Supplementary Figure Legends

Supplementary Figure 1: Knockdown of DNMT1. Knockdown of DNMT1 in cultured human keratinocytes using two separate shRNA constructs. QRT-PCR was used to determine DNMT1 mRNA levels; error bars=s.d., n= 3.

Supplementary Figure 2: Loss of DNMT1 in epidermal tissue does not increase apoptosis. TUNEL staining on control (CTL) and DNMT1i epidermal tissue at weeks 1, 2, and 3 post-grafting. TUNEL staining for apoptosis is shown in orange and Hoechst staining for nuclei is shown in blue. DNase I treated epidermal tissue=positive control for TUNEL staining. Scale bar = 30μm.

Supplementary Figure 3: DNMT1 is necessary for epidermal proliferation. a, Loss of proliferative capacity in DNMT1i epidermis. Keratinocytes knocked down for DNMT1 or controls were used to regenerate human interfollicular epidermis and grafted onto immune compromised CB.17 *scid/scid* mice. Grafts were harvested at weeks 1, 2, and 3 post-grafting and stained for Ki67 (green) to assess proliferation. Differentiation protein keratin 10 (K10) is shown in orange. Hoechst staining for the nuclei is shown in blue. Scale bar = 50μm. b, Quantitation of Ki67 positive cells in the epidermis over a 3 week period. 250 basal nuclei were counted for each time point. N= 9 grafted mice for each experimental group; error bars=s.d.

Supplementary Figure 4: Loss of DNMT1 results in premature differentiation of marked basal layer cells. DNMT1i and control marked cells in the basal layer were counted and scored for co-staining with the differentiation protein keratin 1. By week 2, up to 11% of the DNMT1i marked basal cells stained for keratin 1 whereas no staining could be detected for control marked cells. 250 basal nuclei were counted for each time point. N= 9 grafted mice for each experimental group.

Supplementary Figure 5: DNMT1 knockdown in cultured epidermal progenitor cells does not result in increased apoptosis. Cells were knocked down for DNMT1 or control and stained using an Annexin V antibody conjugated to FITC. The percent of cells staining positive for the apoptotic marker Annexin V is shown; error bars represent s.d., n= 2.

Supplementary Figure 6: Partial rescue of DNMT1i proliferation defects by Cdk4-cyclin D1. a, Over-expressing Cdk4 and Cyclin D1 in the presence of DNMT1i partially restores the clonogenic colony forming capacity of epidermal cells. 100 cells were seeded onto 30mm plates

and stained with crystal violet 2 weeks later. b, Quantitation of clonogenic assays with colonies > 5 mm^2 (n=3/group) ; error bars represent s.d.

Supplementary Figure 7: A wild-type but not a catalytically inactive point mutant of DNMT1 rescues the proliferation defects and suppresses premature expression of p15/p16 in DNMT1 deficient epidermal cells. Wild-type (wt) or DNA methylation inactive point mutant (mut) DNMT1 constructs were transduced into keratinocytes. These modified keratinocytes were also transduced with a DNMT1 shrna construct targeting the 3' UTR which recognizes the endogenous but not the retrovirally delivered DNMT1 expression constructs. QRT-PCR was performed to determine the levels of p15/p16. Expression of wt but not mut DNMT1 restored the p15/p16 levels (left panel). Proliferation defects were also rescued when wt but not mut DNMT1 was introduced in DNMT1 deficient cells (right panel); error bars represent s.d., n= 3.

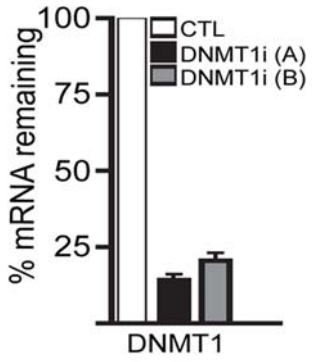
Supplementary Figure 8: Diagram of methylation peaks in undifferentiated and differentiated epidermal cells. a, Diagram of the differentially methylated genes described in Fig 4c. Genes are shown in relation to the transcriptional start site (TSS). The tiled region on the MeDIP promoter array is shown in grey, which on average is tiled 3500bp upstream and 750bp downstream of a gene's TSS. Methylated peaks can be found in undifferentiated cells (shown in red) which are erased upon epidermal differentiaion (no detectable yellow peaks). Genes such as *POU2F3* contain a CpG island, which is shown in green. b, Diagram of differentially methylated genes in CpG island shores. Methylated peaks are found from several hundred (*LPHN1*) to a couple of kb (*TP53INP2* and *TMEM45B*) away from CpG islands in undifferentiated cells which are erased upon epidermal differentiation. c, Methylation status does not change upon epidermal differentiation of imprinted gene, *H19* (Note presence of both yellow and red peaks). d, Absence of methylation peaks in undifferentiated and differentiated epidermal cells in the tiled region of *CDKN2A* and *CDKN2B*.

Supplementary Figure 9: DNMT1 and GADD45A/B controls the methylation status of S100P. Bisulfite sequencing was performed on DNA isolated from DNMT1i and control cells grown in the absence of calcium (self-renewing conditions) and GADD45A/Bi and control cells grown in the presence of calcium (differentiating conditions). The upstream promoter region of S100P which ranges -507 to -139 from the transcriptional start site was analyzed for methylation status. Loss of DNMT1 resulted in demethylation of S100P. GADD45A/B knockdown prevented the erasure of methylation marks seen during epidermal differentiation. (Black circles=methylated site; white circles=unmethylated site; n= 8 clones sequenced for each group).

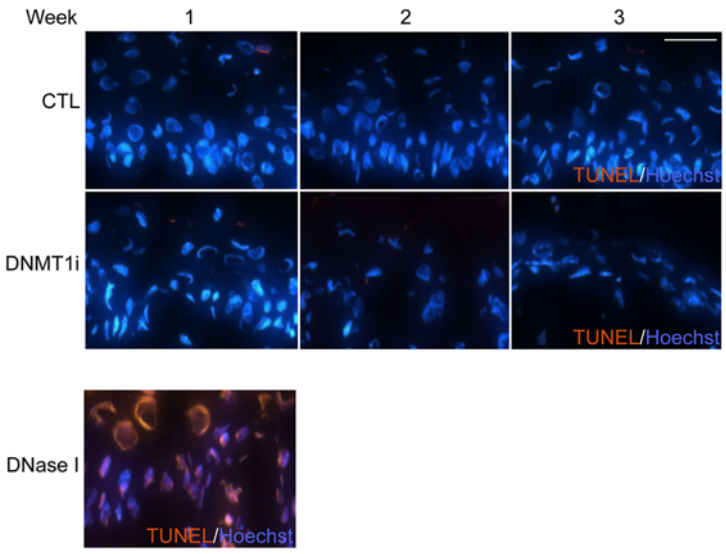
Supplementary Figure 10: UHRF1 is necessary for progenitor maintenance. a, UHRF1 protein distribution in adult human epidermis. UHRF1 (orange), differentiation keratin 1 (K1: green), Hoechst nuclear stain (blue), dotted line denotes basement membrane. b,c, Time course of UHRF1 down-regulation during differentiation at the mRNA and protein level; error bars represent s.d., n= 3. d, Knockdown of UHRF1 in cultured human keratinocytes using two separate shRNA constructs. Levels of knockdown were assayed by QRT-PCR; error bars represent s.d., n= 3. e, Knockdown of UHRF1 results in de-repression of epidermal differentiation genes; error bars represent s.d., n= 3. f, Effects of UHRF1 loss on clonogenic growth. g, Quantitation of colonies > 5 mm^2 (n=3/group); error bars represent s.d.

Supplementary Figure 11: Loss of DNMT1 or expression of GADD45A/B accelerates muscle differentiation. a, DNMT1 is down-regulated during muscle differentiation. (GM: primary human myoblasts grown in self-renewing conditions; DM: primary human myoblasts in differentiation medium for 3 days) b, Knockdown of DNMT1 in cultured human myoblasts using two separate shRNA constructs. Levels of knockdown were assayed by QRT-PCR. c, Myoblasts were knocked down for DNMT1 expression along with controls and induced to differentiate for 24 hours. Loss of DNMT1 results in increased expression of muscle differentiation structural genes, skeletal actin (ACTA1), myosin heavy chain (MYH8), and tropomyosin (TPM1). d, Expression of GADD45A or B results in the upregulation of muscle differentiation genes, ACTA1, MYH8, and TPM1. All error bars represent s.d., n= 3.

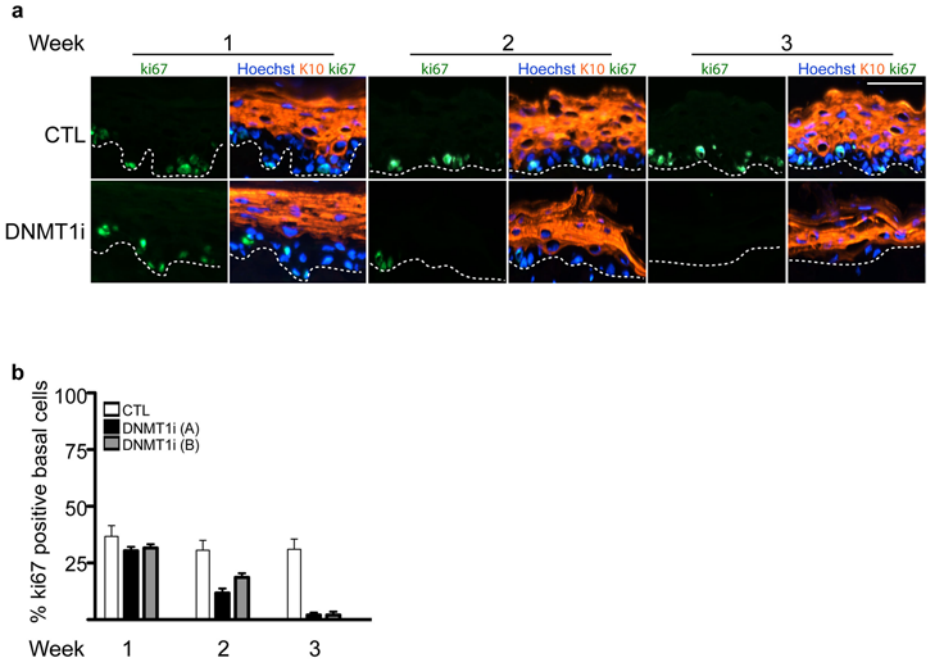
Sen_SFig1



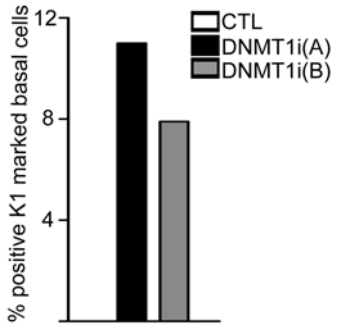
Sen_SFig2

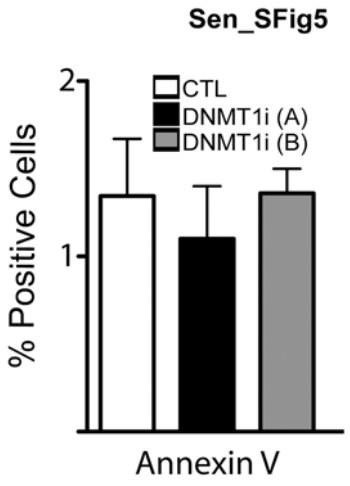

Week
1
2
3
CTL
DNMT1i
DNase I
TUNEL/Hoechst
TUNEL/Hoechst
TUNEL/Hoechst

Sen_SFig3

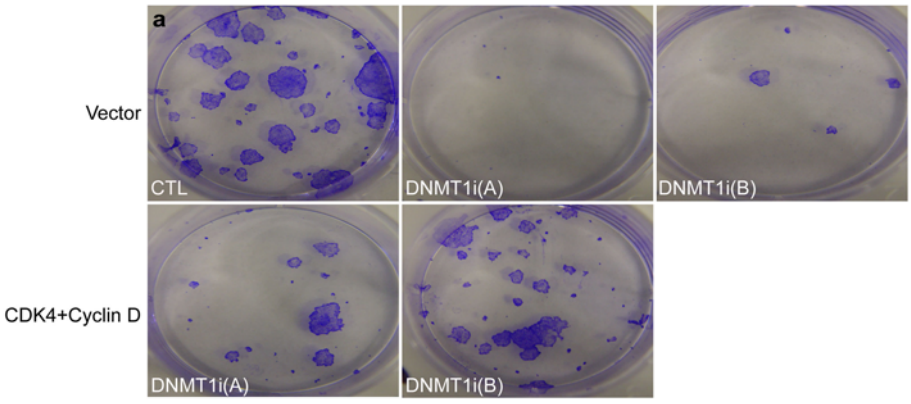

a
Week
1
2
3
ki67
Hoechst K10 ki67
CTL
DNMT1i
b
% ki67 positive basal cells
100
75
50
25
CTL
DNMT1i (A)
DNMT1i (B)
Week
1
2
3

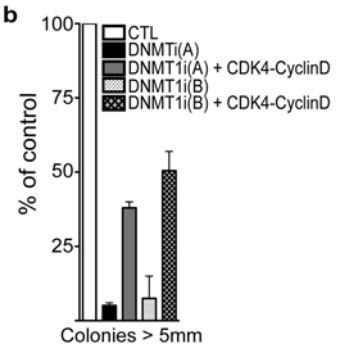
Sen_SFig4



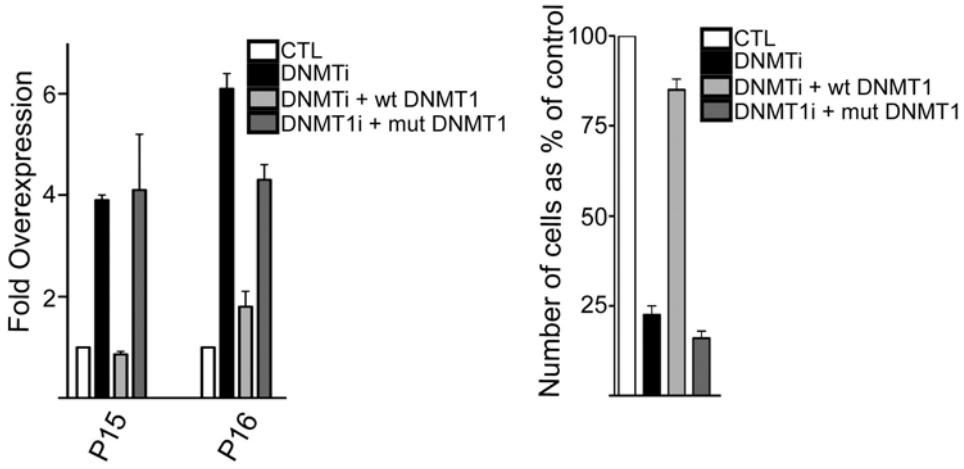

Sen_SFig5
CTL
DNMT1i (A)
DNMT1i (B)
% Positive Cells
2
1
Annexin V

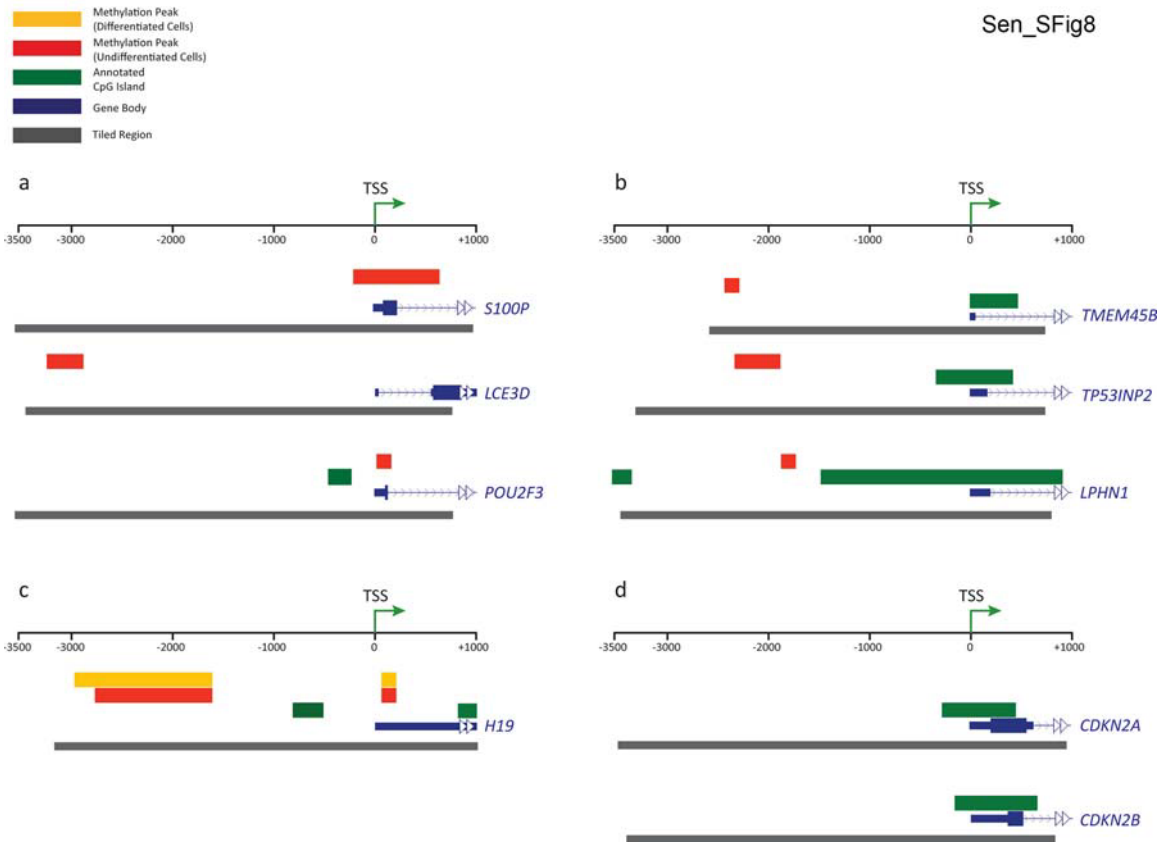
Sen_SFig6

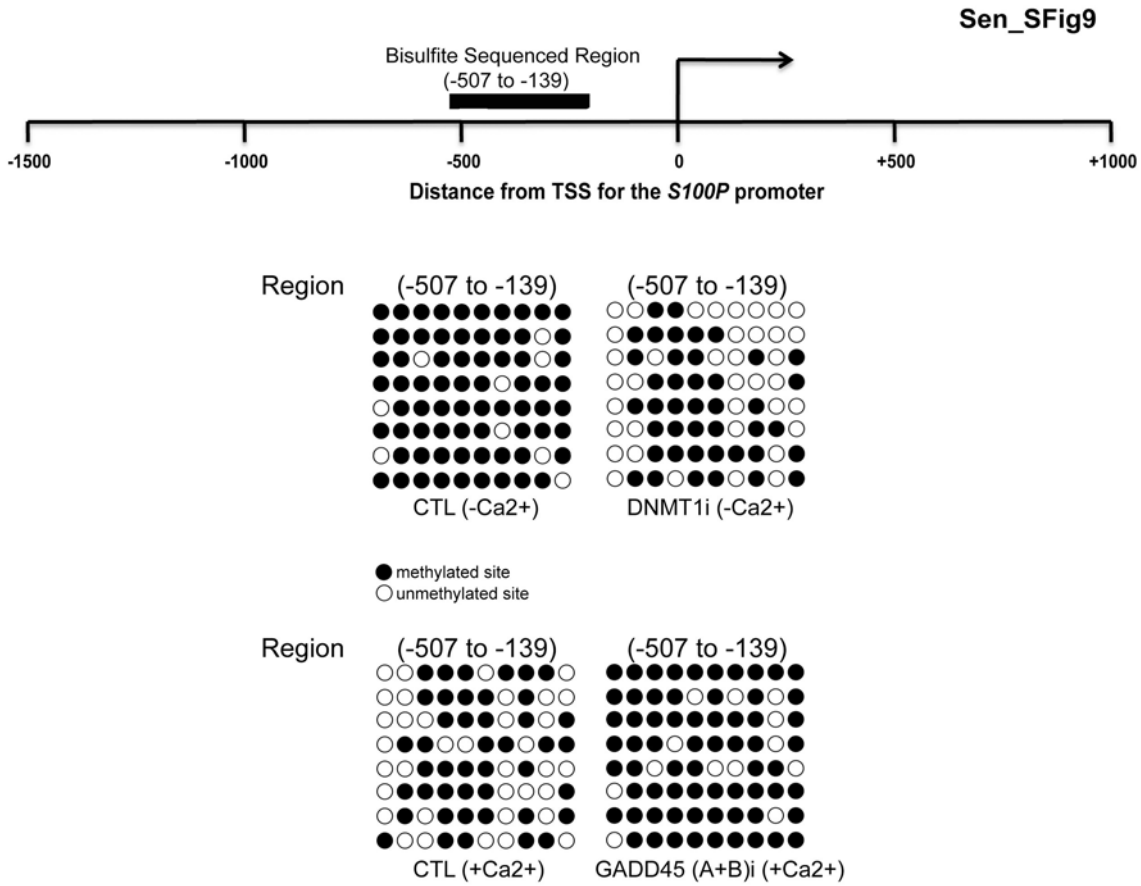

a
Vector
CDK4+Cyclin D
CTL
DNMT1i(A)
DNMT1i(B)
DNMT1i(A)
DNMT1i(B)


b
100
75
50
25
% of control
CTL
DNMTi(A)
DNMT1i(A) + CDK4-CyclinD
DNMT1i(B)
DNMT1i(B) + CDK4-CyclinD
Colonies > 5mm

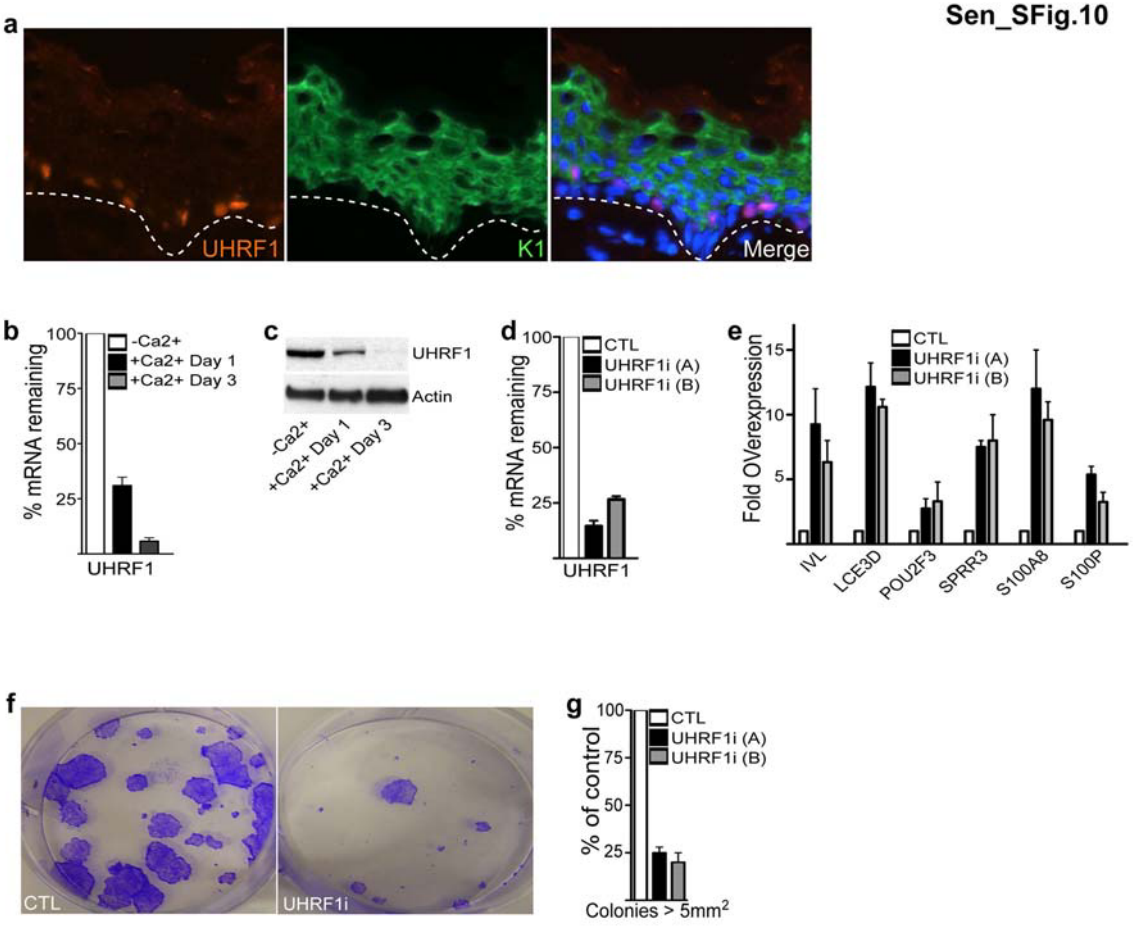
Sen_SFig7


Fold Overexpression
6
4
2
P15
P16
CTL
DNMTi
DNMTi + wt DNMT1
DNMT1i + mut DNMT1
Number of cells as % of control
100
75
50
25
CTL
DNMTi
DNMTi + wt DNMT1
DNMT1i + mut DNMT1

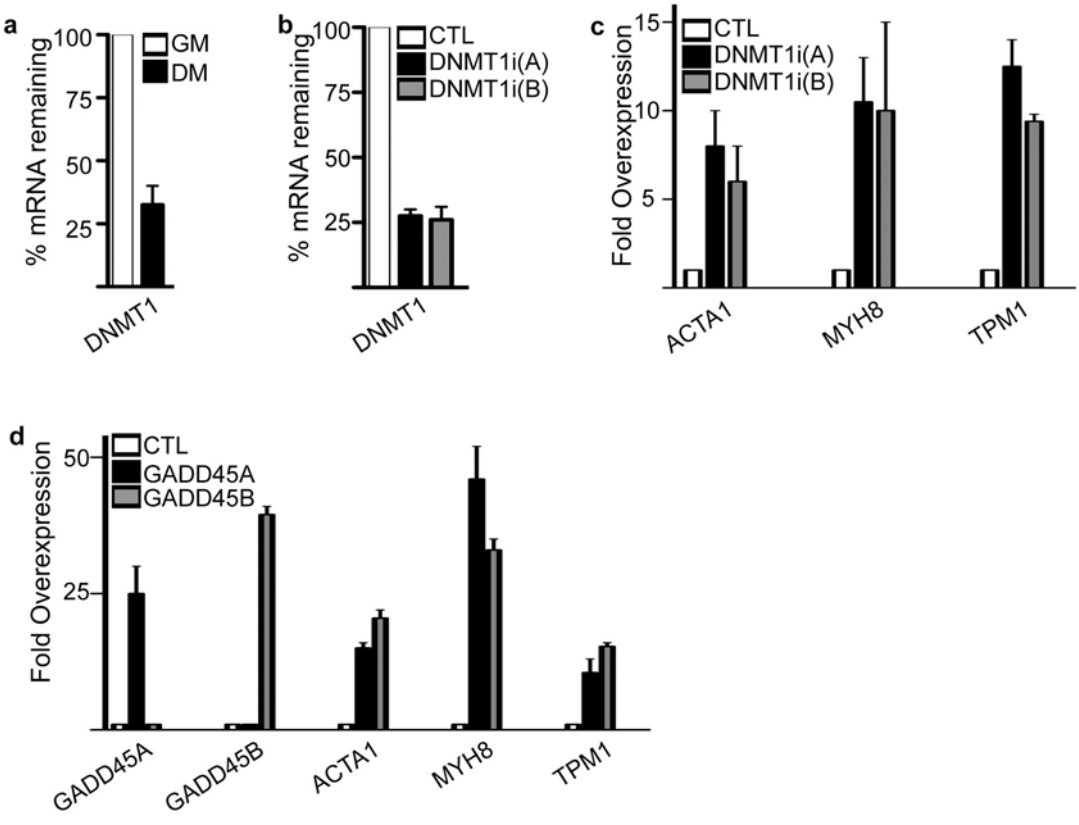

Methylation Peak (Differentiated Cells)
Methylation Peak (Undifferentiated Cells)
Annotated CpG Island
Gene Body
Tiled Region
Sen_SFig8
a
TSS
-3500
-3000
-2000
-1000
0
+1000
S100P
LCE3D
POU2F3
b
TMEM45B
TP53INP2
LPHN1
c
H19
d
CDKN2A
CDKN2B


Sen_SFig9
Bisulfite Sequenced Region
(-507 to -139)
-1500
-1000
-500
0
+500
+1000
Distance from TSS for the S100P promoter
Region
(-507 to -139)
(-507 to -139)
CTL (-Ca2+)
DNMT1i (-Ca2+)
methylated site
unmethylated site
Region
(-507 to -139)
(-507 to -139)
CTL (+Ca2+)
GADD45 (A+B)i (+Ca2+)

Sen_SFig.10


a
UHRF1
K1
Merge
b
% mRNA remaining
100
75
50
25
-Ca2+
+Ca2+ Day 1
+Ca2+ Day 3
UHRF1
c
UHRF1
Actin
-Ca2+
+Ca2+ Day 1
+Ca2+ Day 3
d
% mRNA remaining
100
75
50
25
CTL
UHRF1i (A)
UHRF1i (B)
UHRF1
e
Fold OVerexpression
15
10
5
CTL
UHRF1i (A)
UHRF1i (B)
IVL
LCE3D
POU2F3
SPRR3
S100A8
S100P
f
CTL
UHRF1i
g
% of control
100
75
50
25
CTL
UHRF1i (A)
UHRF1i (B)
Colonies > 5mm2

Sen_SFig11


a
% mRNA remaining
100
75
50
25
GM
DM
DNMT1
b
% mRNA remaining
100
75
50
25
CTL
DNMT1i(A)
DNMT1i(B)
DNMT1
c
Fold Overexpression
15
10
5
CTL
DNMT1i(A)
DNMT1i(B)
ACTA1
MYH8
TPM1
d
Fold Overexpression
50
25
CTL
GADD45A
GADD45B
GADD45A
GADD45B
ACTA1
MYH8
TPM1