**SUPPLEMENTARY MATERIALS AND METHODS**

**Cell lines**

SiHa, LoVo, SW620, HCT116, A549, H1299, SK-MEL-1, Malme-3M, cf-pac-1 and Panc-1 cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), and SNUC4 cell line was purchased from Korean Cell Line Bank (KCLB, Seoul, Korea). CUMC6 and 526mel cell lines were kindly provided by Dr. JW Kim (The Catholic University of Korea, Seoul, Korea) and Dr. MT Lotze (University of Pittsburgh, Pittsburgh, PA, USA), respectively. All cell lines were obtained between 2008 and 2014, and tested for mycoplasma using Mycoplasma Detection Kit (Thermo Fisher Scientific, San Jose, CA, USA). The identities of cell lines were confirmed by short tandem repeat (STR) profiling by IDEXX Laboratories Inc. and used within 6 months for testing. CUMC6, HCT116, H1299, SK-MEL-1, cf-pac-1 and Panc-1 cells were cultured in DMEM (Thermo Scientific, Waltham, MA) containing 100 units/ml of penicillin-streptomycin and 10% fetal bovine serum (FBS). SiHa, LoVo, SW620, SNUC4, A549, 526mel and Malme-3M cells were cultured in RPMI 1640 containing 100 units/ml of penicillin-streptomycin and 10% fetal bovine serum (FBS). All cells were grown at 37°C in a 5% CO2 incubator/humidified chamber.

**Western blot (WB) analysis**

Lysate extracted from a total of 1×105 cells was used to perform Western blot, as described previously (1). Primary antibodies against HDAC-1, -2, -3, -4, AKT, phospho-AKT (S473), TCL-1, Histone-H3, -H4, AcH3-K9, -K14, -K18, -K27, AcH4-K5, -K8 and CYCLIN A (Cell Signaling, Danvers, MA, USA); MCL1, p21, p27, pSer/Thr and HDAC8 (Santa Cruz Biotechnology, Santa Cruz, CA, USA); β-ACTIN and FLAG (Sigma Aldrich, St Louis, MO, USA); NANOG and CHFR (Abnova, Taipei, Taiwan); and HA (MBL, Nagoya, Japan) were used for Western blotting followed by the appropriate secondary antibodies conjugated with horseradish peroxidase. Immunoreactive bands were developed with the chemiluminescence ECL detection system (Elpis Biotech, Daejeon, Korea), and signals were detected using a luminescent image analyzer (LAS-4000 Mini, Fujifilm, Tokyo, Japan). All Western blots (WB) analysis were repeated three times and loading controls for each membrane were verified by performing re-blotting in the stripped membrane. One representative data are shown.

**Luciferase assay**

For luciferase assay, cells were maintained in RPMI with 10% FBS and seeded at 1 × 105 cells/well in 12-well plates 1 day prior to the assay. The reporter constructs, pGL3 basic, pGL3-HDAC1 or pGL3-HDAC1 NANOG Mut together with pCMV-*β*-Gal, an internal control for transfection efficiency, were co-transfected into CaSki cells using Lipofectamine 2000 (Invitrogen). After 24 hours, cells were washed with PBS and lysed with Cell Culture Lysis Reagent (Promega). Luciferase activity was measured with a Turner Biosystems TD-20/20 luminometer after addition of 40 µl of luciferase assay reagent (Promega). *β*-Galactosidase activity was measured with a uQuant microplate reader (BioTek, Winooski, VT, USA) at 570 nm wavelength after addition of β-galactosidase assay reagent containing 1 mM chlorophenol red β-D-galactopyranoside substrate (Roche, Mannheim, Germany). Relative luciferase activity was normalized with the β-galactosidase activity in the cell lysate and calculated as an average of three independent experiments.

**Trypan blue exclusion assay**

For determining cell viability, trypan blue exclusion assay was performed. Briefly, cells were seeded at 1 × 105 cells/well in 12-well plates 1 day prior to the assay. Chemical reagents were treated at the concentrations indicated in figures. After 24h, cells were detached and stained with 0.4% trypan blue. Unstained cells were counted using a hemocytometer. Data are expressed as percentages of unstained cells compared with control cells not exposed to the chemical reagents.

**Immunohistochemistry**

For IHC, all paraffin sections were cut to 5 μm thickness, deparaffinized through xylene, and dehydrated with graded ethanol. Heat-induced antigen retrieval was done for 20 min in an antigen retrieval buffer pH 9.0 (Dako, Carpinteria, CA, USA) for AcH3K14 and pH 6.0 for HDAC1 and AcH3K27 using a pressure cooker (Pascal, Dako). Endogenous peroxidase activity was blocked by addition of 3% H2O2 for 10 minutes. Samples were incubated at room temperature with rabbit polyclonal anti-HDAC1 antibodies (Cell Signaling: Cat #2062) at 1:200 dilution for 2 h, rabbit monoclonal anti-AcH3K14 antibodies (Cell Signaling: Cat #04-1044) at 1:250 dilution for 2 h, and rabbit polyclonal anti-AcH3K27 antibodies (Abcam: ab 4729) at 1:1000 dilution for 1 h, respectively. The antigen-antibody reaction was detected with EnVision+ Dual Link System-HRP (Dako) and visualized with DAB+ (3, 3’-Diaminobenzidine; Dako). The sections were lightly counter-stained with hematoxylin. The evaluation of immunostaining was done using image analyzing software version 4.5.1.324 (Visiopharm, Hoersholm, Denmark). The intensity of staining was categorized as 0, 1+, 2+, and 3+ according to the distribution pattern across the TMA cores. The final histoscore was calculated by multiplying the intensity and percentage of staining resulting in a score of 0 to 300. NANOG staining pattern was previously evaluated in the same cohort (2).

**ChIP and quantitative ChIP (qChIP) assays**

The ChIP kit (Millipore) was employed according to the manufacturer’s instructions and ChIP assay was performed as described previously (2). Briefly, cells (1 × 107 per assay) were bathed in 1% formaldehyde (Sigma-Aldrich) at 25°C for 10 minutes for cross-linking of proteins and DNA and then lysed in SDS buffer containing protease inhibitor. DNA was sheared to 0.2-1 kb fragments by sonication using a Sonic Dismembrator Model 500 (Fisher Scientific, Pittsburgh, PA, USA). Immunoprecipitation was carried out by incubation with 1 *µ*g of anti-FLAG (Sigma-Aldrich), anti-AcH3-K9, -K14, -K27 (Cell Signaling) antibody or mouse IgG (Upstate Biotechnology, Lake Placid, NY, USA) for 16 hours. To reverse the protein-DNA crosslinks, the immunoprecipitated sample and input were incubated at 65°C overnight. After reversal of cross-linking, DNA fragments were purified on spin columns (Upstate Biotechnology). The region flanking the NANOG-binding sites in the HDAC1 promoter region was amplified and the PCR product was resolved on a 2% agarose gel containing ethidium bromide and visualized under ultraviolet light. For qChIP assay, DNA immunoprecipitated by mouse IgG, AcH3-K9, -K14 or –K27 was quantified by real-time qPCR using iQ SYBR Green super mix with CFX96 real-time PCR detection system as described above. Each sample was assayed in triplicate, and the amount of precipitated DNA was calculated as the percentage of input sample. The sequences of the primers used for ChIP and qChIP assays are listed in Supplementary Table 1.

**SUPPLEMENTARY REFERENCES**

1. Noh KH, Kang TH, Kim JH, Pai SI, Lin KY, Hung CF*, et al.* Activation of Akt as a mechanism for tumor immune evasion. Molecular therapy : the journal of the American Society of Gene Therapy **2009**;17:439-47

2. Noh KH, Kim BW, Song KH, Cho H, Lee YH, Kim JH*, et al.* Nanog signaling in cancer promotes stem-like phenotype and immune evasion. The Journal of clinical investigation **2012**;122:4077-93