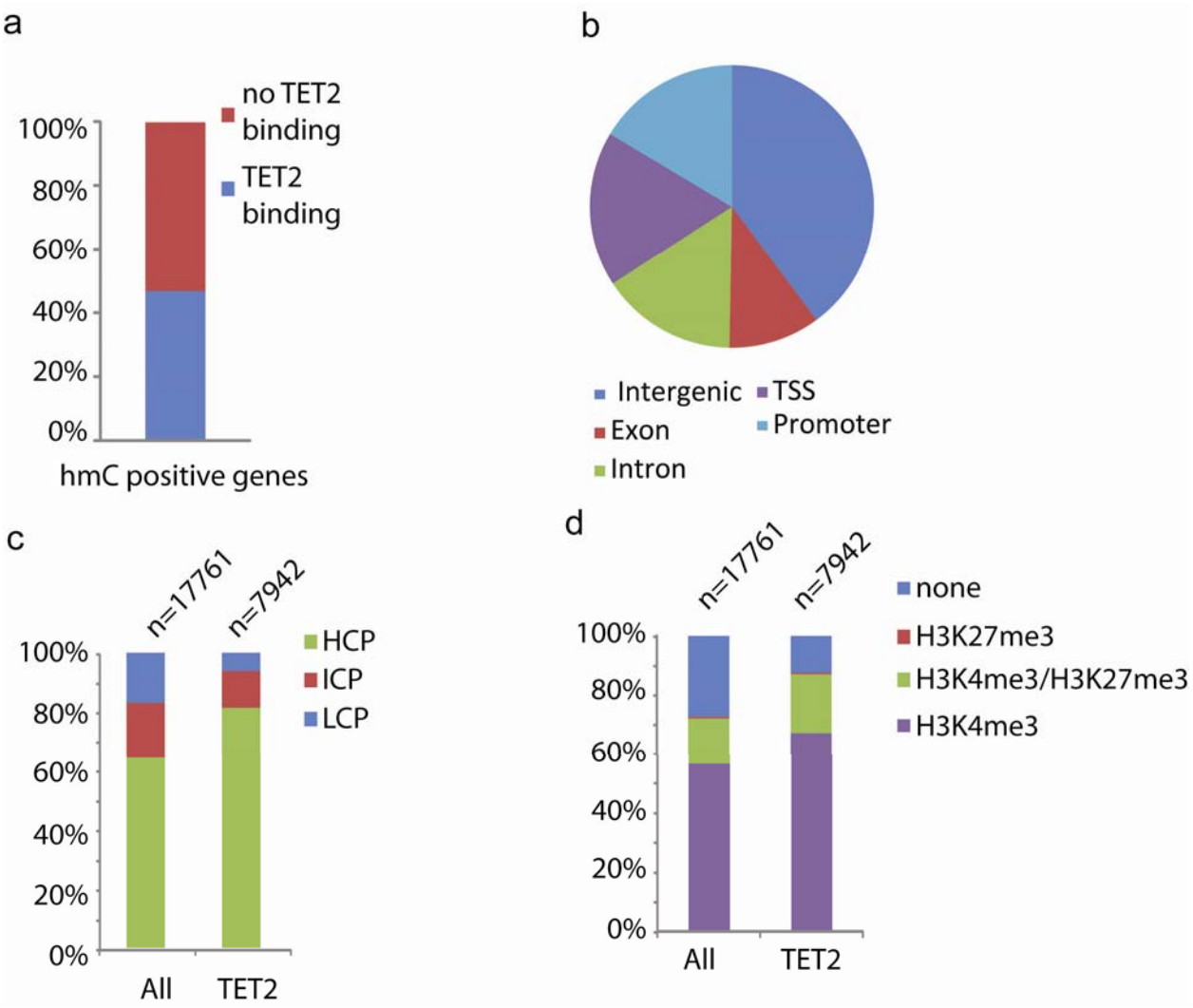
Supplementary Fig. 12. TET2 recruits OGT to associate with DNA.

(a) TET2 mediates OGT to associate with mono-nucleosome *in vitro*. ^{32}P labeled DNA with C or 5mC was used to package mono-nucleosomes. The TET2/OGT complex-bound mono-nucleosome was examined by 4 % native PAGE. **(b)** ^{32}P labeled DNA with 5mC was incubated with recombinant TET2 and/or OGT. Protein-bound DNA was examined by EMSA.



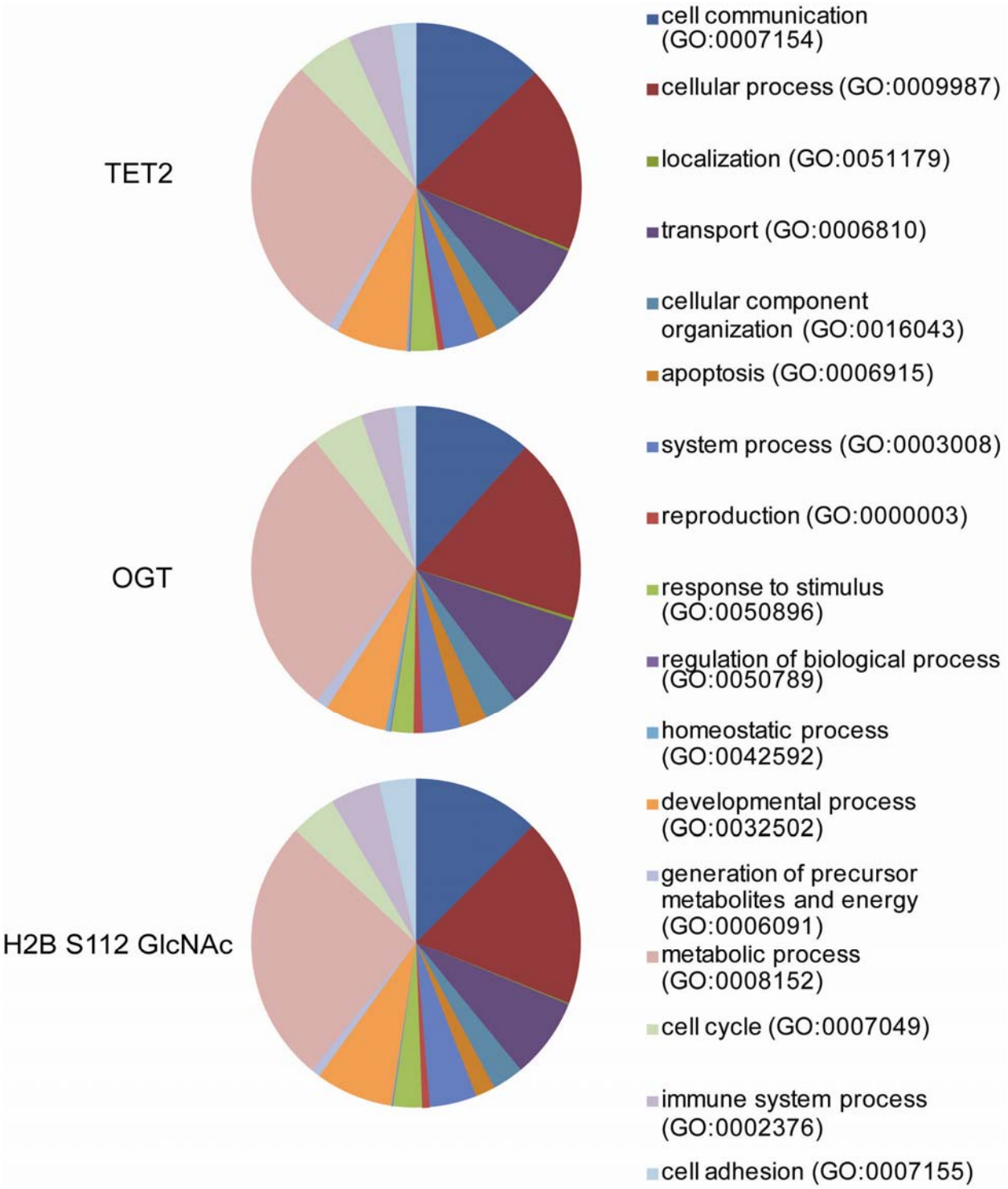
Supplementary Fig. 13. Validation of ChIP-seq results.

ChIP-Seq fragment densities of TET2 (x-axis) are plotted against ChIP-qPCR fold-enrichment of TET2 (percentage of input) (y-axis) at 45 selected loci in mouse ES cells. The 45 selected loci contain 15 loci that are TET2 positive and 15 loci that are TET2, OGT and H2BS112GlcNAc positive (ChIP-seq signal > 5). The others are TET2, OGT and H2B S112 GlcNAc negative. The same methods were used to analyze the ChIP results of OGT and H2B S112 GlcNAc. 30 sites identified as significantly enriched by ChIP-Seq were clearly differentiated from 15 unenriched sites in the plots.


a
100%
80%
60%
40%
20%
0%
no TET2 binding
TET2 binding
hmC positive genes
b
Intergenic
TSS
Exon
Promoter
Intron
c
n=17761
n=7942
100%
80%
60%
40%
20%
0%
HCP
ICP
LCP
All
TET2
d
n=17761
n=7942
100%
80%
60%
40%
20%
0%
none
H3K27me3
H3K4me3/H3K27me3
H3K4me3
All
TET2

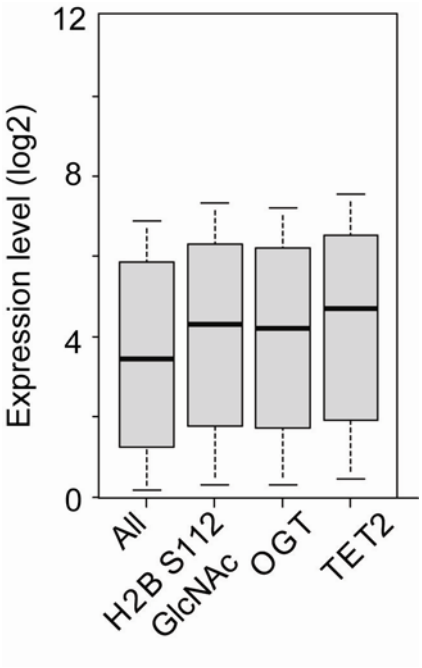
Supplementary Fig. 14. TET2 ChIP-seq result analysis

(a) Overlay of genes positive for 5hmC at the TSS with TET2 target genes in the ChIP-seq analysis. Around 47 % of 5hmC positive genes were also occupied by TET2. 5hmC database were downloaded from NCBI (GSE28682). **(b)** The ChIP-seq data was analyzed by Cisgenome 2.0. The position of TET2 ChIP-seq peaks was illustrated by Pie diagram showing the overall distribution of TET2 at TSS (± 1 kb), promoter (-1 to -10 kb), exon, intron and intergenic regions. **(c)** Based on CpG density, promoters are divided into high-, intermediate- or low CpG density promoters (HCP, ICP or LCP) as defines in before[1] for all genes and for TET2 target genes. **(d)** Histograms showing distribution of H3K4me3 and H3K27me3 on the promoters of all genes or TET2 target genes.


TET2
OGT
H2B S112 GlcNAc
cell communication (GO:0007154)
cellular process (GO:0009987)
localization (GO:0051179)
transport (GO:0006810)
cellular component organization (GO:0016043)
apoptosis (GO:0006915)
system process (GO:0003008)
reproduction (GO:0000003)
response to stimulus (GO:0050896)
regulation of biological process (GO:0050789)
homeostatic process (GO:0042592)
developmental process (GO:0032502)
generation of precursor metabolites and energy (GO:0006091)
metabolic process (GO:0008152)
cell cycle (GO:0007049)
immune system process (GO:0002376)
cell adhesion (GO:0007155)

Supplementary Fig. 15. Gene ontology analysis of TET2, OGT and H2B S112 GlcNAc target genes.

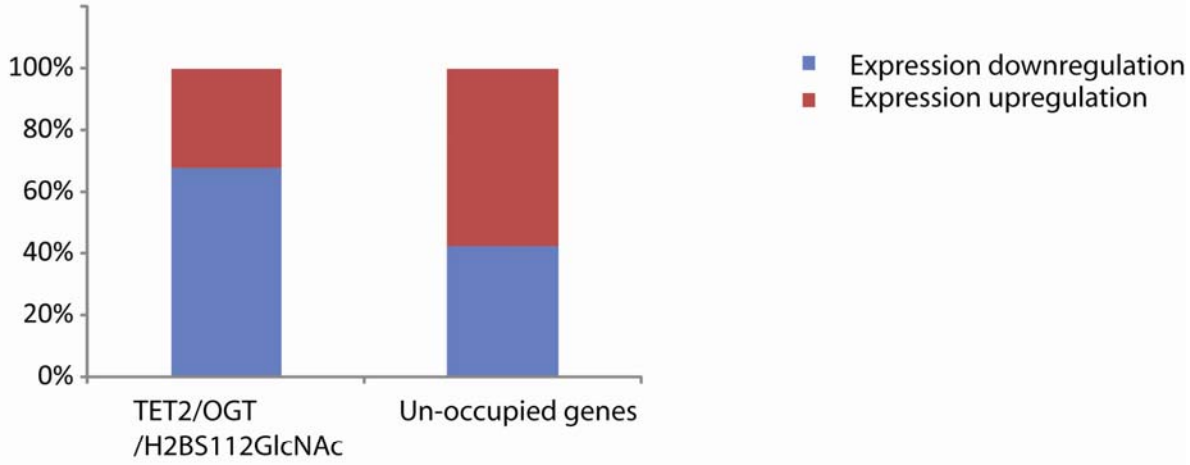
Genes occupied by TET2 at TSS (± 1 kb) was submitted to Panther website[2] for gene ontology analysis. The same analyses were performed for OGT or H2B S112 GlcNAc occupied genes. Gene ontology results were shown in pie diagram.



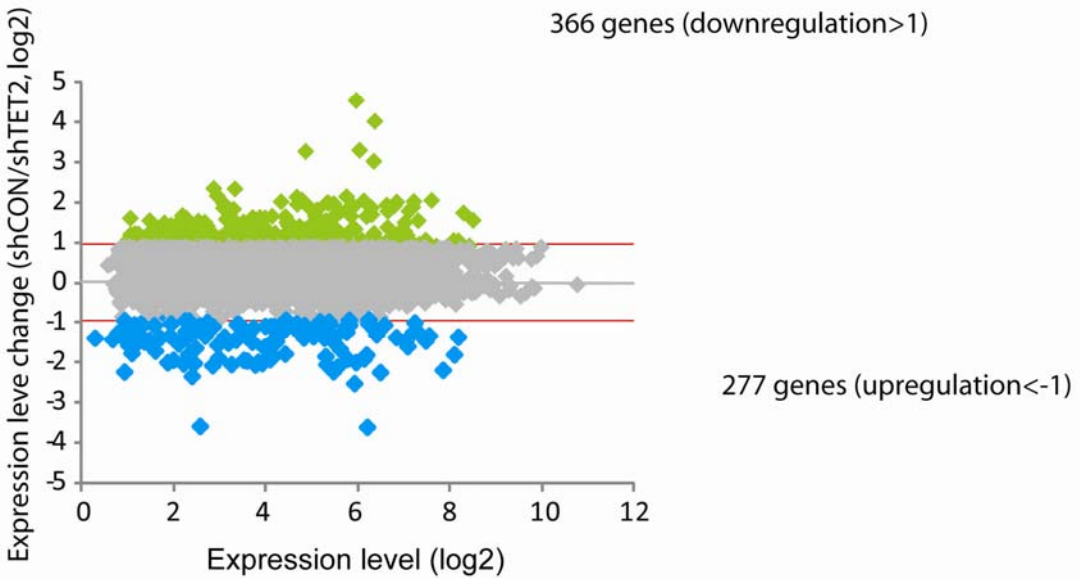
Supplementary Fig. 16. The expression of TET2, OGT or H2B S112 GlcNAc occupied genes is higher than average gene expression.

Gene expression values were obtained from the microarray analysis of wild type ES cells. The expression of TET2, OGT or H2B S112 GlcNAc occupied genes was compared with that of all genes in ES cells. The data were presented by Boxplot using Program R. Boxplot shows median, 25^{th} and 75^{th} percentile expression levels in ES cells.

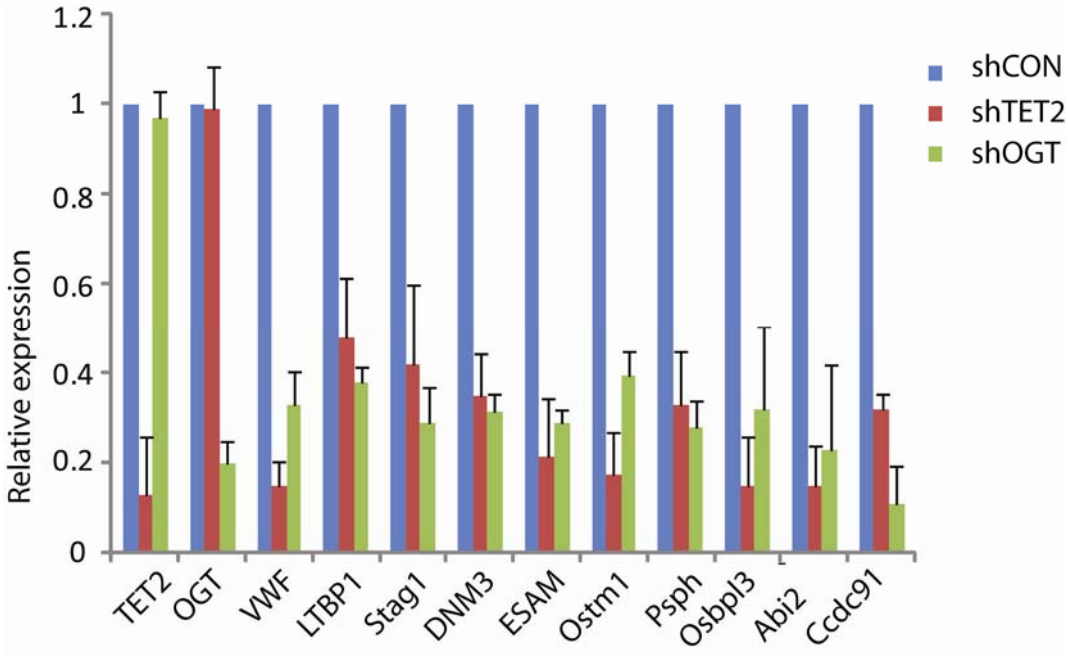
a


100%
80%
60%
40%
20%
0%
TET2/OGT
/H2BS112GlcNAc
Un-occupied genes
Expression downregulation
Expression upregulation

b

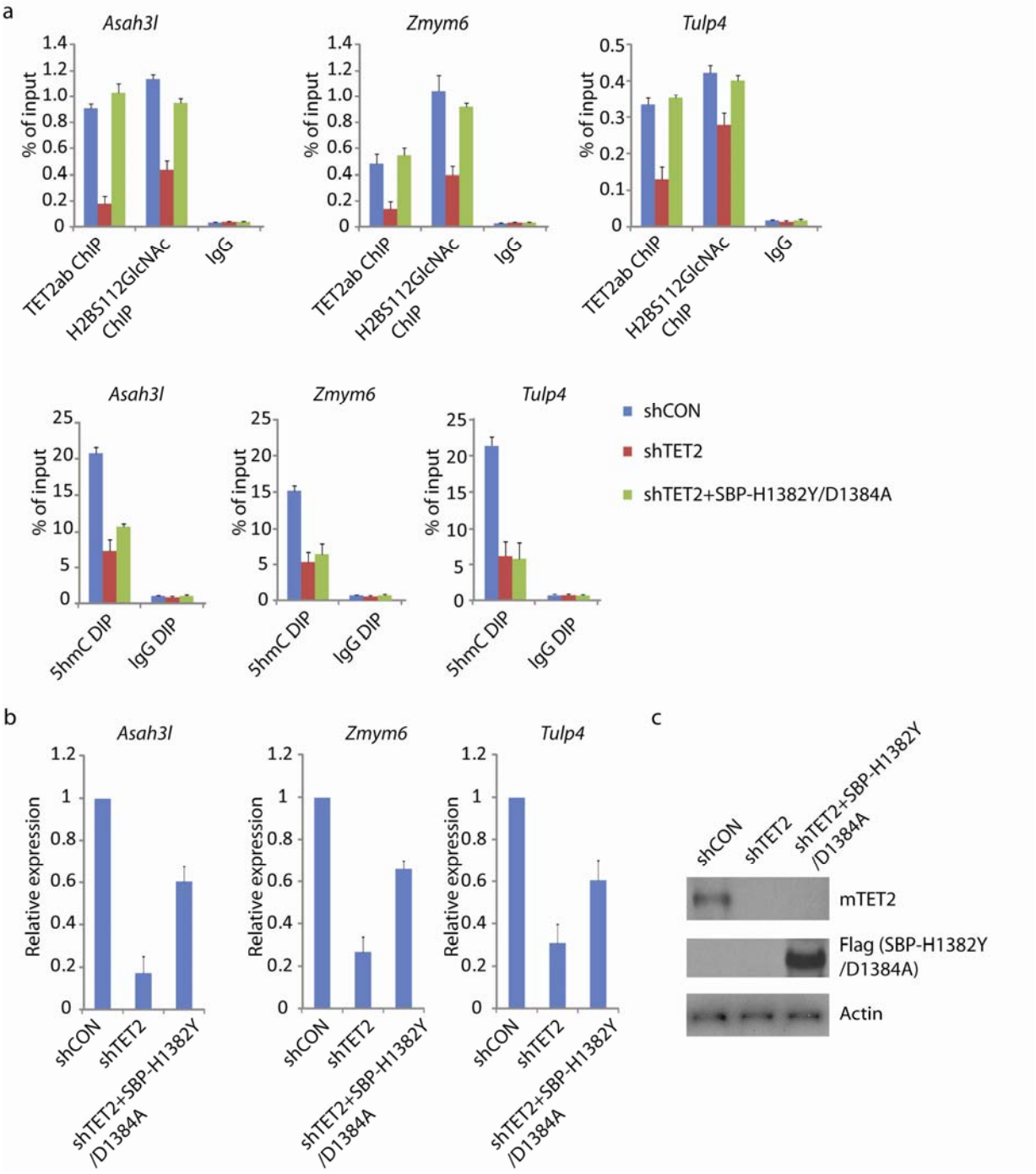

366 genes (downregulation>1)
277 genes (upregulation<-1)
Expression leve change (shCON/shTET2, log2)
Expression level (log2)
5
4
3
2
1
0
-1
-2
-3
-4
-5
0
2
4
6
8
10
12

Supplementary Fig. 17. The microarray analyses of TET2 knockdown ES cells.
(a) The genes harboring TET2, OGT and H2B S112 GlcNAc were categorized into expression up-regulation and expression down-regulation based on the microarray analyses of shCON and shTET2 treated ES cells (shCON vs shTET2). Around 68 % of genes were down-regulated. Only ~ 42 % of genes that were not harbored by TET2, OGT and H2B S112 GlcNAc were down-regulated. **(b)** A scatterplot illustrating the changes in gene expression scores upon TET2 knockdown. The gene expression level was shown in log2 scale on the x axis. The fold changes of the expression level (log2 scale) in the shTET2 treated ES cells compared to that in the shCON treated ES cells are indicated on the y axis. Green and blue squares mark transcripts that were significantly up- or down-regulated by TET2 knockdown (absolute value of the expression log 2 fold change >1 and FDR <0.05).



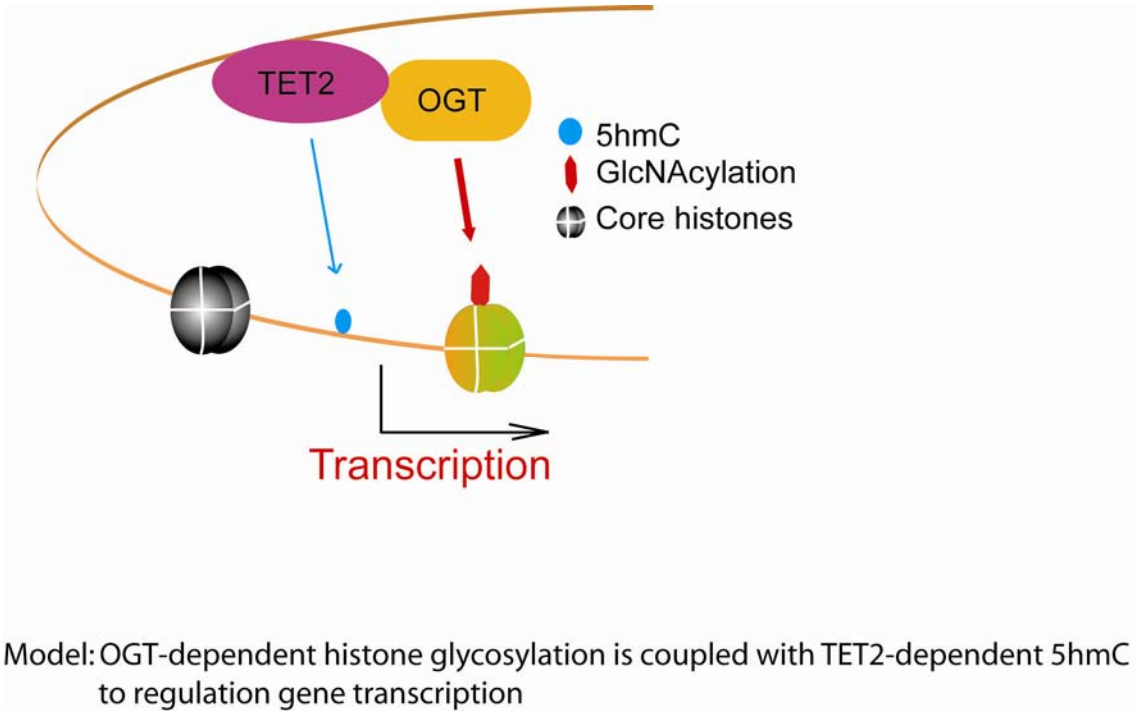
Supplementary Fig. 18. Validation of microarray analyses in TET2 knockdown ES cells by qRT-PCR.

We examined the expression changes of a set of genes in TET2 knockdown or OGT knockdown ES cells by qRT-PCR. These genes are common target genes of TET2 and OGT, and are also in the group that has been showed down-regulated expression in TET2 knockdown ES cells (Supplementary Fig. 17b). The expression of genes that is impaired in TET2 knockdown cells is also repressed in OGT knockdown cells. All error bars denote s.d., n=3.


a
Asah3l
Zmym6
Tulp4
% of input
TET2ab ChIP
H2BS112GlcNAc ChIP
IgG
5hmC DIP
IgG DIP
shCON
shTET2
shTET2+SBP-H1382Y/D1384A
b
Relative expression
shCON
shTET2
shTET2+SBP-H1382Y /D1384A
c
mTET2
Flag (SBP-H1382Y /D1384A)
Actin

Supplementary Fig. 19. Gene expression was partially rescued by the H1382Y/D1384A mutant in TET2 knockdown ES cells.

(a) ChIP or hmeDIP analyses were performed in control, TET2 knockdown cells or TET2 knockdown cells expressing SBP-H1382Y/D1384A (shCON, shTET2, shTET2+SBP-H1382Y/D1384A) using indicated antibodies. The H1382Y/D1384A mutant restored H2B S112 GlcNAc but not 5hmC at the indicated gene loci. **(b)** qRT-PCR results illustrated the expression of indicated genes. **(c)** Cell lysates from control, TET2 knockdown cells or TET2 knockdown cells expressing SBP-H1382Y/D1384A were examined by Western blot with indicated antibodies. Since the H1382Y/D1384A mutant is a human TET2 mutant, anti-mTET2 antibody does recognize the H1382Y/D1384A mutant. All error bars denote s.d., n=3.



Supplementary Fig. 20. A model to explain the mechanism by which TET2 regulates OGT.

Reference

1. Mikkelsen, T.S., *et al.* Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* **448**, 553-560 (2007).
2. Thomas, P.D., *et al.* PANTHER: a browsable database of gene products organized by biological function, using curated protein family and subfamily classification. *Nucleic Acids Res* **31**, 334-341 (2003).

Supplemental Table 7: DNA sequence used in 5ghmC assay and EMSA assay

1, DNA sequence used in 5ghmC *in vitro* assay.

AGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGG
CGGCTTTGTTGAATAAATCGAACTTTTGCTGAGTTGAAGGATCAGATCACGCATCTT
CCCGACAACGCAGACCGTTCCGTGGCAAAGCAAAAGTTCAAAATCACCAACTGGTC
CACCTACAACAAAGCTCTCATCAACCGTGGCTCCCTCACT

2, DNA sequence used in DNA and mono-nucleosome EMSA assay (based on Widom 601 sequence, 157 mer)

GCATGATCGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGC
ACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACT
CCCTAGTCTCCAGGCACGTGTCAGATATATACATCCGATTAAGT