Supplementary Methods

Cell culture

All cell lines were grown in monolayers in appropriate media: Dulbecco’s modified Eagle’s medium (D-MEM) for EJ28, RERF-LC-AI and 293T cells; Eagle’s Minimum Essential Medium (E-MEM) for CCD-18Co, 253J, 253J-BV, HT1376, SCaBER, UMUC3 and SBC5 cells; Leibovitz’s L-15 for SW480 and SW780 cells; McCoy’s 5A medium for RT4, T24 and HCT116 cells; RPMI1640 medium for 5637, A549, H2170 and ACC-LC-319; ATCC-formulated Eagle’s Minimum Essential Medium (Catalog No. 30-2003) for MRC-5 cells supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic solution (Sigma-Aldrich). LoVo cells were cultured in Ham’s F-12 medium supplemented with 20% fetal bovine serum and 1% antibiotic/antimycotic solution (Sigma-Aldrich). SAEC cells were maintained in small airway epithelial cell basal medium supplemented with 52 µg/ml bovine pituitary extract, 0.5 ng/ml human recombinant EGF, 0.5 µg/ml hydrocortisone, 0.5 µg/ml epinephrine, 10 µg/ml transferrin, 5 µg/ml insulin, 0.1 ng/ml retinoic acid (RA), 6.5 ng/ml triiodothyronine, 50 µg/ml Gentamicin/Amphotericin-B (GA-1000) and 50 µg/ml fatty acid-free bovine serum albumin (BSA). All cells were maintained at 37ºC in humid air with 5% CO₂ condition (SAEC, 5637, 253J, 253J-BV, EJ28, HT1376, J82, RT4, SCaBER, T24, UMUC3, A549, H2170, ACC-LC-319, RERF-LC-AI, SBC5 and 293T) or without CO₂ (SW480 and SW780). Cells were transfected with FuGENE6™ (Roche Applied Science) according to manufacturer’s protocols.

Expression profiling in cancer using cDNA microarrays

We established a genome-wide cDNA microarray with 36,864 cDNAs selected from the UniGene database of the National Center for Biotechnology Information (NCBI).
This microarray system was constructed essentially as described previously (1-3). Briefly, the cDNAs were amplified by RT-PCR using poly (A)^+ RNAs isolated from various human organs as templates; the lengths of the amplicons ranged from 200 to 1100 bp, without any repetitive or poly (A) sequences. Many types of tumor and corresponding non-neoplastic tissues were prepared in 8-μm sections, as described previously (2). A total of 30,000-40,000 cancer or noncancerous cells were collected selectively using the EZ cut system (SL Microtest GmbH) according to the manufacturer’s protocol. Extraction of total RNA, T7-based amplification, and labeling of probes were performed as described previously (2). A measure of 2.5-μg aliquots of twice-amplified RNA (aRNA) from each cancerous and non cancerous tissue was then labeled, respectively, with Cy3-dCTP or Cy5-dCTP. Detailed expression profiling data of lung cancers, shown in this study, were based on the data reported previously by Dr. Takefumi Kikuchi (1).

**Amino acid analysis**

The excised protein bands blotted on the PVDF membrane were individually inserted in clean 6 mm × 32 mm glass tubes containing 50 pmol of norvaline as internal standard and dry up by a centrifugal concentrator (Micro Vac MV-100, TOMY) with a rotary vacuum pump (ULVAC). The tubes were placed in a glass vial containing 200 μl of constant-boiling HCl and a piece of phenol crystal and the vial was sealed after evacuation for a few minutes using by a Mininert valve (Pierce). The hydrolyze samples for 20 h at 110°C were derivatized in situ by 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) for fluorophore detection. The AQC-amino acids were separated by ion-pair chromatography (4).

**Flow cytometry assays (FACS)**

To examine the effect of SETD8 expression on the cell cycle progression, SBC5 and
SW780 cells were treated with siSETD8 or control siRNAs (siEGFP and siNC), and cultured in a CO₂ incubator at 37°C for 72 h. 1 x 10⁵ cells were collected by trypsinization, and stained with propidium iodide (PI) following the manufacturer’s instructions (Cayman Chemical). Cells were analyzed by FACScan (Beckman Coulter) with MultiCycle for Windows software (Beckman Coulter) for detailed cell cycle status. The percentages of cells in G₀/G₁, S and G₂/M phases as well as those in any sub-G₁ population were determined from at least 20,000 ungated cells.

**In vivo labeling experiments**

Cells were starved for 1 h in methionine-free medium including cycloheximide (100 μg/ml) and chloramphenicol (40 μg/ml). And then cells were labeled with L-[methyl-³H] methionine (10 μCi/ml, Perkin Elmer) for 5 h. FLAG-PCNA was immunoprecipitated by anti-FLAG M2 agarose and detected by fluorography.

**Immunoprecipitation**

Transfected 293T cells or SBC5 cells were lysed with CellLytic™ M Cell Lysis Reagent (Sigma-Aldrich) containing a complete protease inhibitor cocktail (Roche). In a typical immunoprecipitation reaction, 300 μg of whole-cell extract was incubated with an optimum concentration of the following antibodies: anti-FLAG (M2, Sigma-Aldrich), anti-HA (Y-11, Santa Cruz Biotechnology) or anti-PCNA (PC10, Santa Cruz Biotechnology), and 30 μl of Protein A/G Plus-Agarose beads (Santa Cruz Biotechnology) at 4°C for 1 h. After the beads were washed 3 times in 1 ml of TBS buffer (pH 7.6), proteins that bound to the beads were eluted by boiling in Lane Marker Reducing Sample Buffer (Thermo Fisher Scientific).

**In vitro methyltransferase assay**
For the *in vitro* methyltransferase assay, recombinant His-tagged PCNA (Abcam) was incubated with active SETD8 (Millipore) using 2 μCi S-adenosyl-L-[methyl-3H] methionine (SAM; GE healthcare) as the methyl donor in a mixture of 10 μl of methylase activity buffer (50 mM Tris-HCl at pH 8.8, 10 mM DTT and 10 mM MgCl₂), for 1 h at 30°C. Proteins were resolved on a 5-20 % SDS-PAGE gel (Ready Gel; Bio-Rad) and visualized by ponceau S (MP biomedical) staining and fluorography.

**Mass spectrometry**

The immunoprecipitated products were loaded onto SDS-PAGE and stained with SimplyBlue SafeStain (Life Technologies). The excised protein bands were reduced in 10 mM tris(2-carboxyethyl)phosphine (Sigma-Aldrich) with 50 mM ammonium bicarbonate (Sigma-Aldrich) for 30 min at 37°C and alkylated in 50 mM iodoacetamide (Sigma-Aldrich) with 50 mM ammonium bicarbonate for 45 min in the dark at 25°C. Trypsin GOLD (Promega) solution was added with the enzyme to protein ratio at 1/50 (w/w) and incubated at 37°C for 16 h. The resulting peptides were extracted from gel fragments and separated on a 0.1 × 200 mm homemade C₁₈ column using 45 min linear gradient from 2 to 35% acetonitrile in 0.1% formic acid with flow rate at 200 nl/min. The eluting peptides were analyzed with QSTAR Elite QqTOF system (AB Sciex) in the smart information-dependent acquisition (SIDA) mode of the Analyst QS software 2.0 (AB Sciex). The acquired MS and MS/MS peak lists were analyzed with in-house Mascot server ver.2.3.01 (Matrix Science) to identify peptide sequences. We finally accepted the assigned peptides with Expectation value less than 0.05 as the positive identification in Mascot Database search.

**Okazaki fragment maturation assay**

The 3’ end of a specific primer (P3) strand was labeled with ³²P. Labeled primers were annealed with oligonucleotides P1 and P2 (Supplementary Table S3) for substrates
in annealing buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). FLAG-PCNA (WT) or FLAG-PCNA (K248A) protein co-expressed with SETD8 protein in 293T cells was immunoprecipitated with anti-FLAG M2 agarose and used as an enzyme source. FLAG-PCNA (WT) or FLAG-PCNA (K248A) was incubated with substrates in 20 mM HEPES (pH 7.5), 70 mM KCl, 5 mM MgCl₂, 1 mM dithiothreitol (DTT), 1 mM ATP and 200 μM dNTP at 37°C for 10 and 30 min. Reaction samples were resolved by 10% polyacrylamide gel. After gel fixation with fixation buffer (5% methanol and 5% acetic acid), the gel was analyzed by autoradiography.

**Immunohistochemical staining**

Sections of human lung cancer were stained by VECTASTAIN® ABC KIT (Vector Laboratories). Briefly, endogenous peroxidase activity of xylene-deparaffinized and dehydrated sections was inhibited by treatment with 0.3% H₂O₂/methanol. Non-specific binding was blocked by incubating sections with 3% BSA in a humidified chamber for 30 min at ambient temperature followed by overnight incubation at 4°C with a 1:500 dilution of mouse monoclonal anti-SETD8 (ab3798, Abcam) and a 1:5000 dilution of mouse monoclonal anti-PCNA (PC10) antibody. The sections were washed twice with PBS (-), incubated with 5 μg/μl goat anti-mouse biotinylated IgG in PBS (-) containing 1% BSA for 30 min at ambient temperature, and then incubated with ABC reagent for 30 min. Specific immunostaining was visualized by 3,3’-diaminobenzidine. Slides were dehydrated through graded alcohol to xylene washing and mounted on cover slips. Hematoxylin was used for nuclear counterstaining.

**Coupled cell cycle and cell proliferation assay**

A 5’-bromo-2’-deoxyuridine (BrdU) flow kit (BD Biosciences) was used to determine cell cycle kinetics and to measure the incorporation of BrdU into DNA of proliferating cells (5-7). The assay was performed according to the manufacturer’s
protocol. Briefly, cells (2 x 10^5 per well) were seeded overnight in 6-well tissue culture plates and treated with an optimized concentration of siRNAs in medium containing 10% FBS for 72 h, followed by addition of 10 μM BrdU, and incubations continued for an additional 30 min. Both floating and adherent cells were pooled from triplicate wells per treatment point, fixed in a solution containing paraformaldehyde and the detergent saponin, and incubated for 1 h with DNase at 37°C (30 μg per sample). FITC-conjugated anti-BrdU antibody (1:50 dilution in Wash buffer; BD Biosciences) was added and incubation continued for 20 min at room temperature. Cells were washed in Wash buffer and total DNA was stained with 7-amino-actinomycin D (7-AAD; 20 μL per sample). This was followed by flow cytometric analysis using FACScan (Beckman Coulter, Brea, CA, USA). Total DNA content (7-AAD) was determined using CXP Analysis Software Ver. 2.2 (Beckman Coulter).

**Immunocytochemistry**

Cultured cells were fixed in 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) at room temperature for 30 min, permeabilized in ice-cold methanol at -20°C for 10 min and 0.1% Triton X-100 (Sigma-Aldrich) for 3 min on ice, and blocked with BLOCK ACE® (Yukijirushi, Sapporo, Japan) for 1 h at room temperature. Fixed cells were incubated with primary antibodies overnight at 4°C. Then incubated with Alexa Fluor conjugated second antibody (Molecular Probes, Life Technologies) and observed using a Leica confocal microscopy (8).

**Reference**


