1 Supplementary material
2
3 Methods

4 **Cell culture and transfection.** The human lung cancer cell H1299, human embryonic kidney HEK
5 293T and mouse embryonic fibroblast NIH3T3 were obtained from and maintained as instructed by the
6 ATCC. All transfections were carried out using Lipofectamine 2000 (Invitrogen) according to the
7 manufacturer's instructions. To ectopically express hNaa10p or DNMT1, cells were seeded 1 day
8 before transfection of the plasmid encoding indicated protein and harvested 24 h post transfection. To
9 knock down hNaa10p or DNMT1, $2x10^6$ cells were seeded in 15-cm dish 1 day before transfection of
10 10 nM corresponding siRNA using Lipofectamine 2000 every 24 hours for three times. After that, cells
11 were reseeded and transfected for the fourth time the next day. For experiments with 5-aza-2-
12 deoxycytidine (5'-AzadC, A3656, Sigma) treatment, cells were incubated in medium containing 5 μM
13 of 5'-AzadC for 24 hr, followed by replacement of the medium with fresh one containing same conc of
14 5'-AzadC. The procedure was repeated for 4 times.

15 **Plasmids and siRNAs.** For mammalian expression, full-length hNaa10p cDNA was inserted into pSF
16 vector. CMV-myc-DNMT1 was provided as a gift by Dr. YS Chang (1). To generate the C-terminal
17 V5-tagged hNaa10p, DNMT1 and serial deletion mutants, corresponding PCR-amplified cDNA
18 fragment is inserted into pCDNA3.1/V5-His©TOPO® (Invitrogen). pCDNA3.1/V5-His vector
19 containing GFP and the NLS of large T antigen is used for construction of GFP-fused hNaa10p
20 fragments. pcDNA3.1/V5-His-hNaa10p-R82A was generated using the QuikChange Site-Directed
21 Mutagenesis kit (Stratagene). si-h*NAA10*-1, si-h*NAA10*-2, si-DNMT1, si-Tax (2) and Silencer® Select
22 Negative Control #1 siRNA (scramble) were generated/purchased from Ambion (sequences are
23 provided in Supplementary Information, Table S1). The plasmids encoding si-h*NAA10*-1-resistant
24 hNaa10p derivatives used in Figs. 4, 6C, 7E, Supplementary Figs. S6A, S10, S12C and S12D were
25 generated by PCR-based site-directed mutagenesis using si-h*NAA10*-1 resistant primers
26 (Supplementary Table S1) and cloned into pCDNA3.1/V5-His©TOPO® (Invitrogen). To express His-
27 Xpress-hNaa10p in *E coli*, the PCR-amplified hNaa10p cDNA was inserted into pET100/D-TOPO®
28 (Invitrogen). pGL2-E-cadherin-420/+32 was kindly provided by Dr. KL Jang (3). shRNA oligos
29 against hNaa10p and DNMT1 were annealed and cloned into pLKO.1-puro between AgeI and EcoRI
30 site. pLKO.1-puro plasmid was obtained from the National RNAi Core Facility located at the Institute

of Molecular Biology / Genomic Research Center, Academia Sinica, supported by the National Research Program for Genomic Medicine Grants of NSC (NSC 97-3112-B-001-016). The sequences of shRNA oligos are listed in the supplementary Table 1.

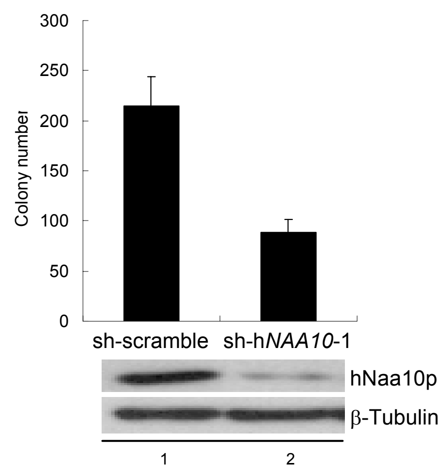
Antibodies. The primary Abs used for IP are polyclonal anti- hNaa10p raised by immunizing rabbits with full-length hNaa10p expressed in and purified from E. coli XA-90 according to standard protocols, anti-DNMT1 (GTX13537, GeneTex) and anti-V5 (V8012, Sigma). For Western, anti-hNaa10p (rabbit polyclonal generated in our laboratory and sc-33820, Santa Cruz Biotechnology), anti-Flag M2 (F1804, Sigma), anti-DNMT1 (ab16632, Abcam and M0231, NEB), anti-E-cadherin (610182, BD Biosciences), anti-GFP (sc-8834, Santa Cruz Biotechnology), anti-β-tubulin (MAB3408; Millipore), anti-Xpress (R910-25, Invitrogen) and anti-V5 Abs (R960-25, Invitrogen) were used.

In vivo acetylation assay. In vivo acetylation assay was performed following Chen et al.(4) with some modifications. Briefly, cells with or without expressing Flag-hARD1 were incubated in complete DMEM containing 1 mCi/ml sodium [^{3}H] acetate (5 Ci/mmol, PerkinElmer) for 2 hours. Cells were then lysed in NP-40 buffer containing protease inhibitor mixture and the lysates were subjected to immunoprecipitation with anti-DNMT1 (M0231, NEB). Immunoprecipitated mixtures were separated by 10% polyacrylamide gel electrophoresis and subjected to autoradiography.

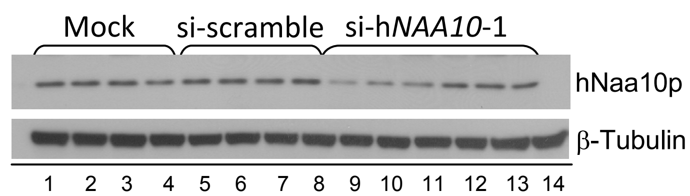
In vitro auto-acetylation assay. His-hNaa10p recombinantly purified from *E. coli* was co-incubated with [^{3}H]acetyl-CoA (Amersham) in buffer containing 50 mM Tris–HCl, pH 8.0, 10% glycerol, 1 mM DTT, 1 mM PMSF, and 0.1 mM EDTA for 30 minutes at 30°C. The proteins were then separated by SDS-PAGE. The gel was stained with Coomassie blue, dried, and subjected to autoradiography.

References

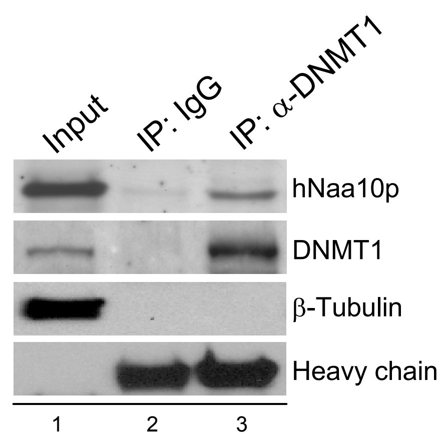
1. Tsai, C.N., Tsai, C.L., Tse, K.P., Chang, H.Y., and Chang, Y.S. 2002. The Epstein-Barr virus oncogene product, latent membrane protein 1, induces the downregulation of E-cadherin gene expression via activation of DNA methyltransferases. *Proc Natl Acad Sci U S A* 99:10084-10089.
2. Suzuki, M., Sunaga, N., Shames, D.S., Toyooka, S., Gazdar, A.F., and Minna, J.D. 2004. RNA interference-mediated knockdown of DNA methyltransferase 1 leads to promoter demethylation and gene re-expression in human lung and breast cancer cells. *Cancer Res* 64:3137-3143.
3. Jung, J.K., Arora, P., Pagano, J.S., and Jang, K.L. 2007. Expression of DNA methyltransferase 1 is activated by hepatitis B virus X protein via a regulatory circuit involving the p16INK4a-cyclin D1-CDK 4/6-pRb-E2F1 pathway. *Cancer Res* 67:5771-5778.
4. Chen, W.Y., Juan, L.J., and Chung, B.C. 2005. SF-1 (nuclear receptor 5A1) activity is activated by cyclic AMP via p300-mediated recruitment to active foci, acetylation, and increased DNA binding. *Mol Cell Biol* 25:10442-10453.



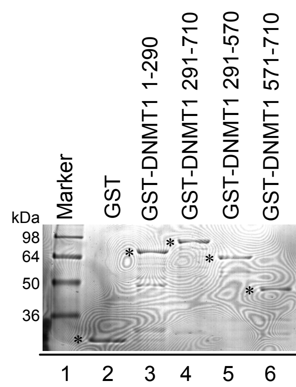
Supplementary Figure S1 Stably depleting hNaa10p by lentivirus infection of shRNA reduces the colony formation ability of lung cancer H1299 cells. Western in the lower panel shows the protein level of hNaa10p. β-tubulin is used as a loading control.



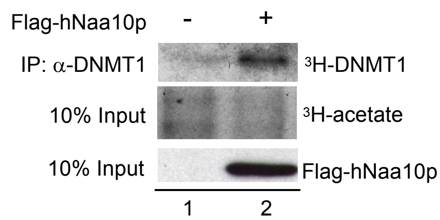
Supplementary Figure S2 Examination of the end-point protein level of hNaa10p in the siRNA-treated xenografts shown in Fig. 2E. The xenograft tumors were excised and whole lysates were extracted for Western blot analysis.



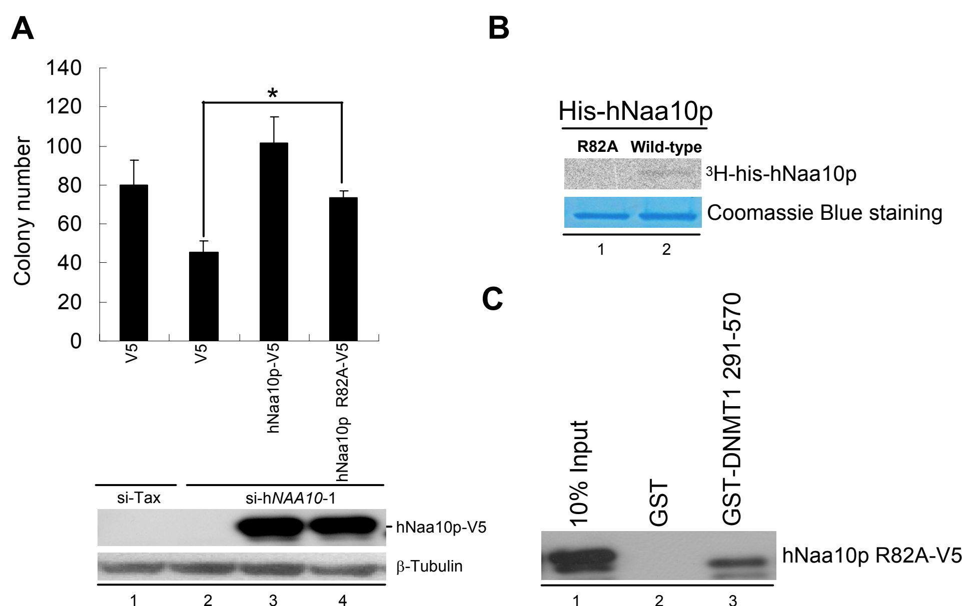
Supplementary Figure S3 Anti-DNMT1 Ab (lane 3), but not IgG (lane 2), precipitates the endogenous hNaa10p, DNMT1, but not β-tubulin, as revealed by Western.



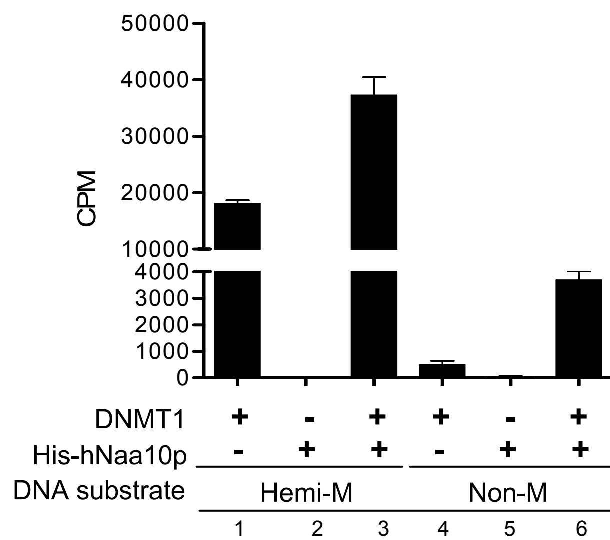
Supplementary Figure S4 The SDS protein gel that shows the expression level of all GST-DNMT1 derivatives used in Fig. 3. The expected band corresponding to each protein is indicated by a star.



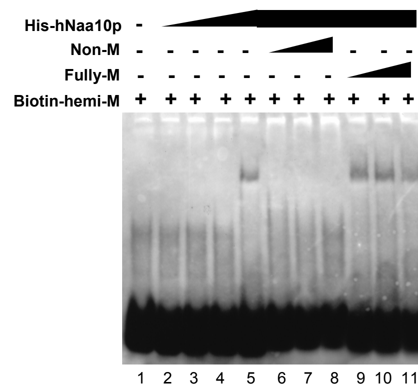
Supplementary Figure S5 hNaa10p stimulates DNMT1 acetylation in cells. 293T cells in the absence (lane 1) or presence (lane 2) of overexpressed Flag-hNaa10p were cultured with medium containing ^{3}H-labeled acetate, followed by collection of lysates for IP with DNMT1 Ab. The pulled down proteins were resolved by SDS-PAGE and the gel was then exposed to X-ray film. The protein level of Flag-hNaa10p is shown in the Western blot (bottom). In the presence of Flag-hNaa10p, the *in vivo* acetylation level of DNMT1 is increased (compare lane 2 to 1).



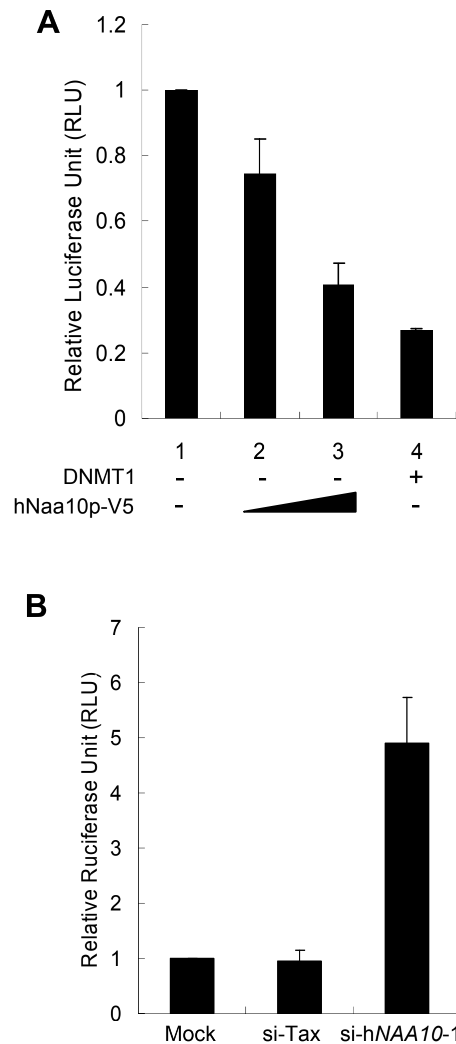
Supplementary Figure S6 Functional analysis of the acetylase-dead mutant hNaa10p R82A in colony formation **(A)**, auto-acetylation **(B)** and DNMT1 interaction **(C)**. **(A)** H1299 cells transiently transfected with si-Tax (lane 1) or si-h*NAA10*-1 (lane 2 to 4) were transfected with V5-vector (lanes 1 and 2), plasmid encoding hNaa10p-V5 (lane 3) or hNaa10p R82A-V5 (lane 4), followed by colony formation assay (upper panel) and Western (lower panel). The data show hNaa10p R82A rescues the colony formation inhibited by depleting the endogenous hNaa10p, although not to the same degree of that by WT hNaa10p. *P-value<0.05. **(B)** The WT hNaa10p (lane 2) or mutant hNaa10p R82A (lane 1) protein recombinantly expressed in and purified from *E. coli* was incubated with ^{3}H-Acetyl-CoA and the mixtures were further separated by SDS-PAGE, followed by autoradiography. The result indicates that R82A mutant fails to auto-acetylate itself. **(C)** GST or GST-DNMT1 aa 291-570 was incubated with in vitro translated hNaa10p R82A-V5, followed by Western using V5 Ab. The result shows that the R82A mutant still interacts with the DNMT1 fragment.



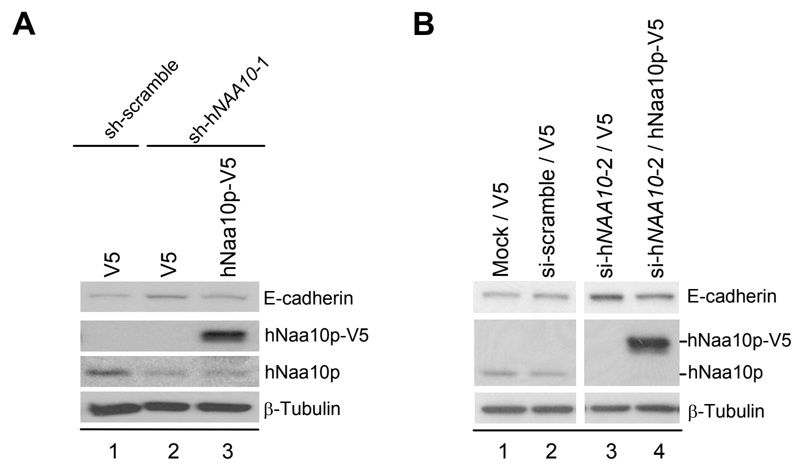
Supplementary Figure S7 hNaa10p stimulates *de novo* DNA methylation. Recombinantly purified DNMT1 and His-hNaa10p were used, alone or in combination, for the in vitro DNMT assay with ^{3}H-AdoMet and hemi-methylated (Hemi-M, lane 1 to 3) or non-methylated (Non-M, lane 4 to 6) DNA substrate. Data are presented as mean ± SD from three independent experiments.



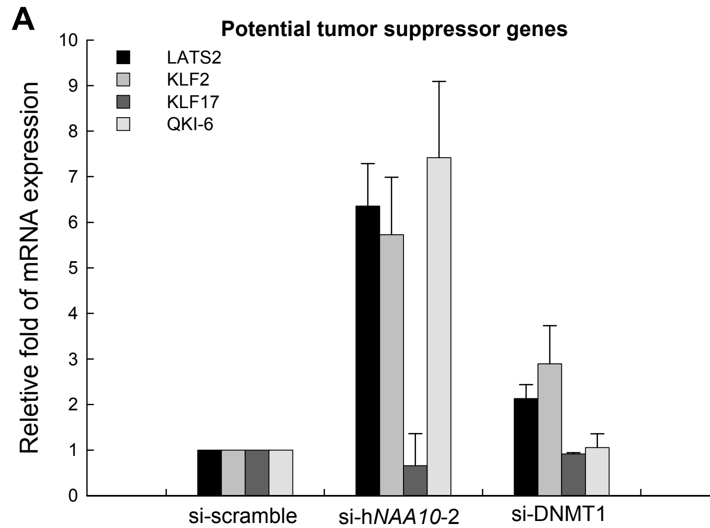
Supplementary Figure S8 hNaa10p binds to DNA in vitro. The *E. coli*-purified hNaa10p was incubated with biotin-labeled hemi-methylated DNA oligo in the absence (lane 2 to 5) or presence of competitive non-methylated (lane 6 to 8) or fully-methylated (lane 9 to 11) DNA oligo and the mixtures were further separated by 10% native PAGE. Lane 1, the biotin-labeled hemi-methylated DNA oligo alone.

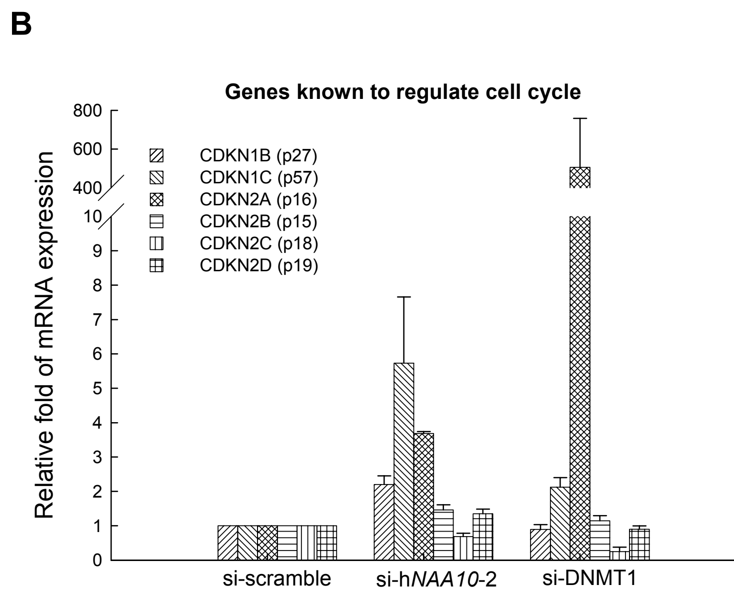


Supplementary Figure S9 (**A**) Ectopic expression of hNaa10p represses the promoter activity of E-cadherin. H1299 cells were transfected with the luciferase reporter construct driven by the E-cadherin promoter together with vector (lane 1), low amount or high amount of plasmid encoding hNaa10p-V5 (lanes 2 and 3) or DNMT1-V5 (lane 4). The cell extracts were then harvested for luciferase activity analysis. (**B**) hNaa10p knockdown activates the E-cadherin promoter activity. H1299 cells co-transfected with the luciferase reporter construct driven by E-cadherin promoter and mock, si-Tax or si-h*NAA10*-1 were subjected to luciferase activity analysis.

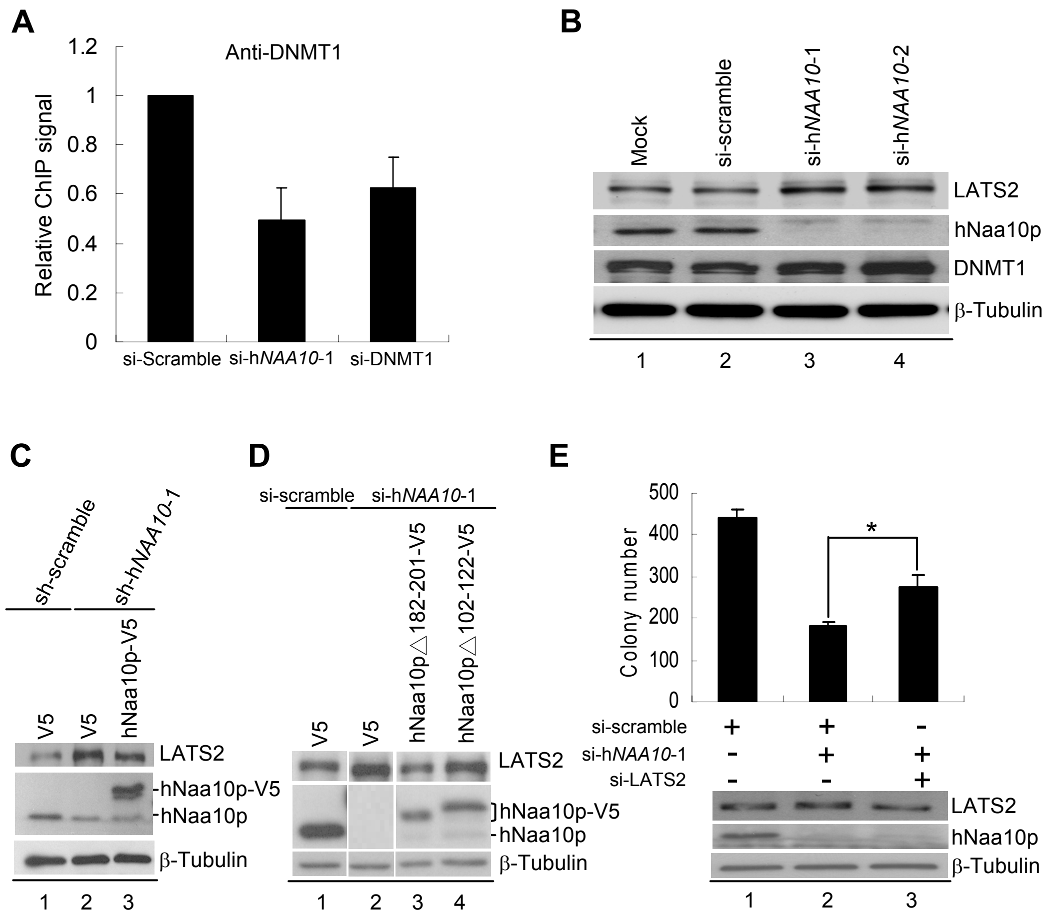


Supplementary Figure S10 Overexpression of siRNA-resistant hNaa10p reduces E-cadherin protein level induced by depleting the endogenous hNaa10p from H1299 cells by lentivirus infection of sh-h*NAA10*-1 **(A)** or transiently transfection of si-h*NAA10*-2 **(B)**. The lanes in (**B**) were run on the same gel but were noncontiguous.





Supplementary Figure S11 Examples of potential tumor suppressor genes (**A**) and genes known to promote cell cycle arrest (**B**) regulated by hNaa10p and DNMT1. Total RNAs were extracted from H1299 cells transfected with si-scramble, si-h*NAA10*-2 or si-DNMT1 for RT-real time PCR. The mRNA level of each gene was measured by normalization to S26 rRNA. Data are presented as mean ± SD from three independent experiments.



Supplementary Figure S12 DNMT1-dependent repression of LATS2 by hNaa10p contributes to the oncogenic potential of hNaa10p. **(A)** Depleting hNaa10p from H1299 cells by si-h*NAA10*-1 diminishes DNMT1 binding to the LATS2 promoter. **(B)** Western shows that depletion of hNaa10p from H1299 cells by si-h*NAA10*-1 or si-h*NAA10*-2 increases the protein level of LATS2. **(C)** Re-expression of hNaa10p represses the LATS2 protein level induced by depleting the endogenous hNaa10p. siRNA-resistant hNaa10p-V5 construct and vector were transfected twice into H1299 cells stably expressing indicated shRNA by lentivirus infection. **(D)** Re-expression of hNaa10p lacking aa102-122 fails to repress LATS2 expression. The siRNA-resistant hNaa10p-V5 derivative construct was co-transfected with indicated siRNA into H1299 cells for 2 times. The lanes were run on the same gel but were noncontiguous. **(E)** Depletion of LATS2 expression partly restores colony formation inhibited by knocking down hNaa10p. The indicated siRNA were transfected alone or in combination into H1299 cells for 4 times, followed by colony formation assay. All data in **(A)** and **(E)** are shown as mean ± SD from three independent experiments. *P-value<0.05.

Supplementary Table S1 The list of siRNAs and DNA primers used in the study.

Experiment	Gene	Sequence
siRNA	si-h*NAA10*-1	UGAACAUGCAGCACUGCAAtt
	si-h*NAA10*-2	CAACUUUCAGAUCAGUGAAtt
	si-DNMT1	GGAUGAGAAGAGACGUAGAtt
Cloning of si-h*NAA10*-resistant plasmid	si-h*NAA10*-1 -resistant	Forward: AGGACCTTATGAATATGCAACATTGTAACCTCCTC
		Reverse: GAGGAGGTTACAATGTTGCATATTCATAAGGTCCT
Cloning of shRNA plasmids	sh*NAA10*-1	Forward: CCGGATGAACATGCAGCACTGCAACTCGAGTTGCAGTGCTGCATGTTCATTTTTTT
		Reverse: ATTAAAAAAATGAACATGCAGCACTGCAACTCGAGTTGCAGTGCTGCATGTTCATT
	shDNMT1	Forward: CCGGGGATGAGAAGAGACGTAGAACTCGAGATCTACGTCTCTTCTCATCCTTTTTA
		Reverse: AATTTAAAAAGGATGAGAAGAGACGTAGATCTCGAGTTCTACGTCTCTTCTCATCC
	shDNMT1-1384	Forward: CCGGAATGAATGGTGGATCACTGGCCTCGAGGCCAGTGATCCACCATTCATTTTTTT
		Reverse: AATTAAAAAAATGAATGGTGGATCACTGGCCTCGAGGCCAGTGATCCACCATTCATT
RT-PCR	h*NAA10*	Forward: GATCAGTGAAGTGGAGCC-3’
		Reverse AGCTCGGAGGTGAATTG-3’
	S26	Forward: TGGATAAATAAACACTAGGAACGC
		Reverse: GGGCTTCAAGAACGGCAA
	E-cadherin	Forward: GCCCTGCCAATCCCGATG
		Reverse: GAACCGCTTCCTTCATAGTCAAACAC
	LATS2	Forward: GCAGGAACTGGTGAACGC
		Reverse: CAGGTAGCCCATCTTGCTGA
	KLF2	Forward: CACCAAGAGTTCGCATCTGAAGG
		Forward: CGTGTGCTTTCGGTAGTGGC
	KLF17	Forward: CTGCTGCTGGTCCTTAGGTG
		Reverse: GTAGAACTTGAAGACTGCCTCTCC
	QKI-6	Forward: CGGAACTCCTCACCCAACTG
		Reverse: GGGAAAGCCATACCTAATACACCAC
	CDKN1B	Forward: CTAACTCTGAGGACACGC
		Reverse: TTCTGTTCTGTTGGCTCTTT
	CDKN1C	Forward: CACTCGGGGATTTCGGGAC
		Reverse: GCTTGGAGAGGGACACGG
	CDKN2A	Forward: ATAGTTACGGTCGGAGGC
		Reverse: CACCAGCGTGTCCAGGAA
	CDKN2B	Forward: TCAACCGTTTCGGGAGGC
		Reverse: GGTGAGAGTGGCAGGGTC
	CDKN2C	Forward: GGCTCAAGTCACCACCGT
		Reverse: GTAGCAGTCTCCTGGCAATCT

	CDKN2D	Forward: GCATCCCGACGCCCTCAA
		Reverse: GGCTGGCACCTTGCTTCA
Bisulfite sequencing	E-cadherin	Forward: TTTAGTAATTTTAGGTTAGAGGGTTAT
		Reverse: AAACTCACAAATACTTTACAATTCC