Supplementary figure legends

**Figure S1.** (A) ZBTB28 expression in human normal adult tissues by semi-quantitative RT-PCR, GAPDH same as previous studies. (B) The specificity of MSP primers for methylated promoter alleles was tested using not-bisulfited DNA. (C) ZBTB28 methylation detection in normal lung and nasal tissues. M, methylation; U, unmethylation.

**Figure S2.** Somatic alterations of ZBTB28 and BCL6 from Public database. Database query was based on amplification, deletion, and mutation alterations of the gene, using data available at http://www.cbioportal.org. Obviously, amplification of BCL6 is common in some cancers, but very rare for ZBTB28. The major genetic alterations of ZBTB28 are deletions and mutations, although they are still rare in multiple cancers.

**Figure S3.** Expression and methylation status of ZBTB28 in multiple tumor tissues. (A) Quantitative reverse transcription-PCR (qRT-PCR) analysis of ZBTB28 mRNA expression in multiple tumor tissue samples and paired adjacent normal tissues, data available at http://mgrc.kribb.re.kr/GENT/overview.php. (B) Methylation of the ZBTB28 promoter in normal and tumor tissues from MethHC database. (C) Downregulation and promoter methylation of ZBTB28 in lung cancer tissue samples and paired non-cancerous samples from Cancer Genome Atlas (TCGA), data accessed via MethHC.

**Figure S4.** Methylation status of ZBTB28 promoter in colon and gastric tumor tissues and normal tissues. Representative data of ZBTB28 methylation in (A) tumor and (B) normal tissues. M: methylated; U: unmethylated.

**Figure S5.** ZBTB28 functions as a tumor suppressor in colon tumor cells. (A) ZBTB28 expression at mRNA and protein levels was examined by
semi-RT-PCR (upper panel) and Western blot (lower panel) in HT29 cells, with GAPDH as a control. (B) Ectopic ZBTB28 expression inhibits HT29 cell growth, as assessed by MTS (B), colony formation (C) or soft agar assays (D). Values are shown as mean±standard error from three independent experiments. (E) Cell cycle distribution of vector- or ZBTB28-transfected HT29 cells as determined by flow cytometry. Representative flow cytometry plots. Histograms of cell cycle alterations. (F) Induction of apoptosis detected in ZBTB28-expressing HT29 cells by AO/EB assay. Histograms of apoptosis rate are shown at the bottom. Values are shown as mean±standard error from three independent experiments. (G) ZBTB28 inhibits tumor growth of HT29 xenograft in vivo. Red and black arrows indicate empty vector control and ZBTB28-overexpressing tumors, respectively. Tumor weight measurements from empty vector control and ZBTB28-overexpressing tumors (n=5). Growth curve of xenograft tumors. Tumor volume was calculated from tumor length and width, measured twice per week.

**Figure S6.** ZBTB28 inhibits tumor growth of A549 xenograft in vivo. (A) Representative images of PCNA immunohistochemistry staining and (B) score of proliferation index.

**Figure S7.** ZBTB28 inhibits tumor cell migration and EMT. (A) Representative wound healing assay. Photographs were taken at 0 and 24 h of ZBTB28-transfected HONE1 and KYSE150 tumor cells. (B) Expression and location of Occludin and Vimentin markers in HONE1 cells as detected by confocal microscopy. Original magnification, 400x.

**Figure S8.** (A-B) Protein-protein association networks predicted an interaction between ZBTB28 and BCL6. (C) Predicted domain structure of ZBTB28 and BCL6 proteins showing the BTB protein-protein interaction domain and Zinc-finger domain (COSMIC, http://cancer.sanger.ac.uk/cosmic). Binding
protein motif prediction showed a highly homologous binding domain between BCL6 and ZBTB28. (D) The expression of p53 and its downstream target genes as detected by qRT-PCR in A549 cells with ZBTB28 overexpression and BCL6 knockdown (** p<0.01, *** p<0.001, ZBTB28 vs ZBTB28+siBCL6).

**Figure S9.** RNA sequencing analyses reveals differentially expressed genes in KYSE150 tumor cells expressing ZBTB28.
Figure S1

A

Normal adult tissues

Fetal tissues

ZBTB26

B

not-bisulfited
DNA

ZBTB26

C

Normal lung tissues

Normal nasal tissues

Markers

LN1, LN2, LN3, LN4, LN5, LN6

Markers

SD1, SD2, SD3, SD4, SD5, SD6, SD7, SD8

Markers

M, U

Markers

M, U
Figure S2

BCL6

Lung Ca

Alteration frequency

HNSCC

GsCa & ESCC

ZBTB28

Alteration frequency

- Mutation
- Amplification
- Deep Deletion
Figure S3

A

B

ZBTB28 methylation

C

Lung Cancer (TCGA)

Expression of ZBTB28 mRNA

Methylation of ZBTB28 promoter

p<0.0001

p<0.0001
Figure S4

A

CRC

Gastric Ca

MSP

B

Normal gastric tissues

Normal colon tissues

MSP
Figure S5

A

HT29

ZBTB28

GAPDH

Vector

ZBTB28

RT-PCR

WB

B

HT29

Absorbance (OD490 nm)

Time (Hours)

Dip G1: 28.35%
Dip G2: 13.83%
Dip S: 57.81%

Vector

ZBTB28

Dip G1: 66.55%
Dip G2: 2.37%
Dip S: 31.07%

E

HT29

% of cells in each phase

G0-G1

G2-M

S

Vector

ZBTB28

F

HT29

Vector

ZBTB28

% of apoptotic cells

Vector

ZBTB28

G

HT29

Vector

ZBTB28

Tumor weight (g)

Tumor volume (mm^3)

<0.05

Vector

ZBTB28
Figure S6

A

Vector
ZBTB28

ZBTB28

PCNA

B

Vector
ZBTB28

PCNA

A549 #2
Figure S7

A

HONE1

Vector  ZBTB28

0h  24h

KYSE150

Vector  ZBTB28

0h  24h

B

ZBTB28  Occludin  merged

HONE1

ZBTB28  Vimentin  merged
Figure S9