SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Materials
Human Gas41 cDNA were cloned into pENTR3C; Human Tip60, DAMP1 and EP400 (1-1300aa) in pENTR or pDONOR were purchased from Open Biosystems. The coding sequences in pENTR were subsequently cloned into p3FLAG, pCDH-Flag, pCAG-Myc, or pGEX-6P1 destination vectors using Gateway techniques (Invitrogen). Point mutations were generated using a site-directed mutagenesis kit (Stratagene). Histone peptides bearing different modifications were synthesized at the W.M. Keck Facility at Yale University or SciLight Biotechnology, LLC. Anti-EP400 (Ab70301) antibody and anti-histone antibodies including anti-H3 (Ab1791), anti-H2A (Ab18255), anti-H3K27ac (Ab4729) and anti-H2AZ (Ab4174) antibodies were obtained from Abcam. Anti-Gas41 (sc-393708) and anti-GST (sc-459) antibodies were from Santa Cruz. Anti-FLAG (M2, F1804) and anti-beta-tubulin (T8328) antibodies were from Sigma. Anti-MYC (05-724) and anti-H3K14ac (07-353) antibodies were from Millipore. SRCAP antibody was kindly provided by Dr. Jun Huang (Zhejiang University, China). pLKO shRNA constructs were purchased from Sigma. The shRNA sequences were: GASs41#1: CGGTAATGTTGCTCGGTATTT; GAS41#2: TGTAAGAATGGATGCTATATA; H2AFV: GCTGGCAGGTAATGCTTCTAA; H2AFZ: GCTTCAAAGAAGCTATTGATT; SRCAP: CCCTCAGGTTGGAGATAAAA; EP400: GCGGAAACTCATGGAGGAAAT.

Cell Culture, Viral Transduction, and RNA Interference
Human WI-38, IMR90 and HEK 293T cells (ATCC) were maintained in DMEM (Cellgro) supplemented with 10% fetal bovine serum (Sigma). The human lung cancer cell lines A549, NCI-H1299, NCI-H1355, NCI-H1972, and NCI-H1993 were maintained in RPMI-1640 supplemented with 10% fetal bovine serum. Human lentiviral transduction was performed as described previously. Briefly, 293T cells were co-transfected with pMD2.G, pPAX2 (Addgene) and pLKO-shRNA or pCDH cDNA constructs. For infections, cells were incubated with viral supernatants in the presence of 8 µg/ml polybrene; after 48 hours, the infected cells were selected with puromycin (1.5 µg/ml) for pLKO clones or blasticidin (10 µg/ml) for pCDH clones for 3-4 days before experiments.

Protein Production
Recombinant Gas41 was cloned into pGEX-6P vector with ampicillin resistance. The WT and mutants proteins with N-terminal GST tag were expressed in E. coli Rosetta 2(DE3)pLysS cells in the presence of 0.1 mM IPTG at 16°C. Overnight induced cells were harvested by centrifugation at 5,000 rpm and resuspended in lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.05% NP-40) followed by sonication. GST-fusion proteins were purified on glutathione Sepharose 4B beads (Pierce, Fisher).

Peptide Pull-Down Assay
One µg of biotinylated histone peptides with different modifications were incubated with 1-2 µg of GST-fused proteins in binding buffer (50 mM Tris-HCl 7.5, 250 mM NaCl, 0.1% NP-40, 1 mM PMSF) overnight. Streptavidin beads (Amersham) were added to the mixture, and the
mixture was incubated for 1 hr with rotation. The beads were then washed three times and analyzed using SDS-PAGE and Western blotting.

**Isothermal Titration Calorimetry (ITC)**
The GAS41 (15-159) samples were dialyzed in the following buffer: 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% glycerol and 2 mM β-mercaptoethanol. Protein concentrations were determined by absorbance spectroscopy at 280 nm. Peptides (>95% purity) were quantified by weight, prior to aliquoting and freeze-drying for individual use. Calorimetric titration curves were analyzed using Origin 7.0 (OriginLab) and the “One Set of Binding Sites” fitting model. All calorimetric experiments were conducted at 15 °C using a MicroCal ITC200 instrument (GE Healthcare). Detailed thermodynamic parameters of each titration are summarized in Supplemental Table S3.

**Co-Immunoprecipitation**
Co-IP was performed essentially as described previously (Li et al. 2014). Cells were lysed in cell lysis buffer containing 50 mM Tris-HCl pH 7.4, 250 mM NaCl, 0.5% Triton X-100, 10% glycerol, 1 mM DTT, and a complete protease inhibitor tablet (Roche). Antibodies conjugated with protein A/G beads (Millipore) or anti-FLAG M2-conjugated agarose beads (Sigma) were incubated with the lysates overnight at 4°C. The beads were then washed 3-6 times with cell lysis buffer, and the bound proteins were eluted in SDS buffer and analyzed by Western blotting.

**Cell Proliferation and Colony Formation Assays**
Cell proliferations were determined by counting live cells using hemocytometer cell counter or by CellTiter-Glo luminescent cell viability assay kit (Progema-G7572). For colony formation assays, H1299 cells treated with shRNAs were seeded in 6 well plates (400 cells/well) and grown in RPMI-1640 plus 10% FBS at 37°C for 10-14 days. Cells were fixed and stained with a mixture of 6.0% glutaraldehyde and 0.5% crystal violet. The colonies were photographed and were counted using ImageJ software. For anchorage-independent cell growth assays, 2×10^4 H1299 cells were suspended in complete RPMI-1640 medium containing 0.35% agar and seeded into six-well plates pre-coated with a base layer of 0.6% agar and grown for 3 weeks. Cells were stained with 0.005% crystal violet blue and photographed. Colony numbers were counted using ImageJ software with size cutoff of 25 μm. Results were quantitated from six to eight views from three independent replicates.

**Tumor xenograft**
All animal studies were in compliance with ethical regulations at the University of Texas MD Anderson Cancer Center. Athymic nude mice (age 6–8 weeks) were obtained from University of Texas MD Anderson Cancer Center and housed under pathogen-free conditions. Three million GAS41 KD H1299 cells stably expressing control pCDH vector, wild-type GAS41, Y74A or W93A mutants were suspended in 50 µl serum-free RPMI 1640, mixed with 50 µl Matrigel, and injected subcutaneously into the mice. The growth of tumors was monitored twice a week until the largest one reached the limit of tumor burden. Tumor sizes were measured using a caliper and tumor volume was calculated according to the following equation: tumor volume \( (\text{mm}^3) = (\text{length (mm)} \times \text{width}^2 \text{ (mm}^2)) \times 0.5 \). Representative data were obtained from all the mice per experimental group. Statistical analyses were performed with two-tailed unpaired Student’s test.
Supplemental Figure 1. GAS41 is overexpressed in cancers and is required for cell proliferation.
(A-E) GAS41 mRNA levels are positively correlated with gene amplification status in lung adenocarcinoma (A), sarcoma (B), bladder urothelial carcinoma (C), uterine carcinosarcoma (D), glioblastoma (E). Data were obtained from the cBioPortal for Cancer Genomics.

(F) GAS41 transcripts are elevated in NSCLC. Data were acquired from the Oncomine database using the Garber Lung dataset (Garber et al. 2001). LCC: large cell carcinoma. LAC: lung adenocarcinoma. LSCC: lung squamous cell carcinoma. (P-values were determined by two-tailed unpaired Student’s test).

(G) Western blot analysis of GAS41 KD efficiency in lung carcinoma A549 cells and lung adenocarcinoma H1355 cells.

(H-I) Cell proliferation of control (shNT) and GAS41 KD (shGAS41-1) A549 cells (H) and H1355 cells (I). Error bars indicate SEM of 4 biological replicated. ***P < 0.001; ****P < 0.0001 (Two-tailed unpaired Student’s test).

(J) Western blot analysis of GAS41 KD efficiency in fetal lung fibroblast WI-38 and IMR90 cells as well as human bronchial epithelial HBEC cells.

(K-M) Cell proliferation of control (shNT) and GAS41 KD (shGAS41-1) WI-38 cells (K), IMR90 cells (L), and HBEC cells (M). Error bars indicate SEM of 4 biological replicated. **P < 0.01, ***P < 0.001 (Two-tailed unpaired Student’s test).
Supplemental Figure 2. Expression of GAS41-regulated cell cycle genes involves both H2A.Z isoforms.

(A) qRT-PCR analysis of GAS41, H2AFZ and H2AFV mRNA expression in H1299 cells treated with control (shNT) or GAS41 (shGAS41) shRNAs. Error bar, SEM of duplicates. N.S.: not significant, *P< 0.05 (Two-tailed unpaired Student’s test).

(B) qRT-PCR analysis of H2AFZ and H2AFV KD efficiency in H1299 cells. N.S.: not significant, *P< 0.05; **P < 0.01; ***P < 0.001 (Two-tailed unpaired Student’s test).

(C) Western blot analysis H2A.Z expression in H2AFZ and H2AFV KD H1299 cells.

(D) Cell proliferation of control (shNT), H2AFZ and H2AFV KD H1299 cells. Error bars indicate SEM of 4 biological replicated. ***P < 0.001 (Two-tailed unpaired Student’s test).

(E) KEGG pathway analysis of down regulated genes in H2AFZ and H2AFV KD H1299 cells. Data were analyzed using DAVID Bioinformatics Resources 6.8. The most significant 5 categories are shown. All categories are shown in Supplemental Table S1.
Supplemental Figure 3. Both the SRCAP and Tip60/p400 complexes are required for GAS41-dependent H2A.Z deposition and gene regulation.

(A-D) Overexpression of H2A.Z in GAS41 KD cells cannot restore H2A.Z occupancy on GAS41 target genes.
(A) Western blot analysis of H2A.Z and GAS41 protein levels in shGAS41-1 expressing H1299 cells rescued with exogenous H2AFZ.
(B) ChIP-qPCR analysis of H2A.Z promoter occupancy of the indicated GAS41 targeting cell cycle-related genes in cells in (A). Error bars indicate SEM of triplicates. \( *P < 0.05; **P < 0.01 \) (Two-tailed unpaired Student’s test).
(C) qRT-PCR analysis of indicated GAS41 target genes expression in cells in (A). Error bars indicate SEM of duplicates. \( **P < 0.01; ***P < 0.001 \) (Two-tailed unpaired Student’s test).
(D) Cell proliferation of shGAS41-1 expressing H1299 cells rescued with H2AFZ. Error bars indicate SEM of 5 biological replicated. \( ****P < 0.0001 \) (Two-tailed unpaired Student’s test).
(E) Western blot analysis of EP400 (left panel) and SRCAP (right panel) KD efficiency in H1299 cells. Arrows, p400 (left panel) and SRCAP (right panel).
(F) qRT-PCR analysis of EP400 (left panel) and SRCAP (right panel) KD efficiency in H1299 cells. \( *P < 0.05; ****P < 0.0001 \) (Two-tailed unpaired Student’s test).
(G) qRT-PCR analysis of selected GAS41 target genes expression in control (shNT), EP400, and SRCAP KD H1299 cells. Error bars indicate SEM of a duplicate experiment. \( *P < 0.05; **P < 0.01 \) (Two-tailed unpaired Student’s test).
(H) ChIP-qPCR of H2A.Z at promoters of the indicated GAS41 target genes in control (shNT), EP400 and SRCAP KD H1299 cells. Error bars indicate SEM of a triplicate experiment. \( *P < 0.05; **P < 0.01; ***P < 0.001 \) (Two-tailed unpaired Student’s test).
(I) Cell proliferation of control (shNT), EP400 and SRCAP KD H1299 cells. Error bars indicate SEM of 5 biological replicated. \( ****P < 0.0001 \) (Two-tailed unpaired Student’s test).
Supplemental Figure 4. Comparison of H3K27ac recognition among the GAS41, YEATS2 and ENL YEATS domains.

(A-C) Complex structures of H3K27ac peptide bound to the YEATS domains of GAS41 (A), YEATS2 (B), and ENL (C). YEATS domains are represented in electrostatic surface view and color-coded by electrostatic potential ranging from -10 kT/e to +10 kT/e. The peptides are depicted as sticks. Arrows highlights the N-to-C orientation of the peptides.

(D) Sequence motif the H3 peptide ranging from residues 1 to 31.
Supplemental Figure 5. The YEATS domain is required for GAS41 chromatin recruitment and for GAS41-dependent tumor growth in mice.

(A) Western blot analysis of Flag-GAS41 protein levels in H1299 cells stably expressing WT GAS41 or the indicated acetyl-histone-binding deficient mutants. Actin was used as a loading control.

(B) Tumors derived from xenografted H1299 cells (n=9-10) as in Fig. 6F were collected 5.5 weeks after subcutaneous transplantation in immunodeficient nude mice.
Supplemental Table S3. Summary of thermodynamic parameters from ITC.

| Protein       | Peptides          | ΔH  (kcal/mol) | ΔS  (cal/mol/deg) | K_D (µM) | N  |
|---------------|-------------------|----------------|------------------|----------|----|
| GAS41 (15-159)| H3_{15-39}K27ac   | -86.5          | -9.5             | 32.7     | 1.0|
|               | H3_{1-15}K14ac    | -10.7          | -14.7            | 13.0     | 1.0|

H3_{15-39}K27ac: APRKQLATKAARK(ac)SAPATGGVKKPH
H3_{1-15}K14ac: ARTKQTARKSTGGK(ac)A

SUPPLEMENTAL REFERENCE

Garber, M. E., Troyanskaya, O. G., Schluens, K., Petersen, S., Thaesler, Z., Pacyna-Gengelbach, M., van de Rijn, M., Rosen, G. D., Perou, C. M., Whyte, R. I., et al. (2001). Diversity of gene expression in adenocarcinoma of the lung. Proc Natl Acad Sci U S A 98, 13784-13789.