SUPPLEMENTARY FIGURES

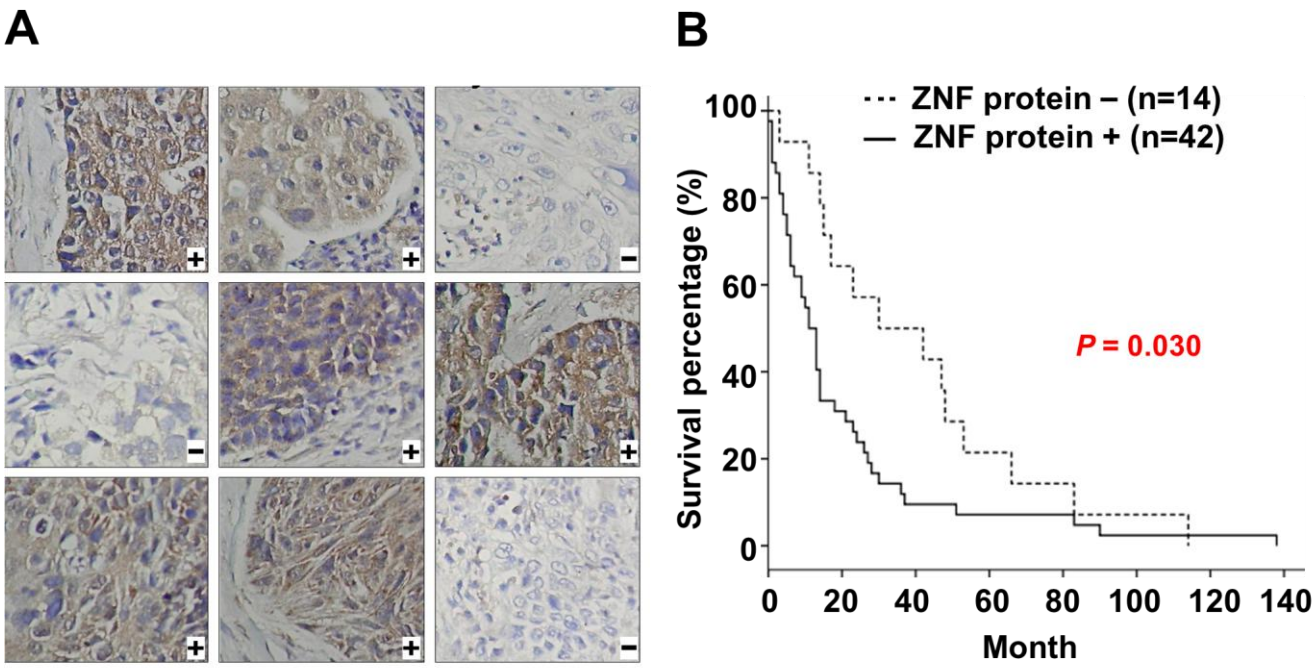


Figure S1. Clinical significance of ZNF322A overexpression in Caucasian lung cancer patients. (**A**) Representative immunohistochemistry images of ZNF322A protein expression in tissue array are shown. (**B**) Overall survival of lung cancer patients with ZNF322A overexpression (solid line) versus patients with ZNF322A normal expression (dotted line). + indicates positive immunoreactivity, as oppose to – for reverse patterns. *P* value for survival analyses (**B**) using log-rank test.

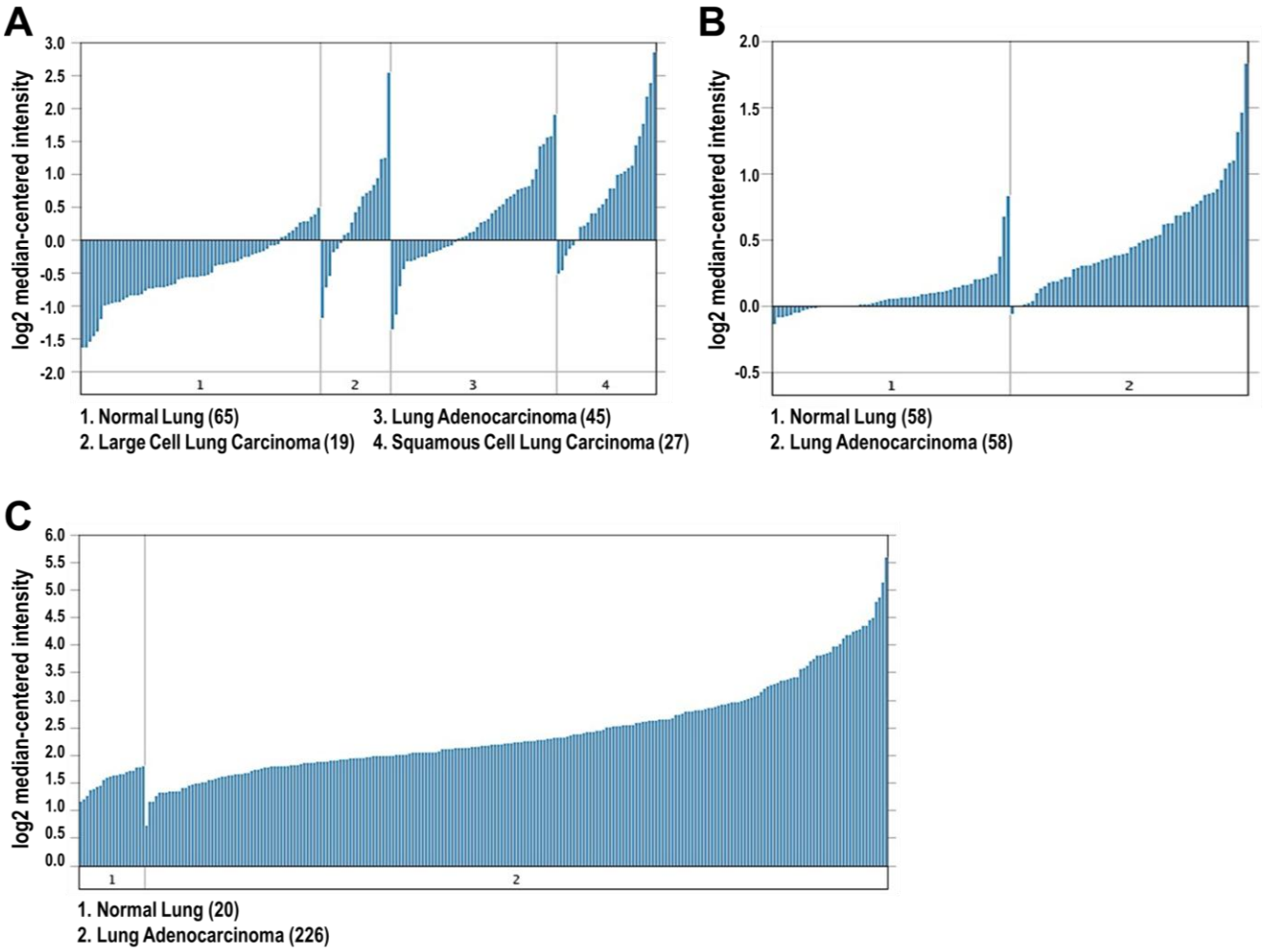


Figure S2. mRNA expression of *ZNF322A* in three published datasets deposited in Oncomine. ZNF322A mRNA expression in normal lung and lung carcinoma specimens from three published datasets was extracted and analyzed in Oncomine and presented as bar charts. Three datasets are derived from (**A**) Hou *et al*, PLoS One, 2010,[16] (**B**) Selamat *et al*., Genome Res, 2012,[17] and (**C**) Okayama *et al*., Cancer Res, 2012.[18] Log2 median-centered intensity of *ZNF322A* mRNA expression was shown as y axis. Number in parentheses indicated the number of samples analyzed in each dataset.

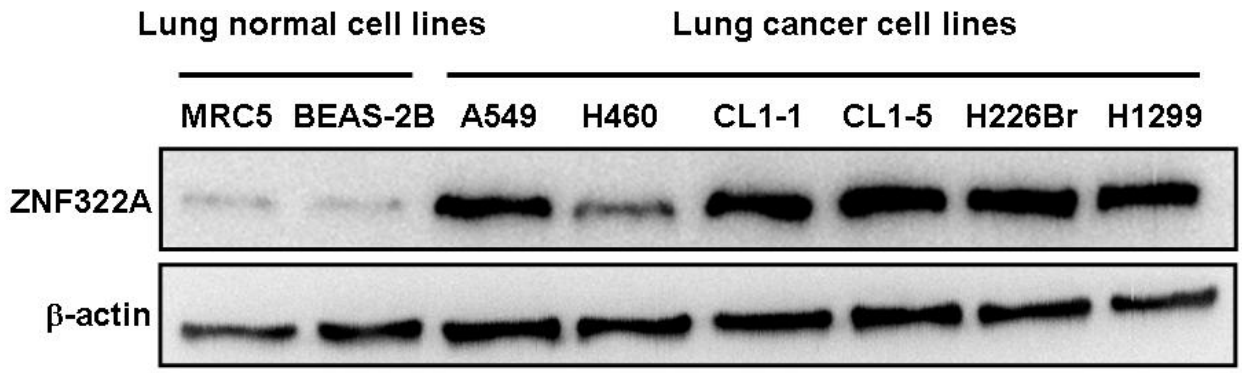


Figure S3. ZNF322A expression level among various lung cell lines. Immunoblotting analyses were performed to examine the protein expression level of ZNF322A in various lung cell lines, including the normal lung cell line MRC5, the immortalized lung cell line BEAS-2B, and lung cancer cell lines A549, H460, CL1-1, CL1-5, H226Br and H1299. β-actin serves as an internal control.

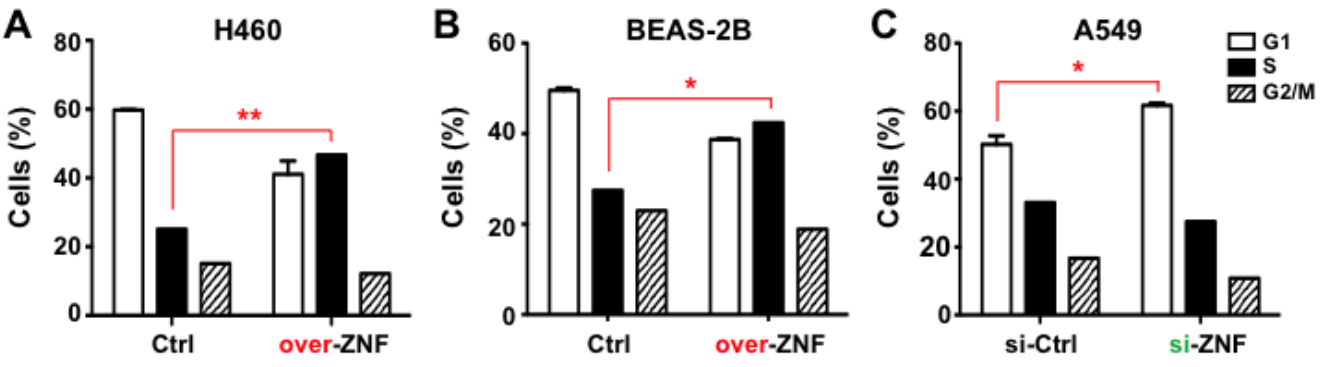


Figure S4. ZNF322A overexpression promotes G1/S transition, while ZNF322A knockdown decreases proportion of cells in S phase. Cell distribution (G1, S, and G2/M) is shown for H460 (**A**), BEAS-2B (**B**) and A549 (**C**) cells. Data are mean ± s.e.m. (n = 3). *, $P < 0.05$; **, $P < 0.01$.

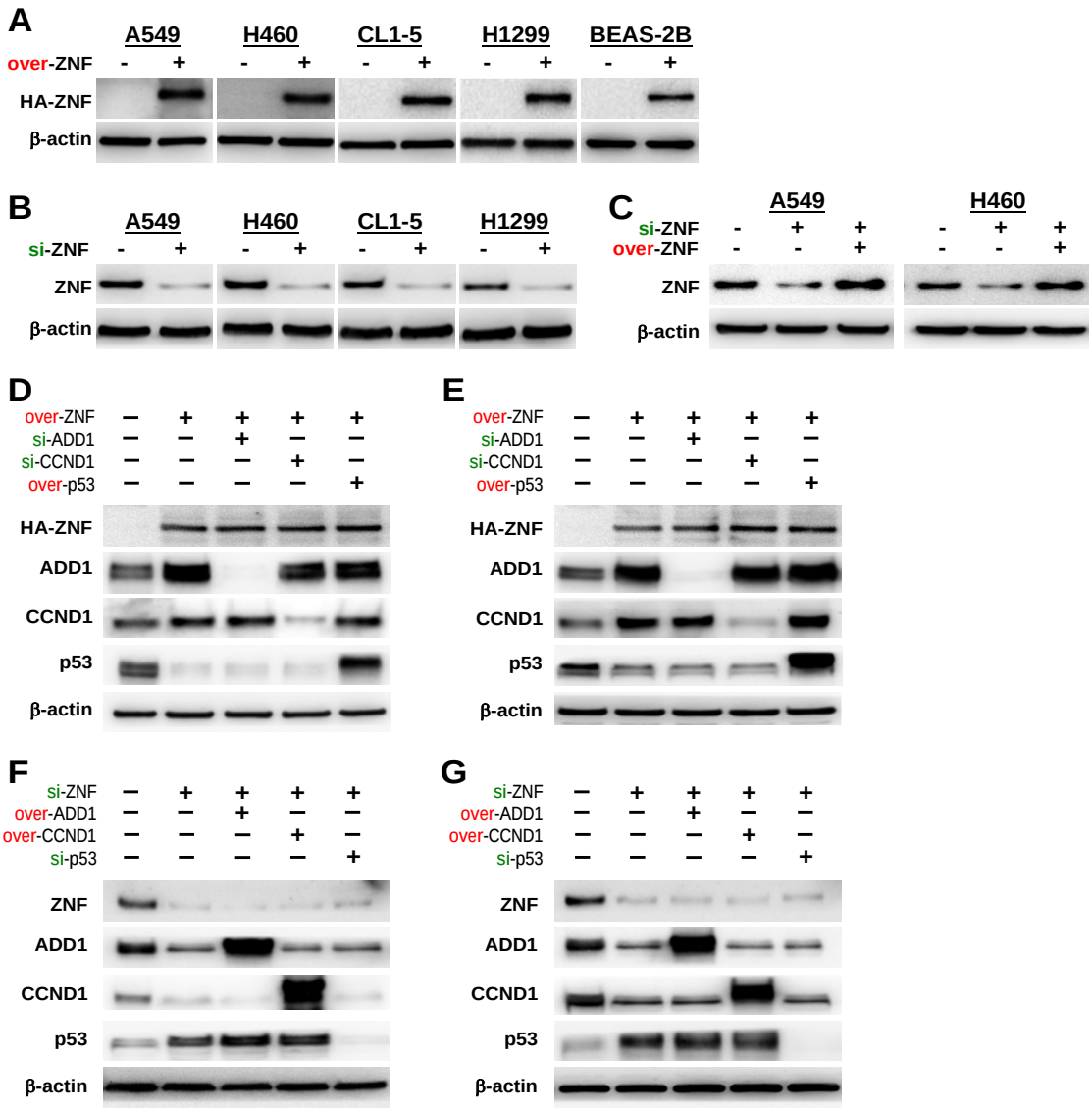
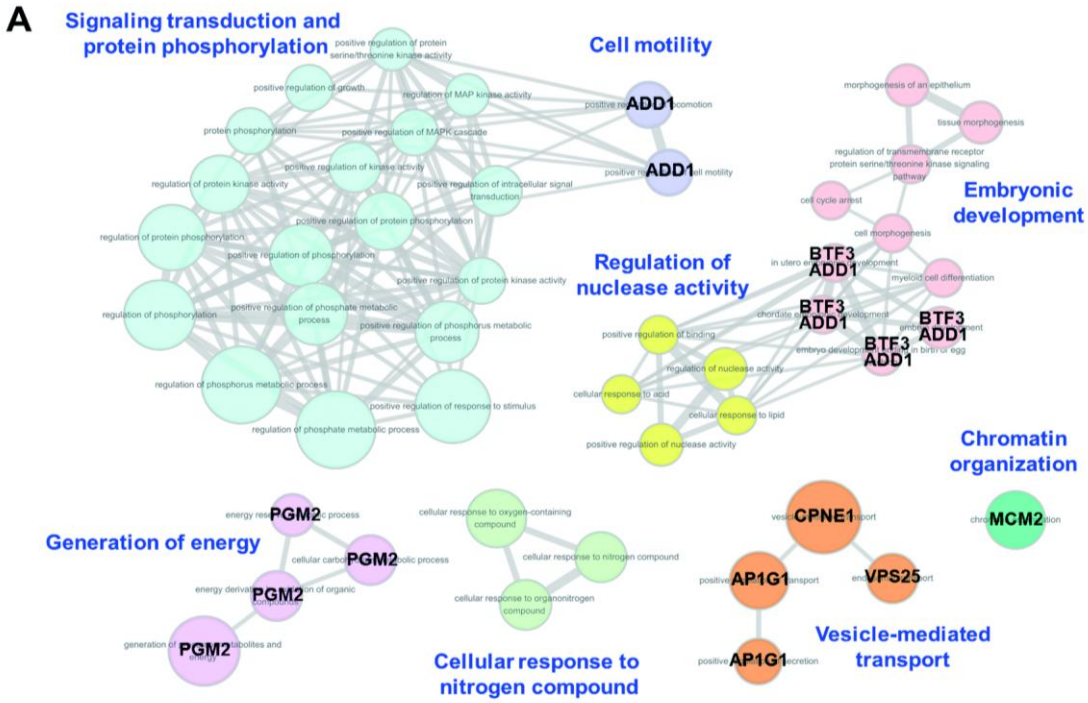


Figure S5. Protein expression level of the manipulated ZNF322A and its downstream targets in various lung cells. (**A**, **B**) *ZNF322A* expression vector (over-ZNF) (**A**) or si-*ZNF322A* oligo (si-ZNF) (**B**) was transfected into A549, H460, CL1-5, H1299 and BEAS-2B cells. (**C**) si-ZNF oligo alone or together with over-ZNF vector were transfected into A549 (left) and H460 (right) cells. (**D**-**G**) over-ZNF vector alone or together with si-*ADD1* oligo, si-*CCND1* oligo or *p53* expression vector (over-p53) were transfected into A549 (**D**) and H460 (**E**) cells. si-ZNF oligo alone or together with *ADD1* expression vector (over-ADD1), *CCND1* expression vector (over-CCND1) or si-*p53* oligo were transfected into A549 (**F**) and H460 (**G**) cells. Cell lysates were subjected to immunoblotting. β-actin serves as an internal control.



B

Top Networks

ID	Associated Network Functions	Score
1	Cancer, Developmental Disorder, Hereditary Disorder	24
2	Cellular Movement, Connective Tissue Development and Function, Cell Death and Survival	19
3	Endocrine System Disorders, Reproductive System Disease, Embryonic Development	15
4	Cell Morphology, Cellular Assembly and Organization, Cardiovascular Disease	2

Diseases and Disorders

Name	p-value	# Molecules
Cancer	5.31E-04 – 4.79E-02	8
Connective Tissue Disorders	1.05E-03 – 8.15E-03	2
Metabolic Disease	1.05E-03 – 4.40E-02	2
Cardiovascular Disease	4.08E-03 – 4.40E-02	2
Developmental Disorder	4.08E-03 – 4.40E-02	3

Figure S6. Protein network and pathway analyses of ZNF322A regulated proteins. (**A**) Differentially expressed proteins upon ZNF322A knockdown were identified by iTRAQ. A total of 1,108 identified proteins were subjected to GO analysis by in-house developed script. Node represents each enriched GO term (FDR q < 0.00001, overlap cutoff > 0.5). Node size is proportional to the total number of genes in each GO term. Edge thickness represents the number of overlapping genes between nodes. GO terms of similar functions are sorted into one cluster, marked with circles and labels. (**B**) Sixty-four significantly differential expressed proteins were analyzed by IPA database and the top four enriched networks (upper) and top five diseases and disorders (lower) regulated by ZNF322A are shown.

A

Gene Symbol	Description	siZNF322A/siControl	
		iTRAQ ratio	Immunoblot ratio
***R* value > 0.6064**			
VPS25	Vacuolar protein-sorting-associated protein 25	3.69	1.89
PGM2	Phosphoglucomutase-2	3.21	2.17
***R* value < -0.6064**			
ADD1	Alpha-adducin	0.34	0.52
KIAA0090	Uncharacterized protein KIAA0090	0.58	0.56
MCM2	DNA replication licensing factor MCM2	0.46	0.45
NSDHL	Sterol-4-alpha-carboxylate 3-dehydrogenase	0.49	0.57
BTF3	Transcription factor BTF3	0.47	0.35
RAD23B	UV excision repair protein RAD23 homolog B	0.47	0.54
CPNE1	Copine-1	0.57	0.52
AP1G1	AP-1 complex subunit gamma-1	0.49	0.41

B

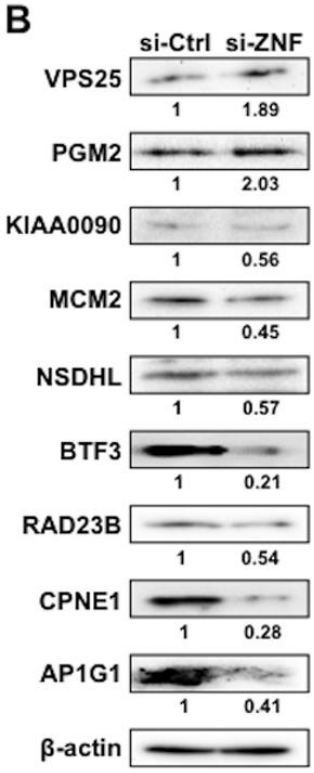


Figure S7. Validation of putative ZNF322A downstream targets identified from proteomic dataset. (**A**) The representative differentially expression proteins analyzed in proteomic analyses of si-*ZNF322A* compared to vector control A549 cells. R value is described as Supplemental Experimental Procedures. The number 0.6064 is the value of 2 σ_s (S.D.) as described in Supplemental Experimental Procedures. (**B**) The relative protein expressions of vacuolar protein-sorting-associated protein 25 (VPS25), phosphoglucomutase-2 (PGM2), uncharacterized protein KIAA0090 (KIAA0090), DNA replication licensing factor MCM2 (MCM2), sterol-4-alpha-carboxylate 3-dehydrogenase (NSDHL), transcription factor BTF3 (BTF3), UV excision repair protein RAD23 homolog B (RAD23B), copine-1 (CPNE1) and AP-1 complex subunit gamma-1 (AP1G1) in A549 cells with ZNF322A knock down (si-ZNF) were measured using immunoblotting. β-actin serves as an internal control. Quantified results are normalized with si-control (si-Ctrl) and indicated below each immunoblot.

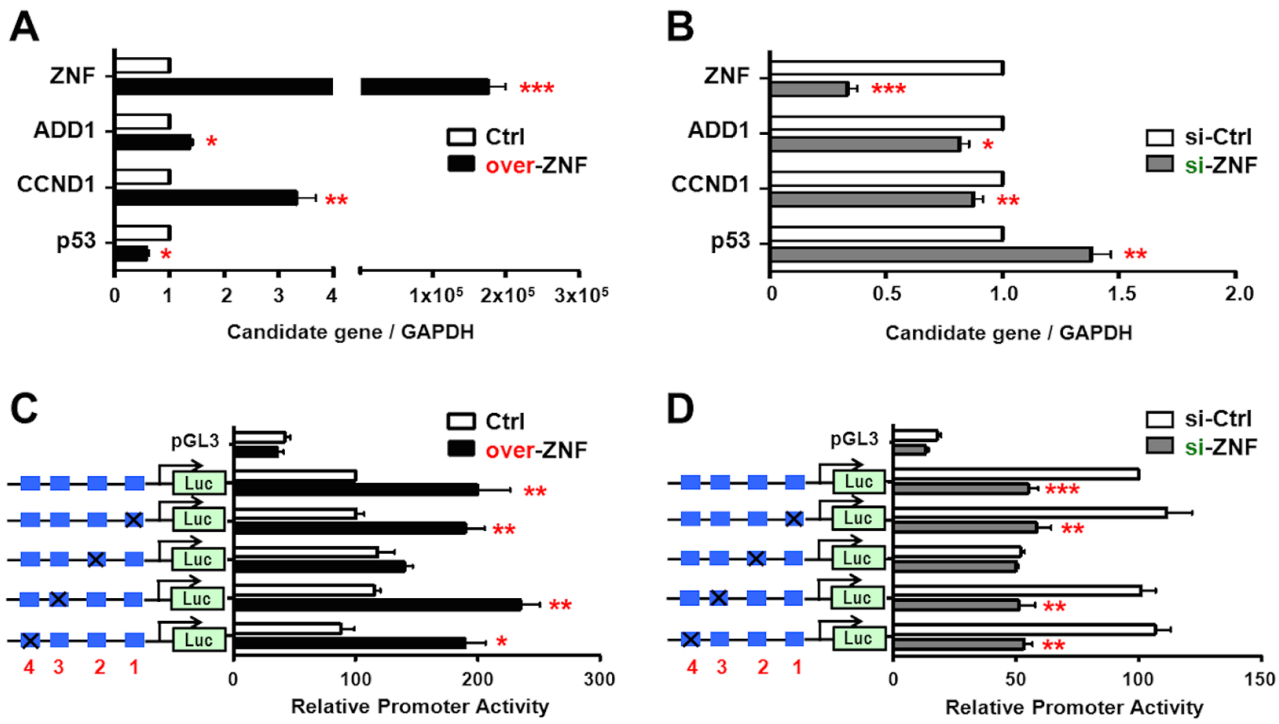


Figure S8. ZNF322A positively regulates ADD1 expression through second AP-1 element at transcriptional level. (**A**, **B**) ZNF322A positively regulates ADD1 and CCND1 expression but negatively regulates p53 expression. (**A**) H460 cell lysates transfected with empty vector (Ctrl) or *ZNF322A* expression vector (over-ZNF) were subjected to mRNA expression analyses as indicated. (**B**) A549 cell lysates transfected with si-control (si-Ctrl) or si-*ZNF322A* (si-ZNF) oligo were detected for mRNA (right) expression. (**C**, **D**) H460 cells transfected with a pGL3-based construct of wild-type AP-1 (blue box) or mutant AP-1 binding site (marked with X symbol) along with ZNF322A overexpression (over-ZNF) (**C**) or ZNF322A silencing (si-ZNF) (**D**). Relative promoter activity is shown for each construct in cells expressing over-ZNF normalized to Ctrl group or si-ZNF compared to si-Ctrl. Data are mean ± s.e.m. (n = 3). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

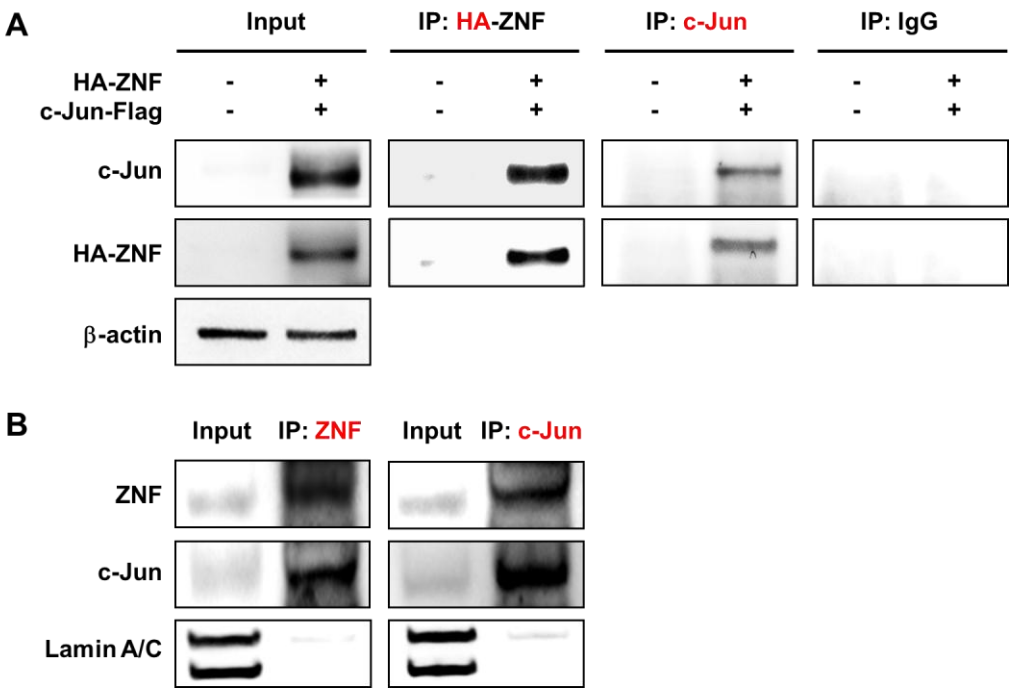


Figure S9. ZNF322A interacts with c-Jun. ZNF322A and c-Jun interaction was examined using immunoprecipitation-Western blot analysis. ZNF322A and c-Jun were immunoprecipitated from (**A**) total cell lysate of H460 cells overexpressing HA-tagged ZNF322A (HA-ZNF) and Flag-tagged c-Jun (c-Jun-Flag) or (**B**) nuclear lysate of A549 cells then subjected to immunoblotting. β-actin and lamin A/C levels are shown as a loading control of total protein lysate and nuclear protein lysate, respectively. IgG serves as a negative control.

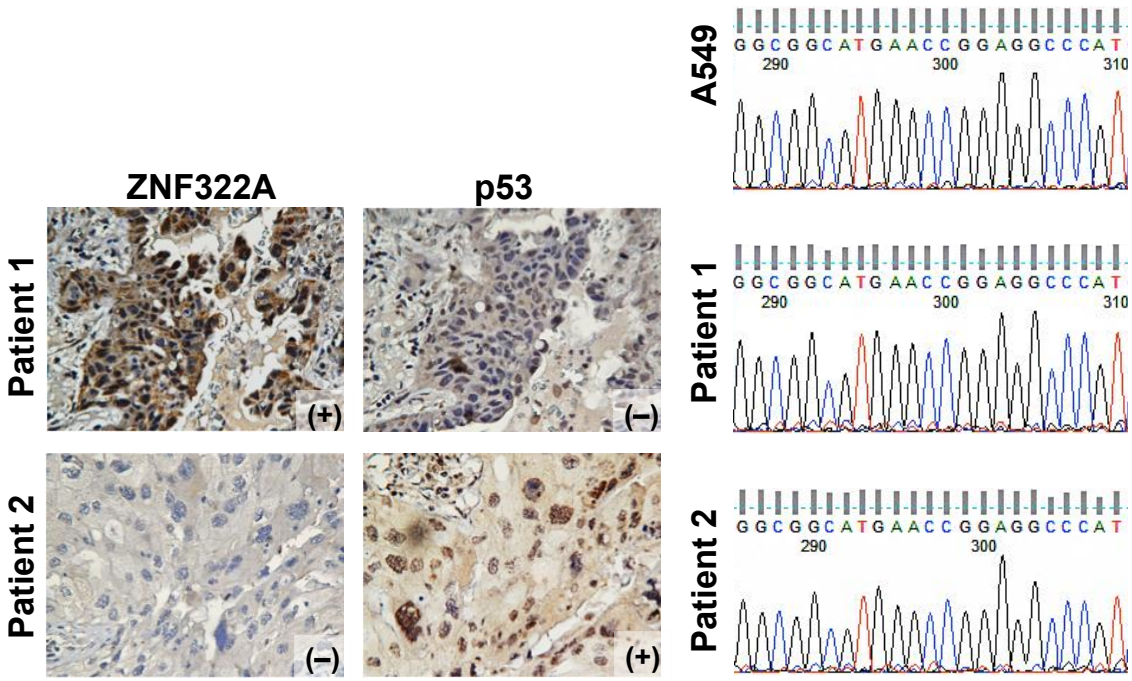


Figure S10. Sequencing results of *p53* gene mutations in lung cancer patients. PCR-sequencing analyses on cDNA covering the mutation hotspot exons 5 to 11 of *p53* gene were conducted in two lung cancer samples analyzed in Fig. 1b and 1c. *p53* sequencing result of A549 lung cancer cell line which contains wild-type *p53* is included as a positive control.

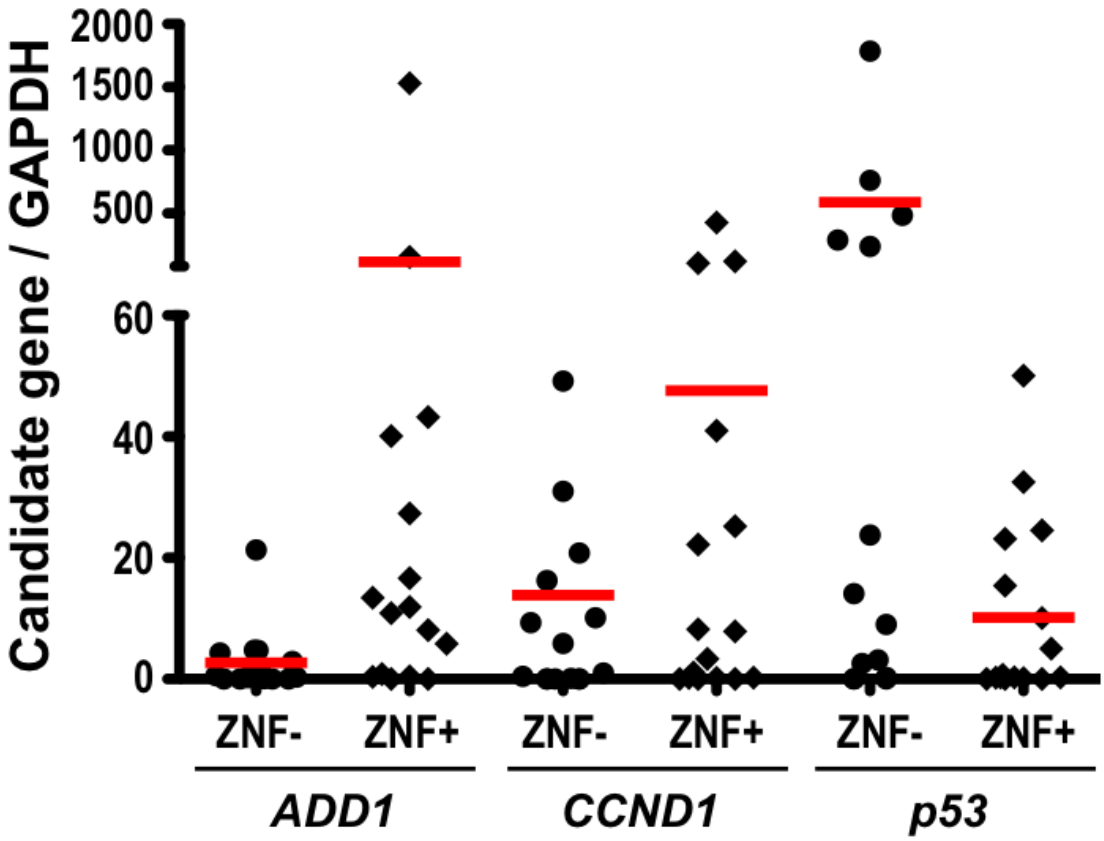


Figure S11. mRNA expression level of *ADD1, CCND1* and *p53* examined in lung cancer patients. Dot plot demonstrates mRNA expression of ratio between indicated genes and *GAPDH* in lung cancer patients with ZNF322A normal expression (ZNF-) (n=16) and patients with ZNF322A overexpression (ZNF+) (n=16). Red line indicates mean.