ACCSCIENCE PUBLISHING

Tumor Discovery

ORIGINAL RESEARCH ARTICLE

Deregulation of Casein Kinase-2 in non-small-cell lung cancer: A subunit-specific analysis

Supplementary File

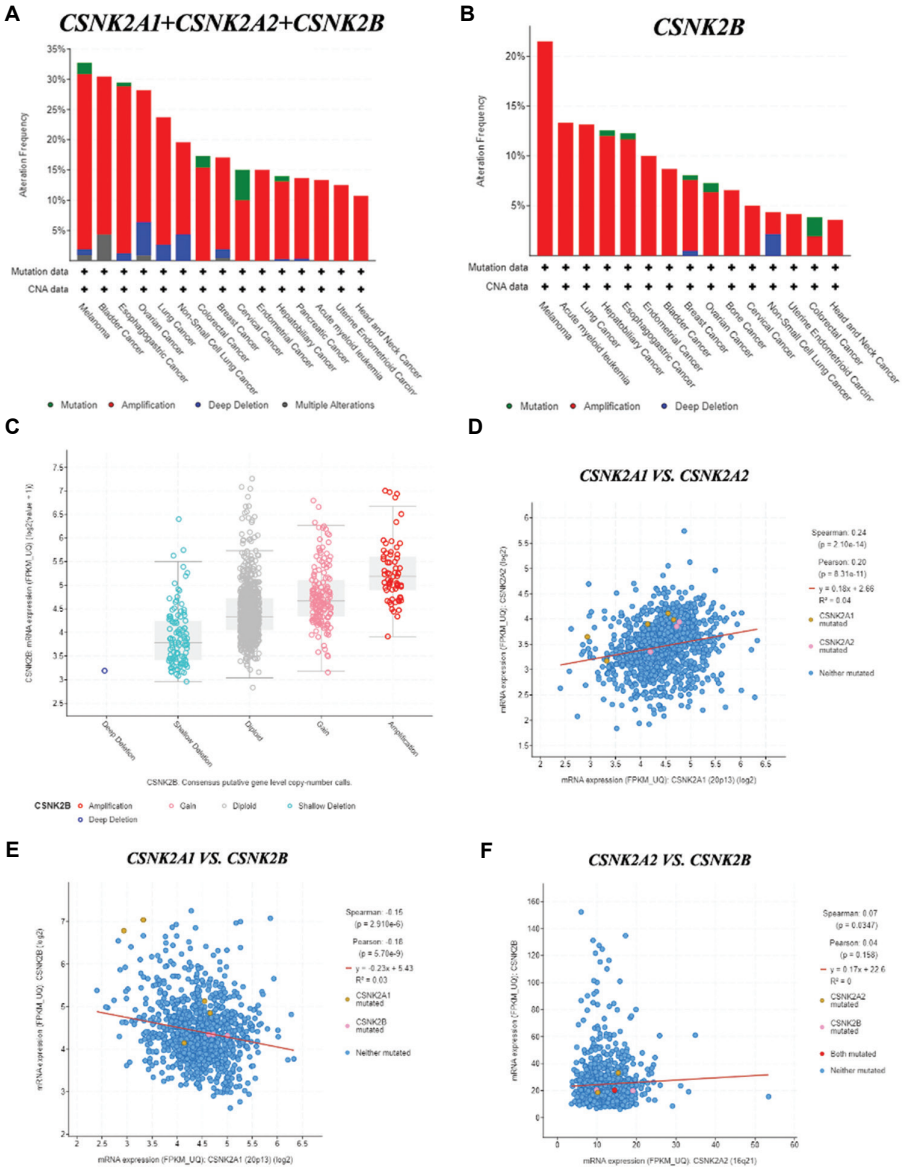

Figure S1. Mutation and CNA burden for *CSNK2A1, CSNK2A2,* and *CSNK2B* in the pan-cancer cohort (ICGC/TCGA, Nature 2020). (A) Cumulative burden across all CK2 subunits, showing only tumors with a cumulative alteration frequency of 10% or higher in the target gene; (B) alteration burden specifically for *CSNK2B*, showing only tumors with an alteration frequency of 3% or higher in the target gene; (C) correlation among CNA and mRNA gene expression for *CSNK2B*; and (D-F) mRNA gene expression correlations among CK2 subunits in the study cohort. All annotations and analyses were obtained from cBioPortal as described in the Materials and Methods section.

Abbreviations: CAN: Copy-number alteration; CK2: Casein kinase-2; ICGC: International Cancer Genome Consortium; TCGA: The Cancer Genome Atlas.

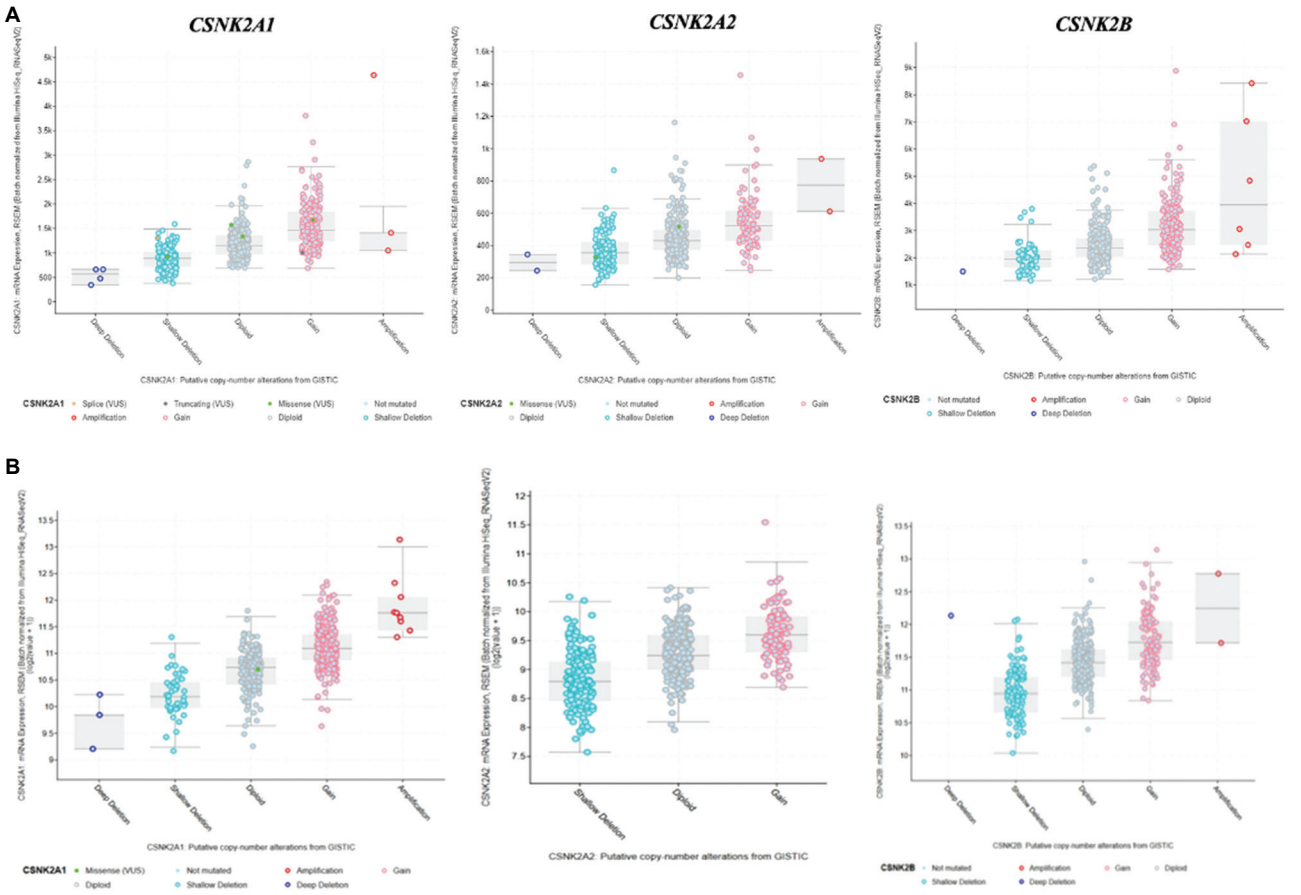

Figure S2. Association between mRNA expression and copy-number alteration (CNA) for each Casein kinase-2 subunit in two major non-small-cell lung cancer (NSCLC) subtypes. (A) Lung adenocarcinoma (LUAD; n = 503) and (B) lung squamous cell carcinoma (LUSC; n = 466) cohorts with complete datasets

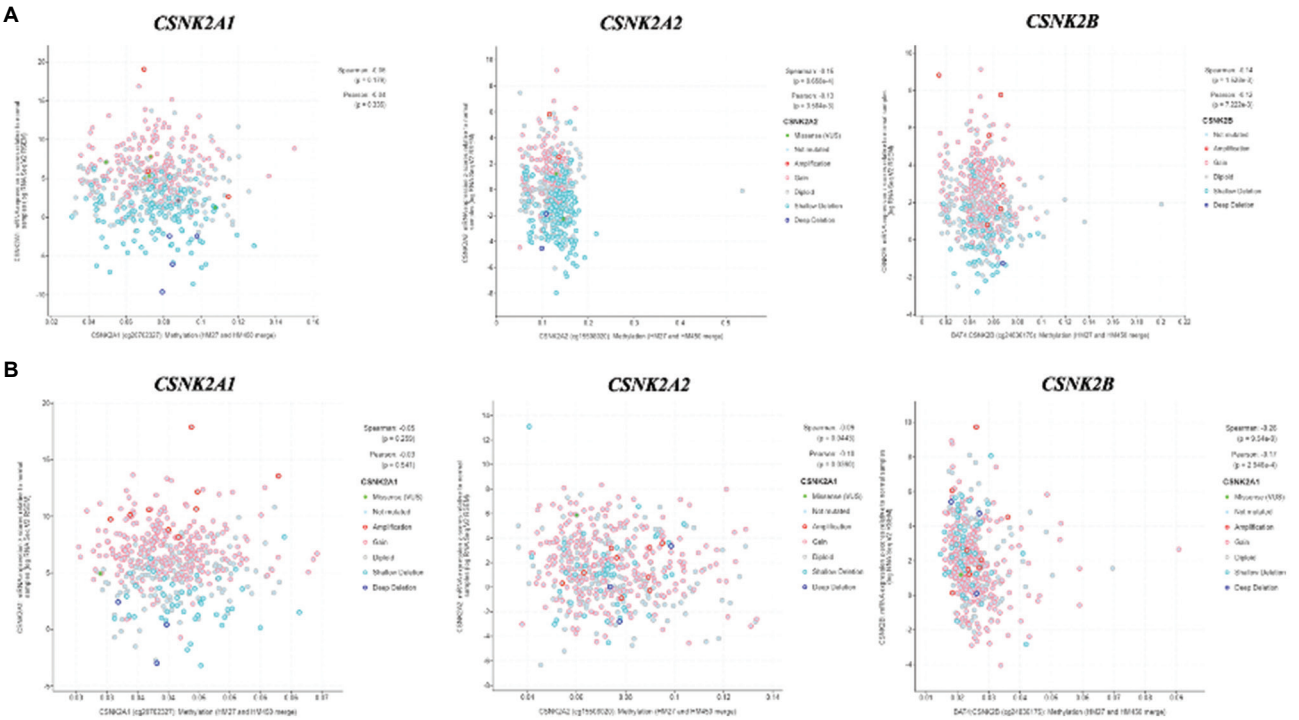

Figure S3. Correlations between Promoter/1stExon/5'UTR methylation and mRNA expression for CK2 subunits in (A) lung adenocarcinoma and (B) lung squamous cell carcinoma samples. Methylation data were obtained from H27&HM450-Merge using probes for TSS/1stExon/5'UTR for *CSNK2A1* (TSS200), *CSNK2A2* (TSS1500/1rstExon), and *CSNK2B* (TSS1500/1stExon/5'UTR).
Abbreviation: CK2: Casein kinase-2.

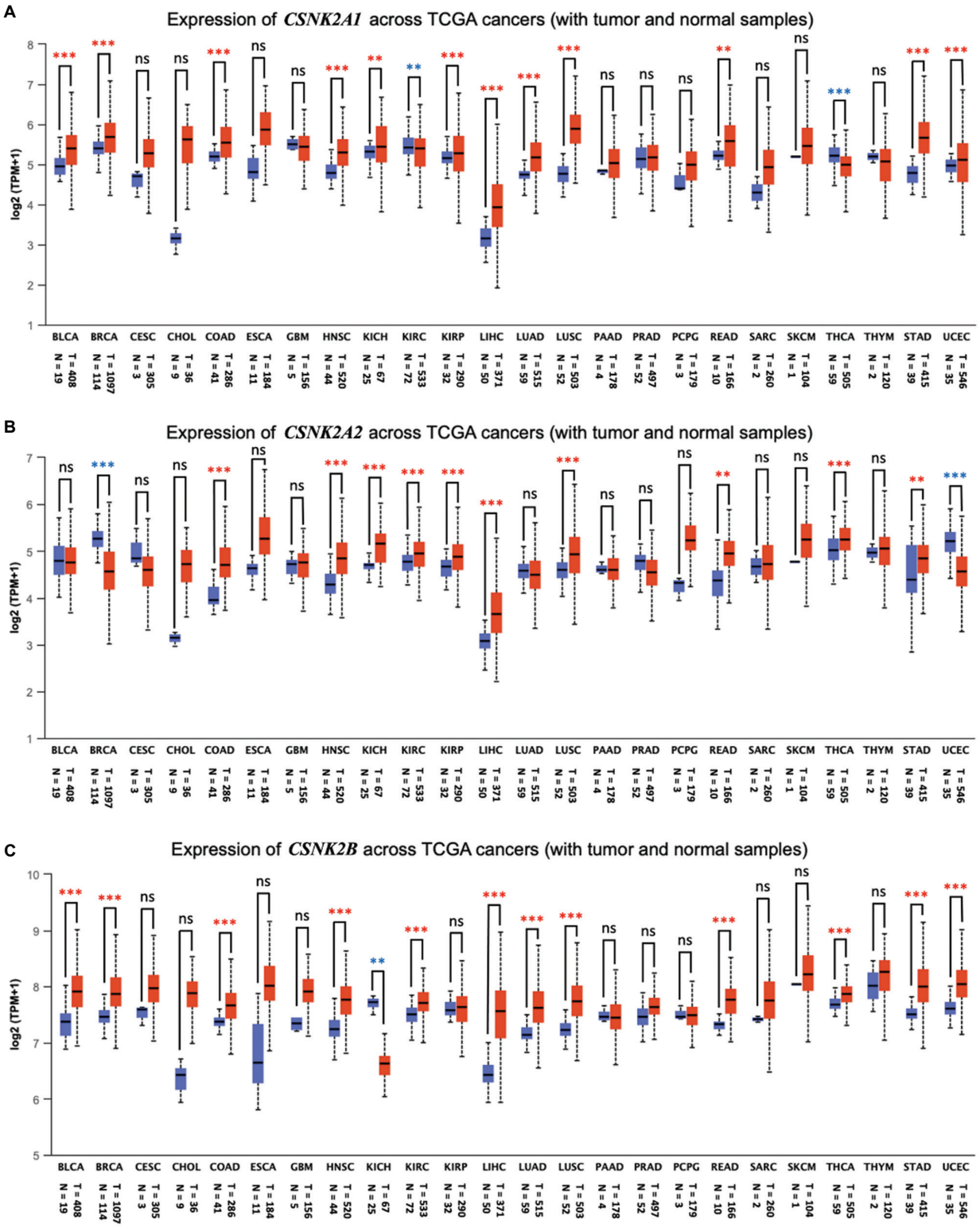

Figure S4. *CSNK2* subunits expression across clinical cancer cohorts. (A) *CSNK2A1,* (B) *CSNK2A2,* and (C) *CSNK2B.* The number of samples is represented below each tumor subtype. Groups with fewer than 15 samples are considered non-significant for statistical comparison. Normal tissue (N) is highlighted in blue, while tumor tissue (T) is in red. Statistical significance is marked with red asterisks for higher expression in tumor tissues and blue asterisks for higher expression in normal tissues. TCGA tumor subtypes are described in the Materials and Methods section.
Notes: ns: Non-significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

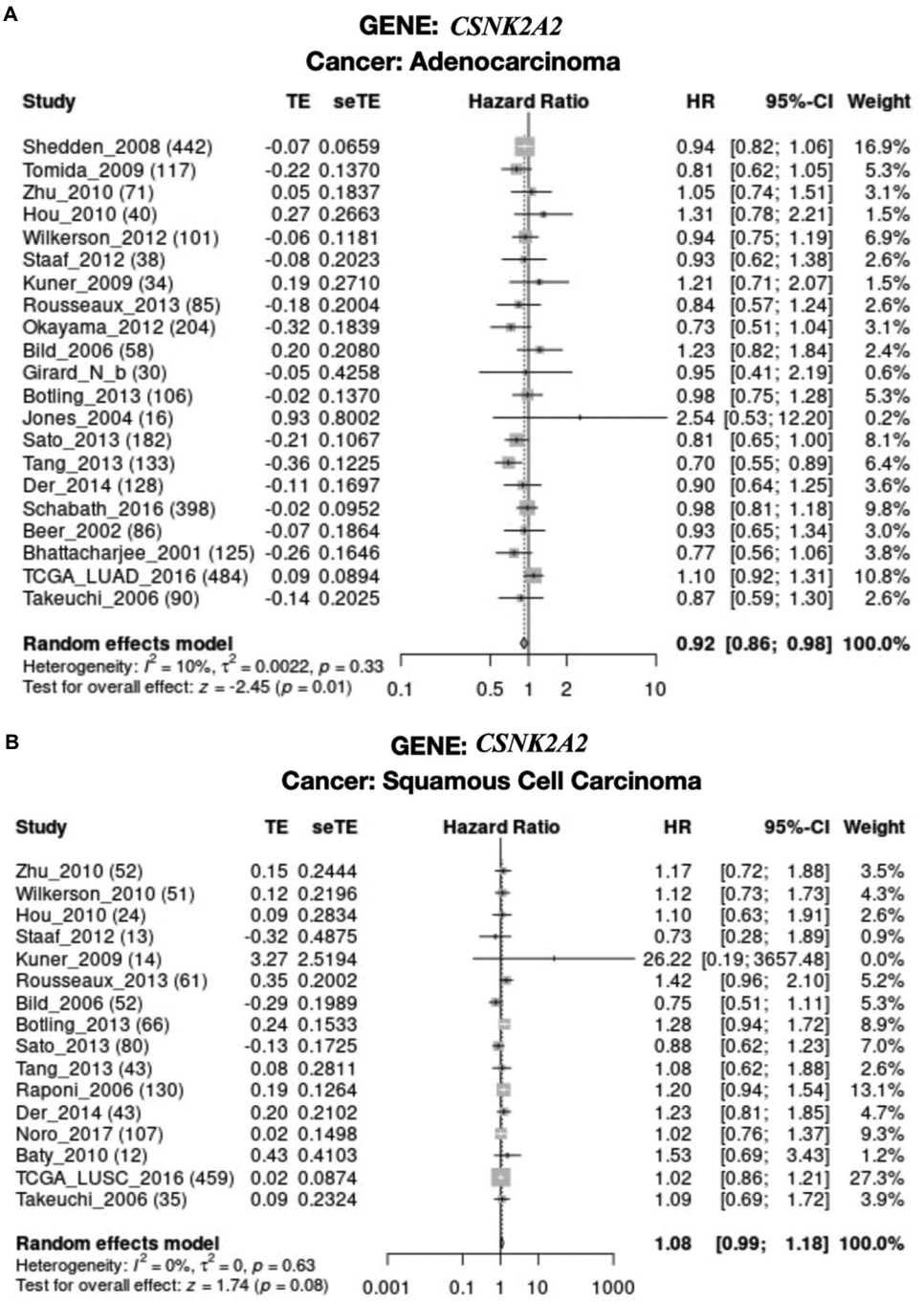

Figure S5. Meta-analysis of *CNSK2A2* deregulation versus overall survival in 20 independent NSCLC cohorts. Global risk and heterogeneity scores are listed at the bottom left of each chart. Curated studies are shown for (A) lung adenocarcinoma and (B) lung squamous cell cancer.
Abbreviation: HR: Hazard ratio; NSCLC: Non-small-cell lung cancer.

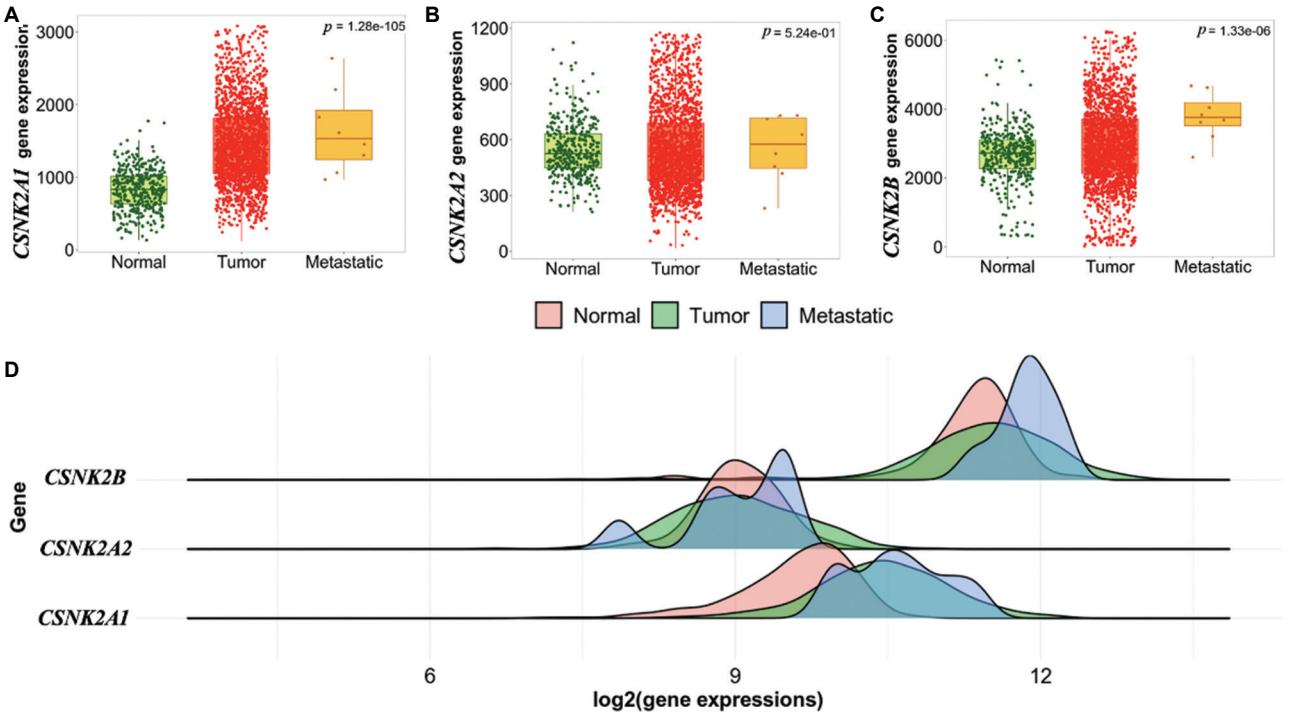

Figure S6. *CSNK2* subunits gene expression in normal tissue, tumor, and lung cancer metastasis. (A) *CSNK2A1*, (B) *CSNK2A2*, and (C) *CSNK2B* expression profiles in normal (green), tumor (red), and metastatic (yellow) lung cancer samples; (D) Expression density plot for *CSNK2* genes in lung cancer, with normal (red), tumor (green), and metastatic (blue). Data analysis and representation were obtained from the TNMplot database

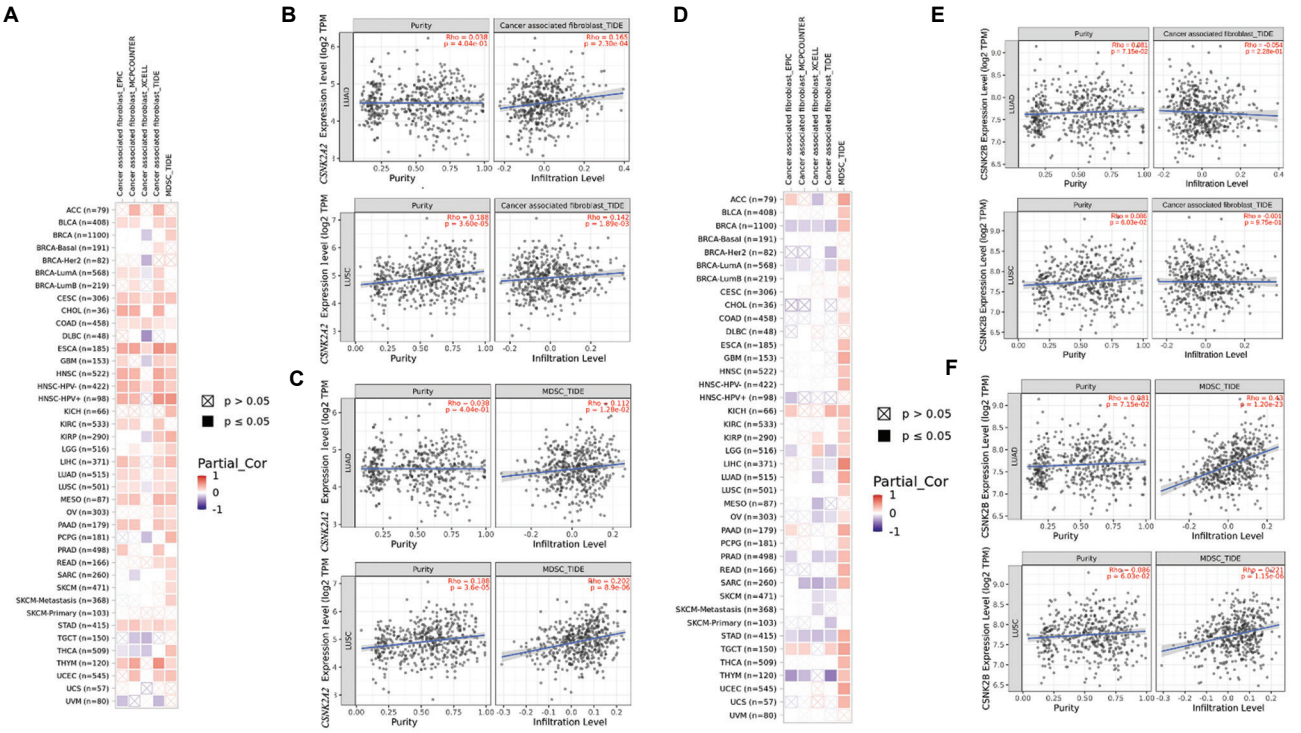

Figure S7. Correlation of *CSNK2A2* and *CSNK2B* gene expression levels with infiltrating cell populations within the tumor microenvironment according to the TIMER2.0 database. (A and D) Correlations with infiltrated populations estimated using four metrics for CAFs (CAF_EPIC, CAF_MCPCOUNTER, CAF_XCELL, CAF_TIDE) and one for MDCS (MDSC_TIDE) across The Cancer Genome Atlas tumor types; Note: n denotes the numbers of clinical samples in each cohort. (B) Detailed correlation analysis between CAFs infiltration metric (CAF_TIDE) and *CSNK2A2* expression level (Log2-TPM) in LUAD and LUSC; (C) MDSC infiltration metric (MDSC_TIDE) versus *CSNK2A2* expression level (Log2-TPM) in LUAD and LUSC; (E) CAF infiltration metric (CAF_TIDE) versus *CSNK2B* expression level (Log2-TMP) in LUAD and LUSC; and (F) MDSC infiltration metric (MDSC_TIDE) versus CSNKB expression level (Log2-TPM) in LUAD and LUSC. Analyses were normalized for sample purity, with significant correlations indicated by color-filled boxes in the heatmap, and color intensity corresponding to the correlation strength.

Abbreviations: CAF: Cancer-associated fibroblasts; MDCS: Myeloid-derived suppressor cells; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell cancer; TPM: Transcripts per million.

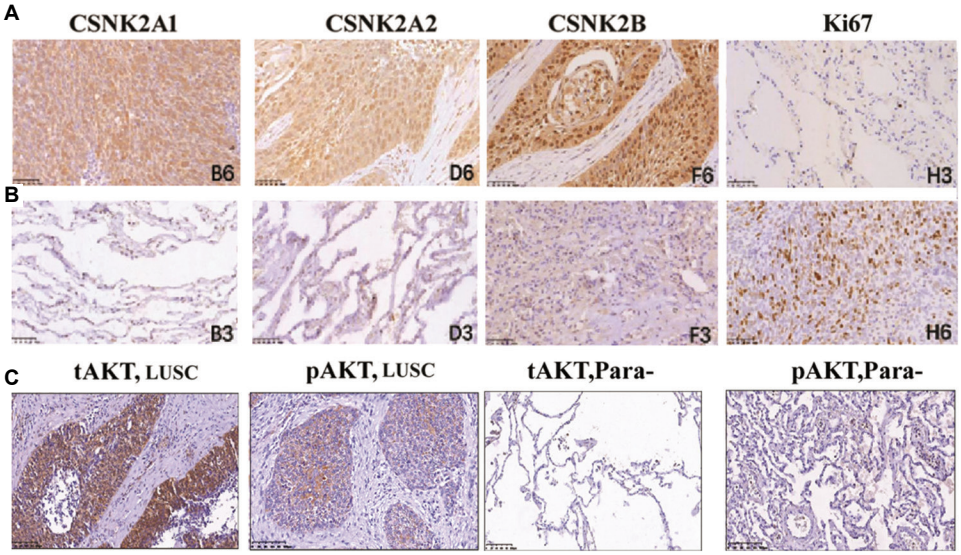

Figure S8. Immunohistochemical detection of CK2 subunits and phosphorylation on CK2 substrates (AKT) in lung squamous cell cancer (LUSC) specimens and corresponding para-neoplastic tissues. Representative images are shown for each CK2 subunit in tumor (A) or para-neoplastic (Para-) tissues (B); (C) Total AKT (tAKT) protein and phosphorylated AKTs129 (pAKT) in the same samples. Images were taken at 200× magnification. Scale bars: 100 μm.
Abbreviations: CK2: Casein kinase-2.

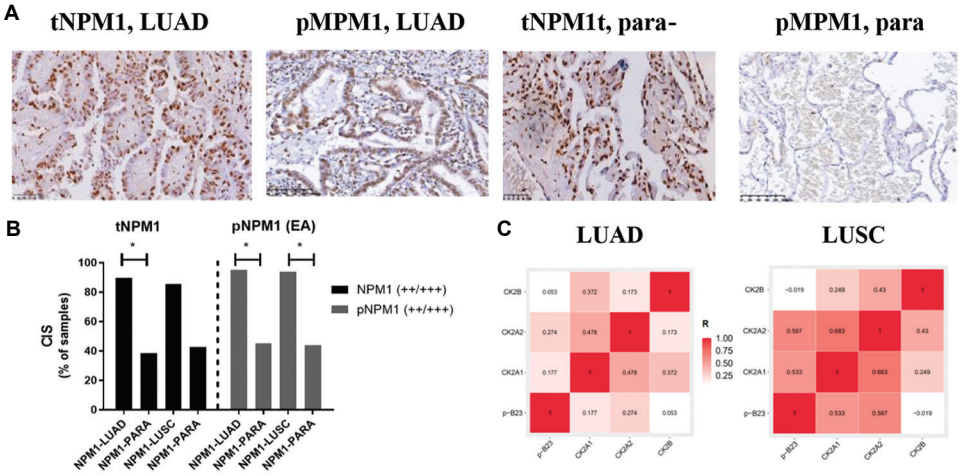

Figure S9. Immunohistochemical detection of CK2 substrate NPM1 (tNPM1) and its phosphorylated form NPM1s125 (pNPM1) in lung adenocarcinoma (LUAD)/lung squamous cell carcinoma (LUSC) specimens and corresponding para-neoplastic (para-) tissues from the same patient. (A) Representative images at 200× magnification. Scale bars: 100 μm; (B) Summarized combined IHC score (CIS) data. Note: Positive-to-strong positive intensity samples: ++/+++, *P<0.05; (C) Visualization of correlation analysis between CSNK2A1/CSNK2A2/CSNKB subunit levels and pNPM1.
Abbreviations: CK2: Casein kinase-2.

Table S1. Correlation of pathophysiological features with protein levels of CSNK2A1 subunit in a non-small-cell lung cancer cohort containing LUAD and LUSC specimens

Pathophysiological features	Cases (*n*=134)	Casein kinase-2A1				Positive rate (%)	z-score	*P*-value
		–	+	++	+++			
Age							−0.495	0.621
≤60	64	14	26	12	12	78.2		
>60	70	16	24	15	15	77.2		
Gender							−1.257	0.209
Male	77	17	24	18	18	77.9		
Female	57	13	26	9	9	77.2		
Smoking							−2.197	0.028*
Yes	41	5	13	14	9	87.8		
No	93	25	37	13	18	73.1		
Histology							−4.894	0.000**
LUAD	103	29	44	17	13	71.8		
LUSC	31	1	6	10	14	96.8		
Tumor size							−3.733	0.000**
≤3 cm	110	27	47	19	17	75.5		
>3 cm	24	3	3	8	10	87.5		
Lymphatic metastasis							−0.979	0.321
Yes	18	4	4	5	5	77.8		
No	116	26	46	22	22	77.6		
Pleural invasion							−0.828	0.407
Yes	12	3	3	1	5	75.0		
No	122	27	47	26	22	77.9		
STAS							−1.059	0.289
Yes	44	12	17	7	8	72.7		
No	90	18	33	20	19	80.0		
Differentiation							−1.785	0.074
Low	46	10	13	7	16	78.3		
High–medium	88	20	37	20	11	77.3		
Clinical stage							−3.257	0.001**
I	104	26	45	17	16	75.0		
II – IV	30	4	5	10	11	86.7		

Notes: Score 1 – 3: – (negative); Score 4 – 5: + (weak positive); Score 6 – 7: ++ (positive); Score ≥8: +++ (strong positive); **P*<0.0; ***P*<0.01.
Abbreviations: STAS: Spread through air spaces; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell cancer.

Table S2. Correlation of relevant pathophysiological features with protein levels of CSNK2A2 subunit in a non-small-cell lung cancer cohort, including LUAD and LUSC specimens

Pathophysiological features	Cases (*n*=134)	Casein kinase-2A2				Positive rate (%)	z-score	*P*-value
		–	+	++	+++			
Age							−0.431	0.667
≤60	64	6	29	14	15	90.6		
>60	70	10	26	27	7	85.7		
Gender							−0.746	0.455
Male	77	11	30	26	10	85.7		
Female	57	5	25	15	12	91.2		
Smoking							−1.341	0.180
Yes	41	5	12	16	8	87.8		
No	93	11	43	25	14	88.2		
Histology							−2.681	0.007*
LUAD	103	15	47	25	16	85.4		
LUSC	31	1	8	16	6	96.8		
Tumor size							−1.281	0.200
≤3 cm	110	15	45	33	17	86.4		
>3 cm	24	1	10	8	5	95.8		
Lymphatic metastasis							−0.224	0.823
Yes	18	2	7	6	3	88.9		
No	116	14	48	35	19	87.9		
Pleural invasion							−0.016	0.987
Yes	12	1	5	5	1	91.7		
No	122	15	50	36	21	87.7		
STAS							−1.471	0.141
Yes	44	7	21	9	7	84.1		
No	90	9	34	32	15	90.0		
Differentiation							−0.470	0.638
Low	46	6	15	19	6	87.0		
High–medium	88	10	40	22	16	88.6		
Clinical stage							−0.913	0.361
I	104	13	45	29	17	87.5		
II – IV	30	3	10	12	5	90.0		

Notes: Score 1 – 3: – (negative); Score 4 – 5: + (weak positive); Score 6 – 7: ++ (positive); Score ≥ 8: +++ (strong positive); *P<0.05; **P<0.01.
Abbreviations: STAS: Spread through air spaces; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell cancer.

Table S3. Immunohistochemistry-based detection and quantification (CIS) of AKTs129 pAKT as an indicator for *in situ* Casein kinase-2 enzymatic activity in (LUAD; *n*=76). Data show the number and percentage of samples displaying each CIS level

Group	Case	p-AKT				z-score	*P*-value
		–	+	++	+++		
LUAD	76	31	15	10	20	−5.474	<0.001
Adjacent	76	70	2	2	2		

Notes: Score 1 – 3: – (negative); Score 4–5: + (weak positive); Score 6–7: ++ (positive); Score≥8: +++ (strong positive).
Abbreviation: pAKT: phosphorylation AKT; LUAD: Lung adenocarcinoma; CIS: Combined immunohistochemistry score.

Table S4. Immunohistochemistry-based detection and quantification (CIS) of AKTs129 pAKT as an indicator for *in situ* Casein kinase-2 enzymatic activity in (LUSC; *n*=36). Data show the number and percentage of samples displaying each CIS level

Group	Case	p-AKT				z-score	*P*-score
		–	+	++	+++		
LUSC	36	10	8	7	11	−4.213	<0.001
Adjacent	36	24	9	2	1		

Notes: Score 1 – 3: – (negative); Score 4 – 5: + (weak positive); Score 6 – 7: ++ (positive); Score≥8: +++ (strong positive).
Abbreviations: pAKT: phosphorylation AKT; LUAD: Lung adenocarcinoma; CIS: Combined immunohistochemistry score.

Table S5. Correlation analysis between AKTs129 pAKT and CK-2 subunits in lung adenocarcinoma (LUAD; *n*=65) and (LUSC; *n*=17)

LUAD	p-AKT	CK2A1	CK2A2	CK2B
p-AKT	1.000			
CK2A1	0.030	1.000		
CK2A2	0.168	0.504**	1.000	
CK2B	−0.176	0.398**	0.217	1.000
LUSC	**p-AKT**	**CK2A1**	**CK2A2**	**CK2B**
p-AKT	1.000			
CK2A1	−0.248	1.000		
CK2A2	−0.024	0.661**	1.000	
CK2B	0.205	0.206	0.260	1.000

Note: *P<0.05, **P<0.01.
Abbreviations: CK2: Casein kinase-2; pAKT: phosphorylation AKT; LUAD: Lung adenocarcinoma; CIS: Combined immunohistochemistry score.

Table S6. Correlation analysis between NPM1 phosphorylation (pNPM1) and CK2 (LUAD; *n*=66) and (LUSC; *n*=16)

LUAD	p-B23	CK2A1	CK2A2	CK2B
p-B23	1.000			
CK2A1	0.177	1.000		
CK2A2	0.274*	0.478**	1.000	
CK2B	0.053	0.372**	0.173	1.000
LUSC	**p-B23**	**CK2A1**	**CK2A2**	**CK2B**
p-B23	1.000			
CK2A1	0.533*	1.000		
CK2A2	0.597*	0.683**	1.000	
CK2B	−0.019	0.249	0.430	1.000

Note: *P<0.05, **P<0.01.
Abbreviations: CK2: Casein kinase-2; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; CIS: Combined immunohistochemistry score.