YMTHE, Volume *28*

Supplemental Information

Integrative Analysis of NSCLC Identifies LINC01234 as an Oncogenic lncRNA that Interacts with HNRNPA2B1 and Regulates miR-106b Biogenesis

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Figure S1

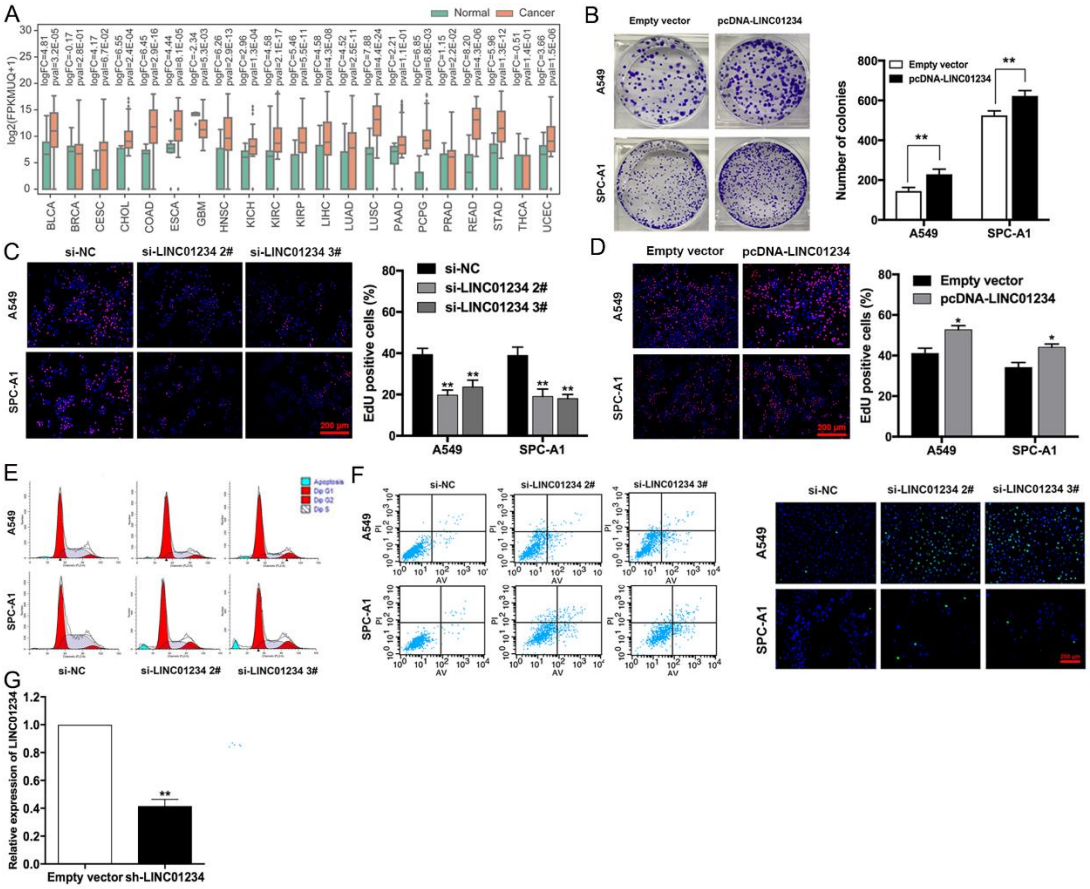


Figure S2

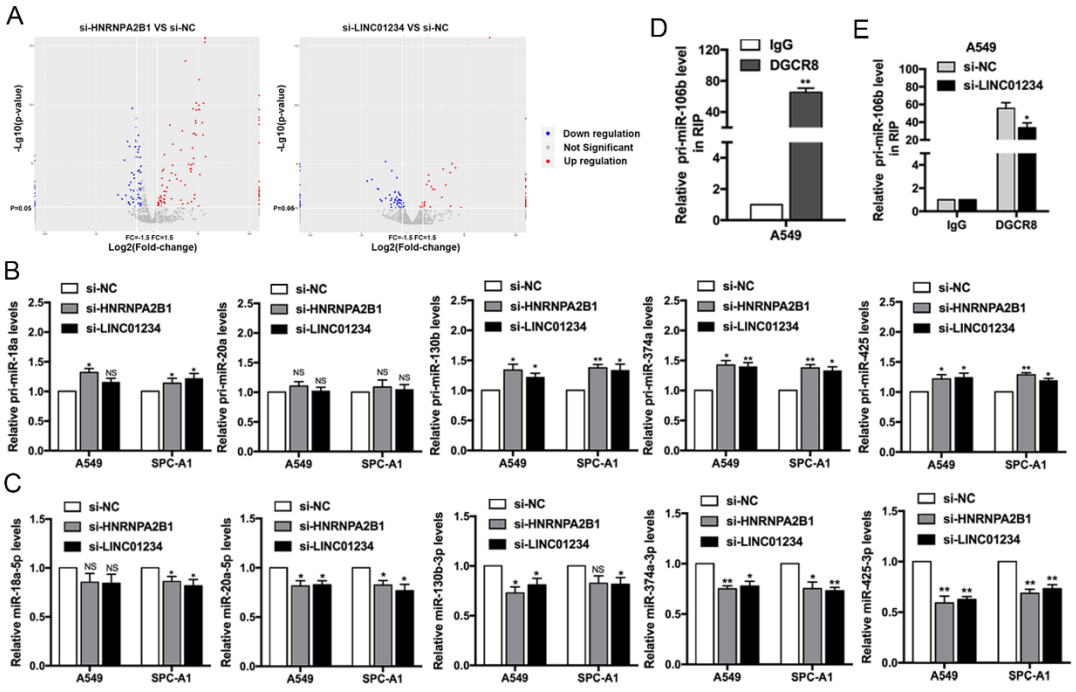


Figure S3

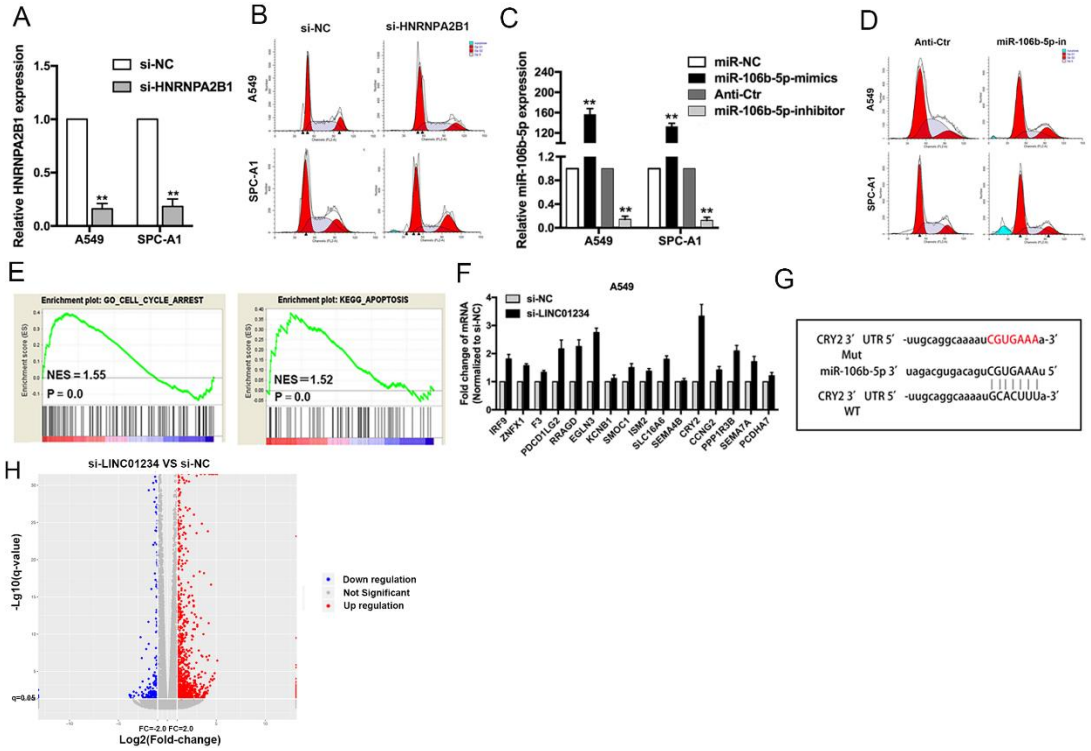
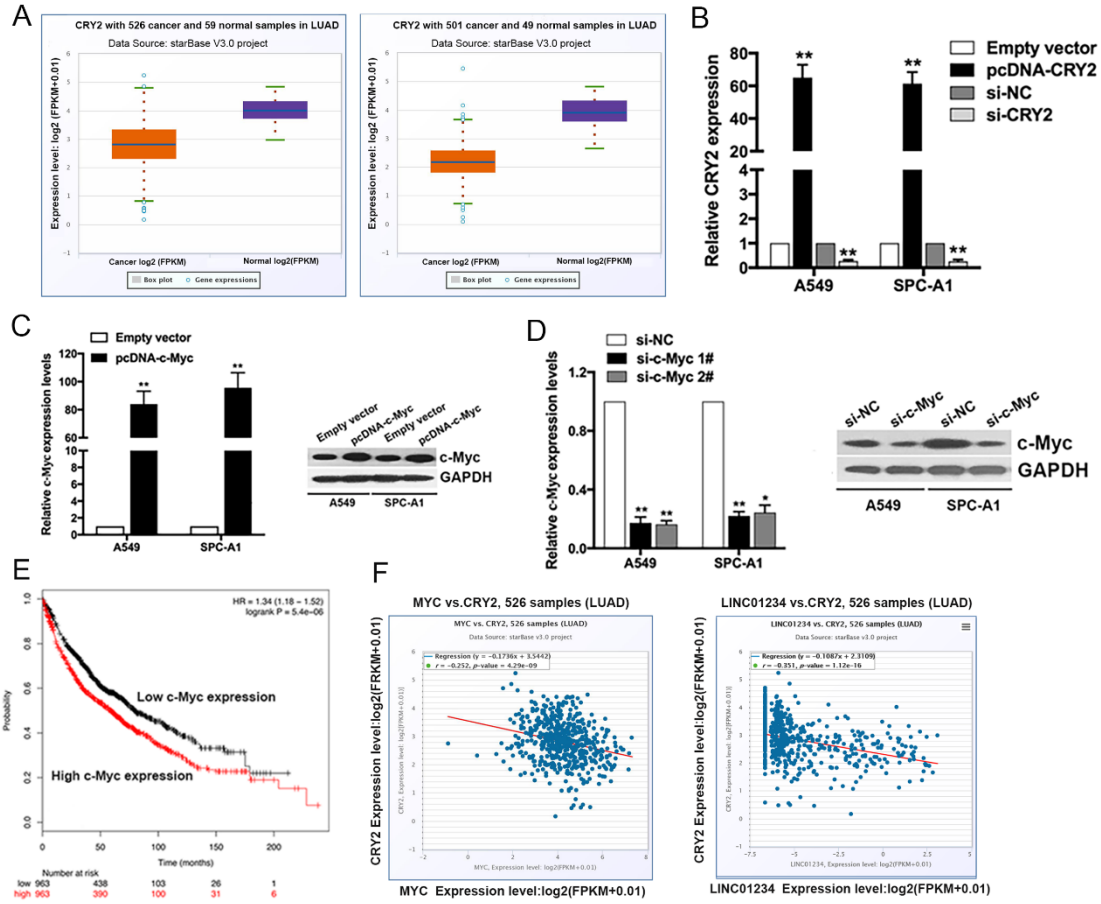


Figure S4



Supplemental Figure Legends

Figure S1. Relative LINC01234 expression levels in human cancers and NSCLC cells. (**A**) LINC01234 expression levels in various cancers (bladder urothelial carcinoma [BLCA], breast invasive carcinoma [BRCA], cervical squamous cell carcinoma and endocervical adenocarcinoma [CESC], cholangiocarcinoma [CHOL], colon adenocarcinoma [COAD], esophageal carcinoma [ESCA], glioblastoma multiforme [GBM], head and neck squamous cell carcinoma [HNSC], kidney chromophobe [KICH], kidney renal clear cell carcinoma [KIRC], kidney renal papillary cell carcinoma [KIRP], hepatocellular carcinoma [LIHC], lung adenocarcinoma [LUAD], lung squamous cell carcinoma [LUSC], pancreatic adenocarcinoma [PAAD], pheochromocytoma and paraganglioma [PCPG], prostate adenocarcinoma [PRAD], rectal adenocarcinoma [READ], stomach adenocarcinoma [STAD], thyroid carcinoma [THCA], and uterine corpus endometrioid carcinoma [UCEC]). (**B**) Colony formation assays were used to evaluate the colony formation capacity of LINC01234-overexpressing A549 and SPC-A1 cells. (**C and D**) EdU assays were used to evaluate the cells proliferation ability of A549 and SPC-A1 cells overexpressing or depleted of LINC01234. (**E and F**) Representative images of cell cycle progression, cell apoptosis evaluated by Flow Cytometry and TUNEL staining. (**G**) qRT-PCR analysis of relative expression of LINC01234 in xenograft tumors. *P < 0.05, **P < 0.01.

Figure S2. Pri-miRNA and miRNA expression in NSCLC cells. (A) Volcano plot illustrating the differentially expressed miRNAs in LINC01234 or HNRNPA2B1 depleted cells. (fold change > 1.5, P-value < 0.05). (B) Pri-miRNA expression levels upon LINC01234 and HNRNPA2B1 depletion as measured by qRT-PCR. (C) Quantification of the expression levels of miRNAs when LINC01234 and HNRNPA2B1 was depleted in NSCLC cells. (D) Immunoprecipitation of DGCR8 and qPCR analysis of associated pri-miR-106b. (E) Immunoprecipitation of DGCR8 and qPCR analysis of associated pri-miR-106b after transfection with LINC01234 siRNA. *P < 0.05, **P < 0.01.

Figure S3. HNRNPA2B1 and miR-106b-5p exert oncogenic function in NSCLC cells. (A) qRT-PCR analysis of HNRNPA2B1 mRNA levels after transfection with siRNA. (B) Flow cytometric cell cycle analysis of NSCLC cells transfected with HNRNPA2B1 siRNA. (C) Relative expression of miR-106b-5p in NSCLC cells transfected with miR-106b-5p mimics or inhibitors. (D) Cell cycle analysis of A549 and SPC-A1 cells transfected with control or miR-106b-5p inhibitors. (E) GSEA analysis showed the LINC01234 regulated genes enriched in cell cycle arrest and apoptosis

signature. (F) The altered mRNA levels of genes were confirmed by qRT-PCR in knockdown LINC01234. (G) Predicted binding sites for miR-106b-5p in CRY2 mRNA 3'UTR. (H) Volcano plot illustrating the differentially expressed genes in LINC01234 depleted cells. (fold change > 2.0, P-value < 0.05). *P < 0.05, **P < 0.01.

Figure S4. c-Myc is a downstream target of CRY2 and promotes LINC01234 transcription in NSCLC cells. (A) Expression levels of CRY2 in LUAD and LUSC from TCGA. (B) qRT-PCR analysis of CRY2 mRNA levels in A549 and SPC-A1 cells under overexpressing or depletion of CRY2. (C-D) qRT-PCR analysis of c-Myc mRNA levels and western blot analysis of c-Myc protein levels after knockdown or overexpression of c-Myc. (E) Kaplan–Meier survival analysis of the association between c-Myc expression level and NSCLC patient overall survival survival. (F) Negative correlation between the expression levels of c-Myc, LINC01234 and CRY2. *P < 0.05, **P < 0.01.

Table S1. Correlation between LINC01234 expression and clinicopathological characteristics of NSCLC patients (n = 95)

Characteristics	**LINC01234**		**P**
	Low no. cases (%)	**High no. case (%)**	**Chi-squared test P-value**
Age (years)			
>65	21	28	0.363
≤65	24	22	
Gender			
Male	27	29	0.843
Female	18	21	
Smoking history			
Smokers	25	30	0.661
Never smokers	20	20	
Histological subtype			
Squamous cell carcinoma	22	18	0.204
Adenocarcinoma	23	32	
Histologic grade			
Well differentiated	20	18	0.668
Moderately differentiated	13	14	
Poorly differentiated	10	13	
Undifferentiated	2	5	
Tumor size			
≤5cm	36	27	0.007**
>5cm	9	23	
Lymph node metastasis			
Negative	31	20	0.005**
Positive	14	30	
T-Status			
T1	13	8	0.059
T2	23	19	
T3	7	18	
T4	2	5	
N-Status			
N0	31	20	0.019*
N1	10	22	
N2	4	8	
TNM Stage			
I	18	6	0.003**
II	17	20	
III	10	24	

*P<0.05 was considered significant

Table S2. Univariate and multivariate analysis of clinicopathological factors for over-survival in NSCLC patients ((n = 95))

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	p value	HR	95% CI	p value
Age (≤65/>65)	0.787	0.441-1.405	0.417			
Gender (Male/Female)	1.457	0.815-2.606	0.204			
Smoking history (no vs yes)	1.550	0.873-2.751	0.135			
Histological subtype (LUAD /LUSC)	1.221	0.686-2.172	0.497			
Histologic grade (Well, mod / Poor, undi)	2.400	1.731-3.328	<0.001*	1.983	1.386-2.838	<0.001*
Tumor size (≤5/>5)	2.308	1.294-4.115	0.005*	1.786	0.889-3.586	0.103
Lymphatic metastasis (no vs yes)	7.364	3.286-16.502	<0.001*	3.195	1.082-9.436	0.036*
TNM stage (III vs I+II)	3.904	2.2-6.925	<0.001*	1.461	0.661-3.227	0.348
LINC01234 expression (high vs low)	4.009	2.053-7.829	<0.001*	2.63	1.272-5.435	0.009*

HR, hazard ratio; 95 % CI, 95 % confidence interval, * Overall P < 0.05.