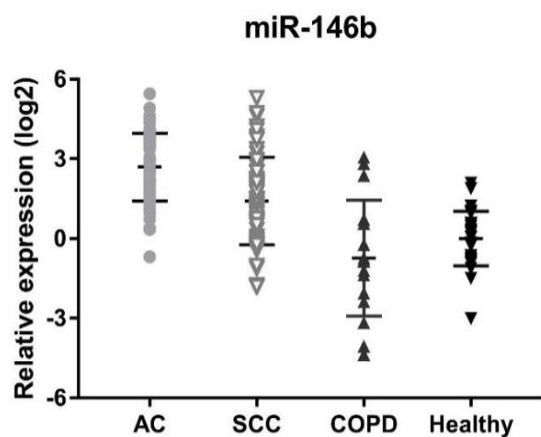


## Supplementary Figures and Tables

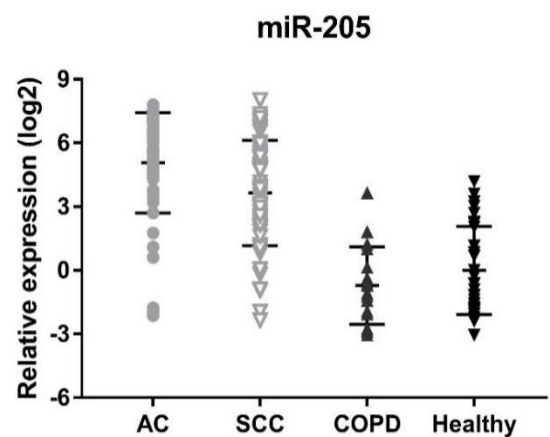
### **Serum microRNA Signature Is Capable of Early Diagnosis for Non-Small Cell**

#### **Lung Cancer**

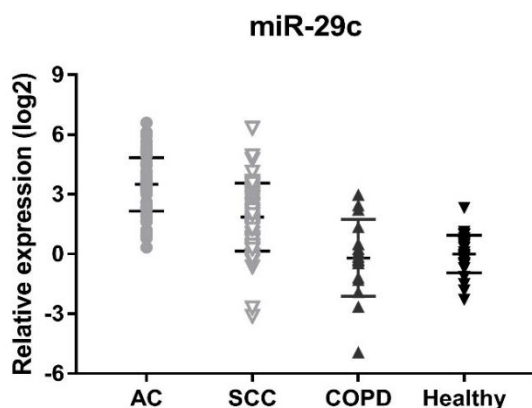
Xia Yang<sup>1</sup>, Qihong Zhang<sup>1</sup>, Ming Zhang<sup>1</sup>, Wenmei Su<sup>2</sup>, Zhuwen Wang<sup>3</sup>, Yali Li<sup>1</sup>,  
Jie Zhang<sup>1</sup>, David G. Beer<sup>3</sup>, Shuanying Yang<sup>1</sup>, ✉, Guoan Chen<sup>4</sup>, ✉



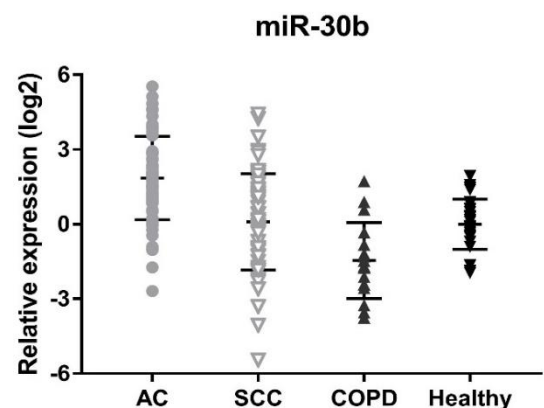
AC vs. SCC,  $p < 0.0001$ ; AC vs. COPD,  $p < 0.0001$ ; AC vs. Healthy,  $p < 0.0001$ ; SCC vs. COPD,  $p < 0.0001$ ; SCC vs. Healthy,  $p < 0.0001$ ; COPD vs. Healthy,  $p = 0.0812$ .



AC vs. SCC,  $p = 0.003$ ; AC vs. COPD,  $p < 0.0001$ ; AC vs. Healthy,  $p < 0.0001$ ; SCC vs. COPD,  $p < 0.0001$ ; SCC vs. Healthy,  $p < 0.0001$ ; COPD vs. Healthy,  $p = 0.3253$ .

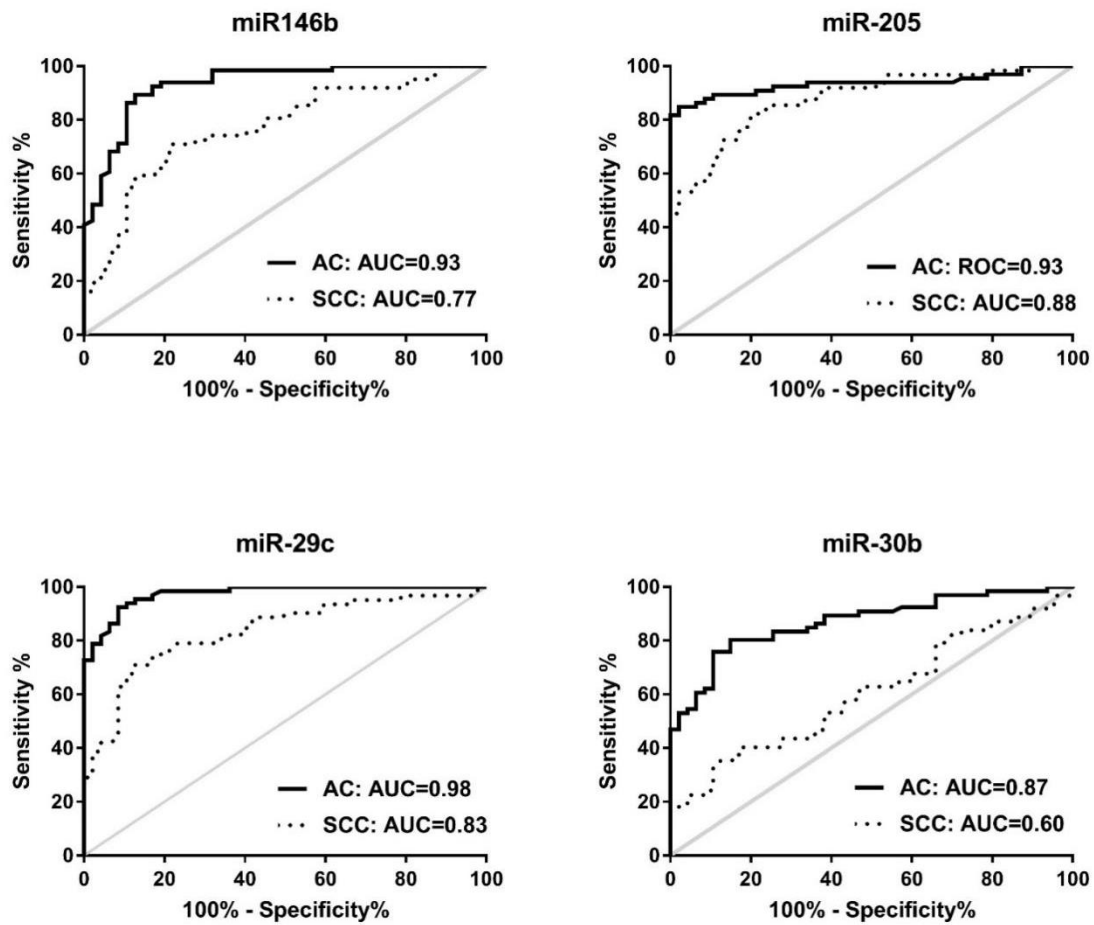


AC vs. SCC,  $p < 0.0001$ ; AC vs. COPD,  $p < 0.0001$ ; AC vs. Healthy,  $p < 0.0001$ ; SCC vs. COPD,  $p < 0.0001$ ; SCC vs. Healthy,  $p < 0.0001$ ; COPD vs. Healthy,  $p = 0.6653$ .

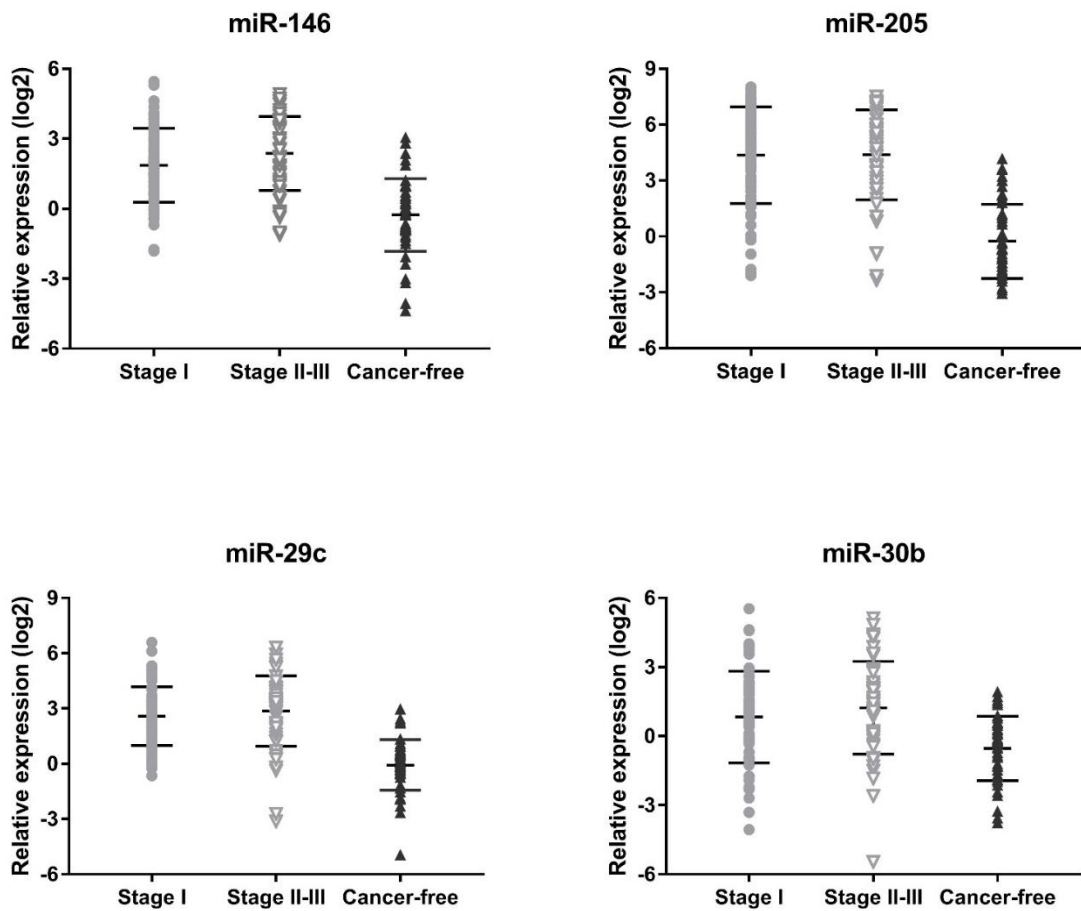


AC vs. SCC,  $p < 0.0001$ ; AC vs. COPD,  $p < 0.0001$ ; AC vs. Healthy,  $p < 0.0001$ ; SCC vs. COPD,  $p = 0.0011$ ; SCC vs. Healthy,  $p = 0.7514$ ; COPD vs. Healthy,  $p = 0.0011$ .

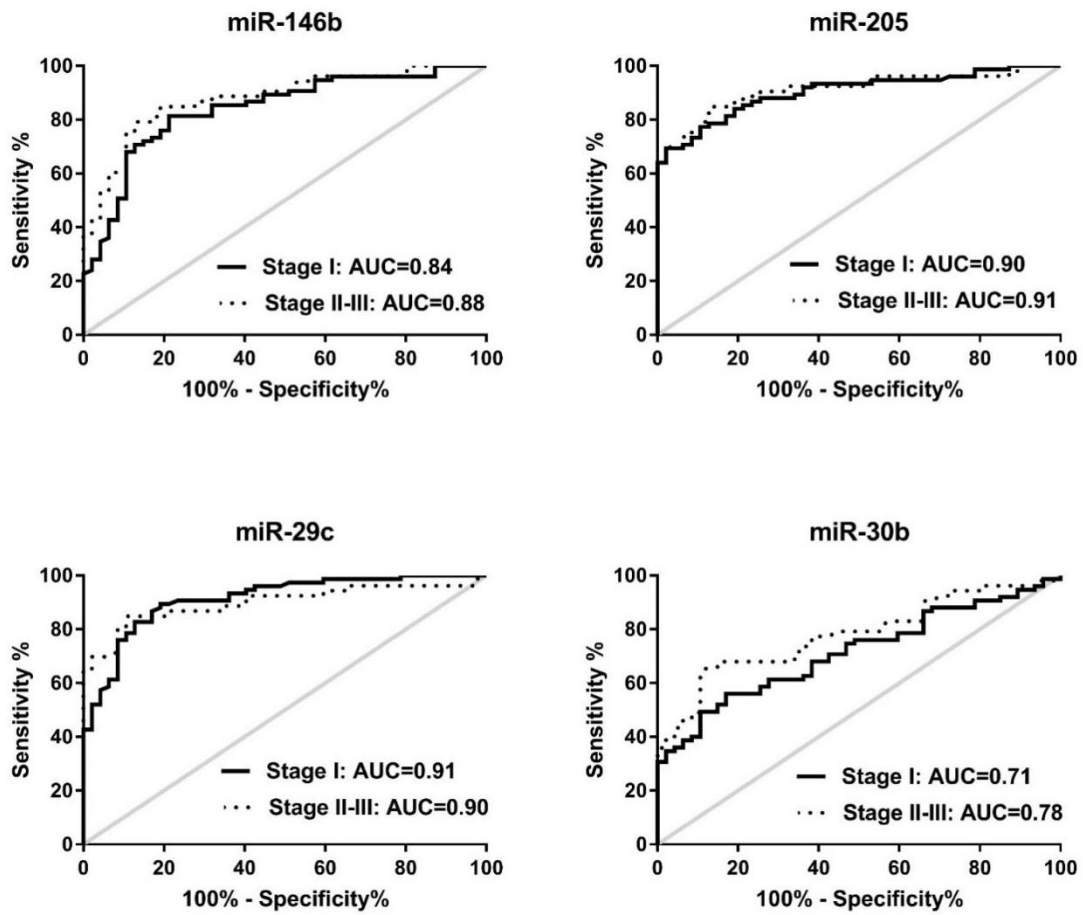
**Figure S1.** The pairwise comparisons of 4 individual miRNAs (miR-146b, miR-205, miR-29c and miR-30b) in 4 subgroups of our study: adenocarcinoma (AC), squamous cell carcinoma (SCC), chronic obstructive pulmonary disease (COPD) or healthy donors (Healthy). Mann-Whitney tests were performed to ascertain statistical significance between the expression levels across each pairwise groups.



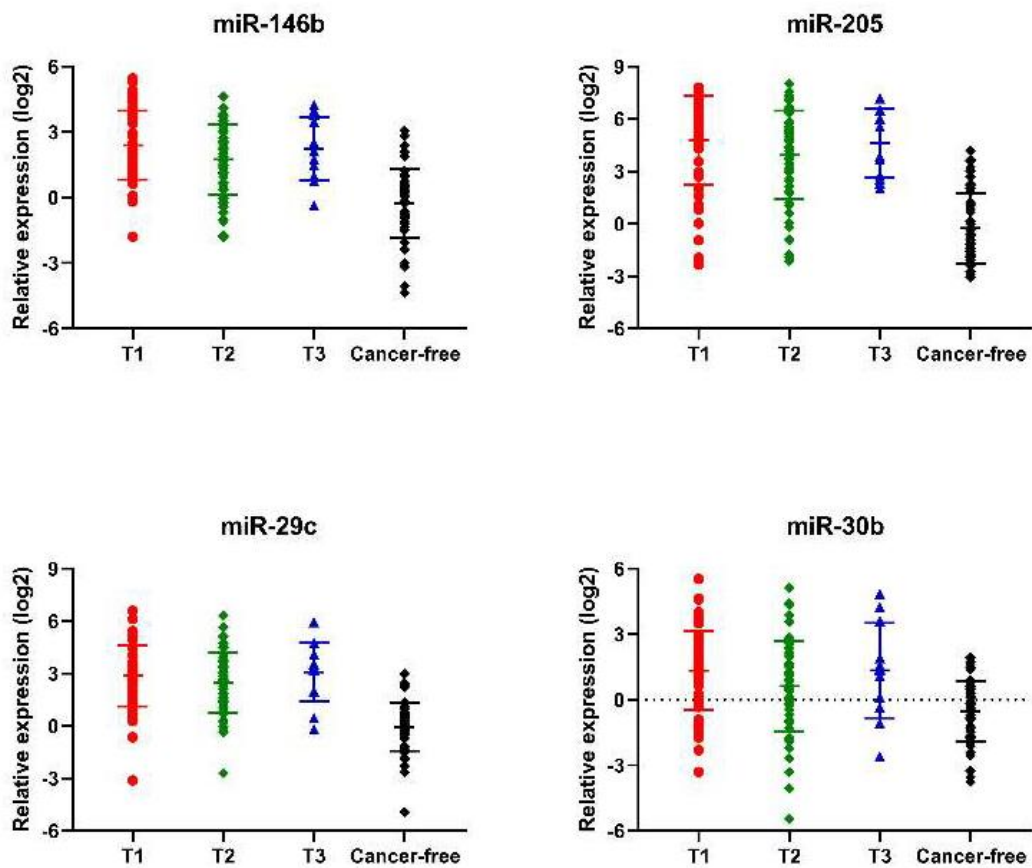
**Figure S2.** Receiver–operator characteristic (ROC) curve analysis of 4 individual miRNAs (miR-146b, miR-205, miR-29c and miR-30b) in distinguishing histological subtypes of NSCLC patients: adenocarcinoma (AC, solid line), or squamous cell carcinoma (SCC, dotted line) from all cancer-free controls.



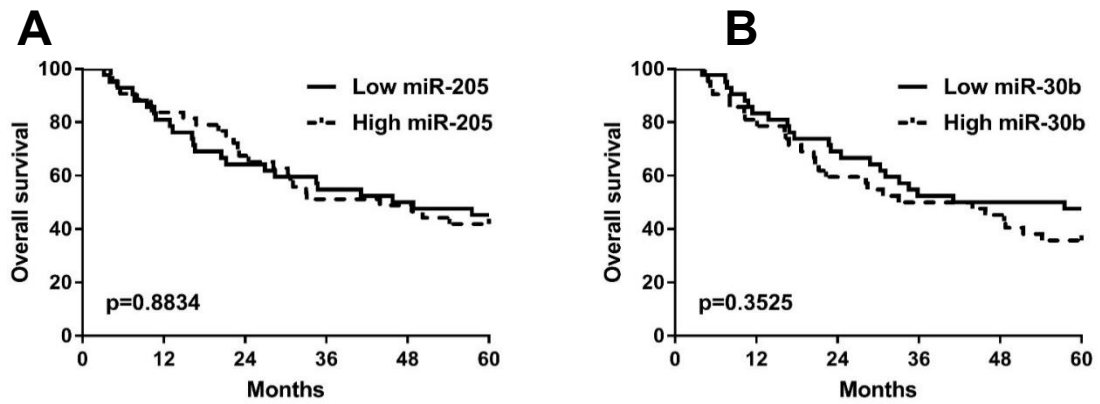
**Figure S3.** The expression levels of 4 individual miRNAs (miR-146b, miR-205, miR-29c and miR-30b) from NSCLC patients with different stages: stage I, stage II-III and cancer-free controls. Mann-Whitney tests were performed to ascertain statistical significance between the expression levels across groups. For all 4 individual miRNAs, there has no significant difference between stage I and stage II-III NSCLC patients (all  $p > 0.05$ ). On the contrast, all 4 miRNAs in serum from either stage I or stage II-III NSCLC patients showed significantly higher expressions than those from cancer-free controls (all  $p < 0.05$ ).



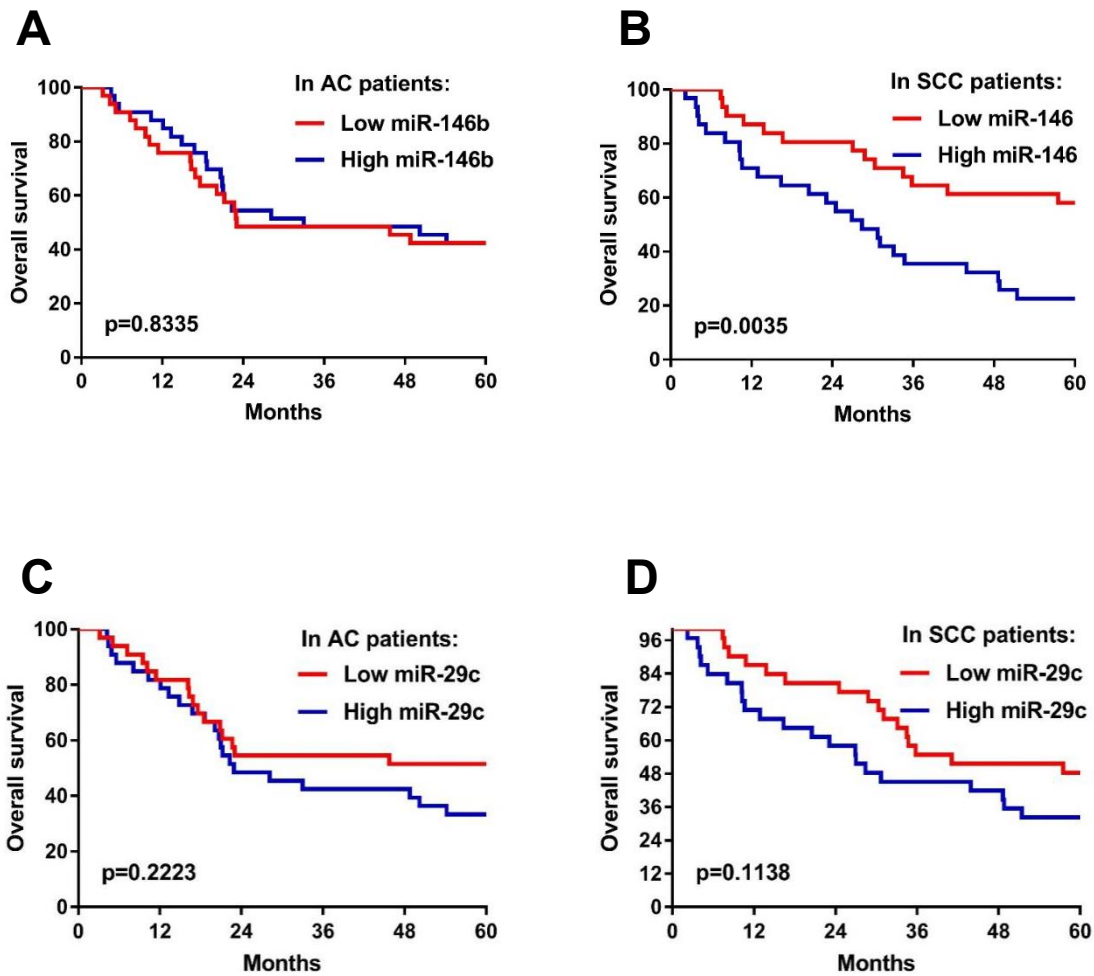
**Figure S4.** Receiver–operator characteristic (ROC) curve analysis of 4 individual miRNAs (miR-146b, miR-205, miR-29c and miR-30b) in distinguishing NSCLC patients with different stages: stage I (solid line) or stage II-III (dotted line) from all cancer-free controls.



**Figure S5.** The expression levels of 4 individual miRNAs (miR-146b, miR-205, miR-29c and miR-30b) from NSCLC patients with different T status: T1, T2, T3 and cancer-free controls. Mann-Whitney tests were performed to ascertain statistical significance between the expression levels across groups. For all 4 individual miRNAs, there has no significant difference between T1, T2 or T3 NSCLC patients (all  $p > 0.05$ ). On the contrast, all 4 miRNAs in serum from either T1, T2 or T3 NSCLC patients showed significantly higher expressions than those from cancer-free controls (all  $p < 0.001$ ).

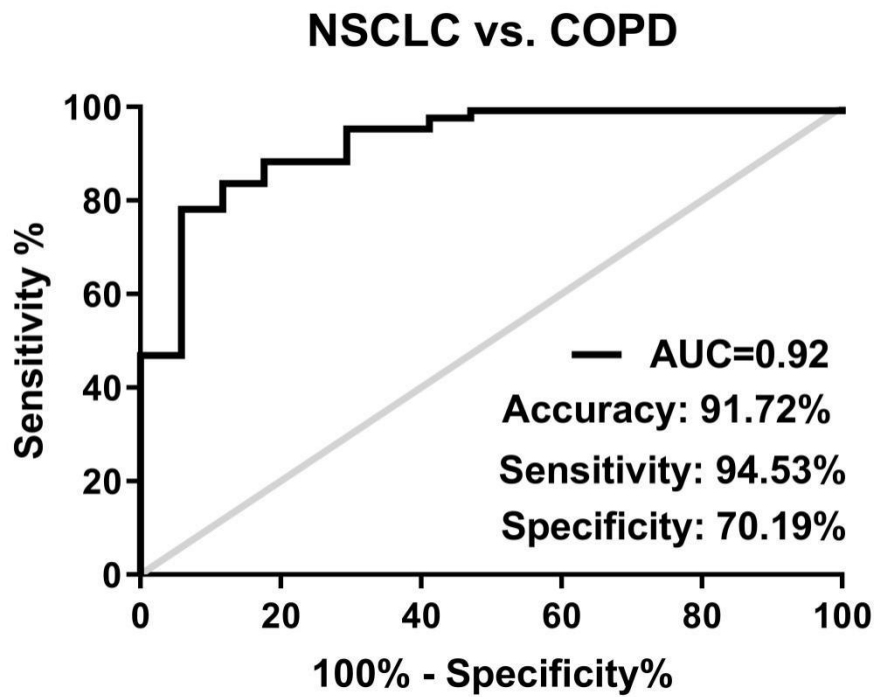


**Figure S6.** Kaplan-Meier survival curves analysis of 5-year overall for NSCLC patients stratified according to high (dotted line) or low (solid line) serum expression levels of miR-205 (A) and miR-30b (B). The p-values were calculated using the log-rank test between patients with high- and low- ( $\log_2$ ) fold changes.



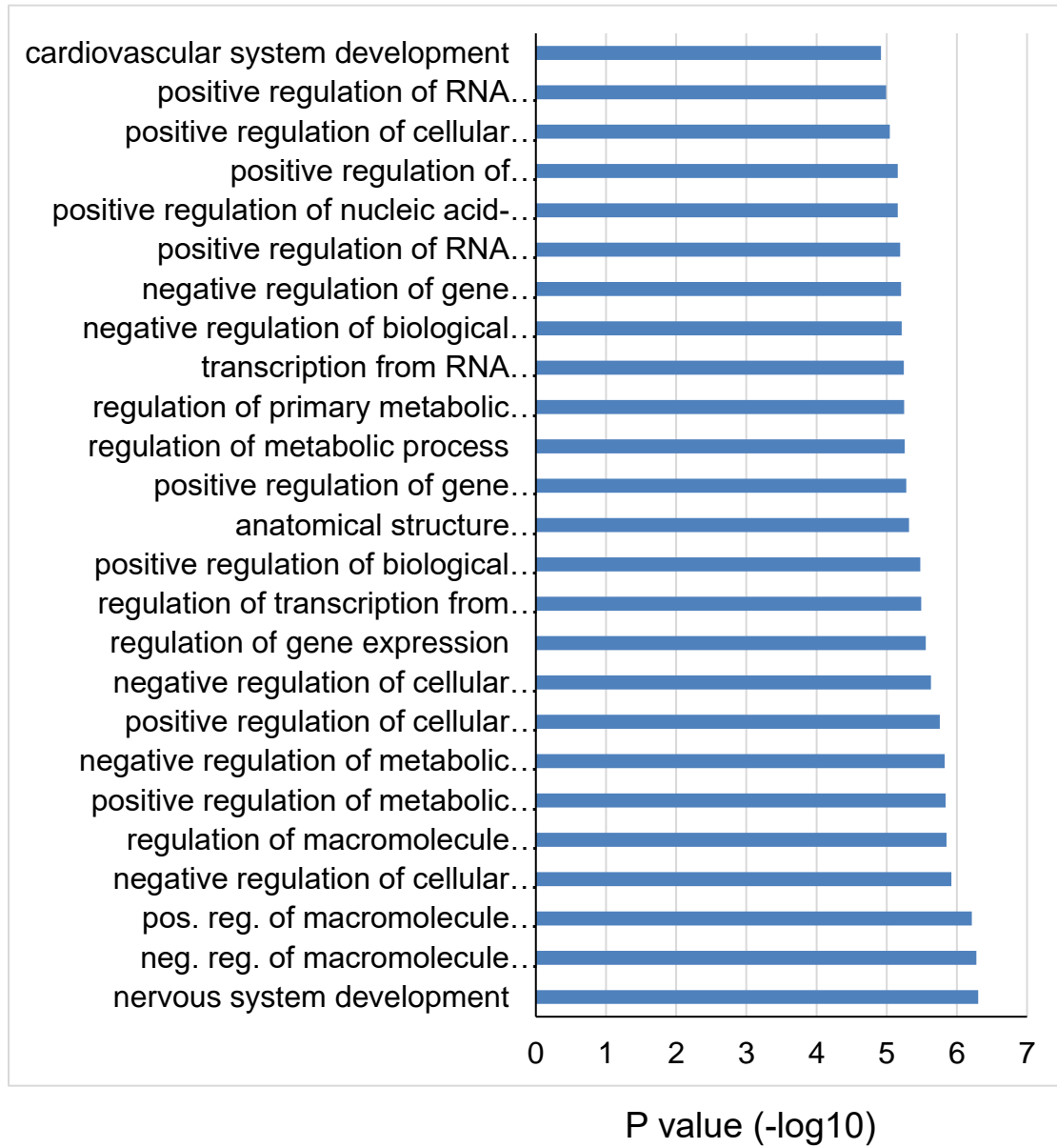
**Figure S7.** Kaplan-Meier survival curves analysis of 5-year overall for different histological subtypes of NSCLC patients, stratified according to high (blue-colored line) or low (red-colored line) serum expression levels of miR-146b (A-B) and miR-29c (C-D) as well as the survival curves of different stages of NSCLC patients: stage I, II and III. The p-values were calculated using the log-rank test between patients with relative groups' (log<sub>2</sub>) fold changes.





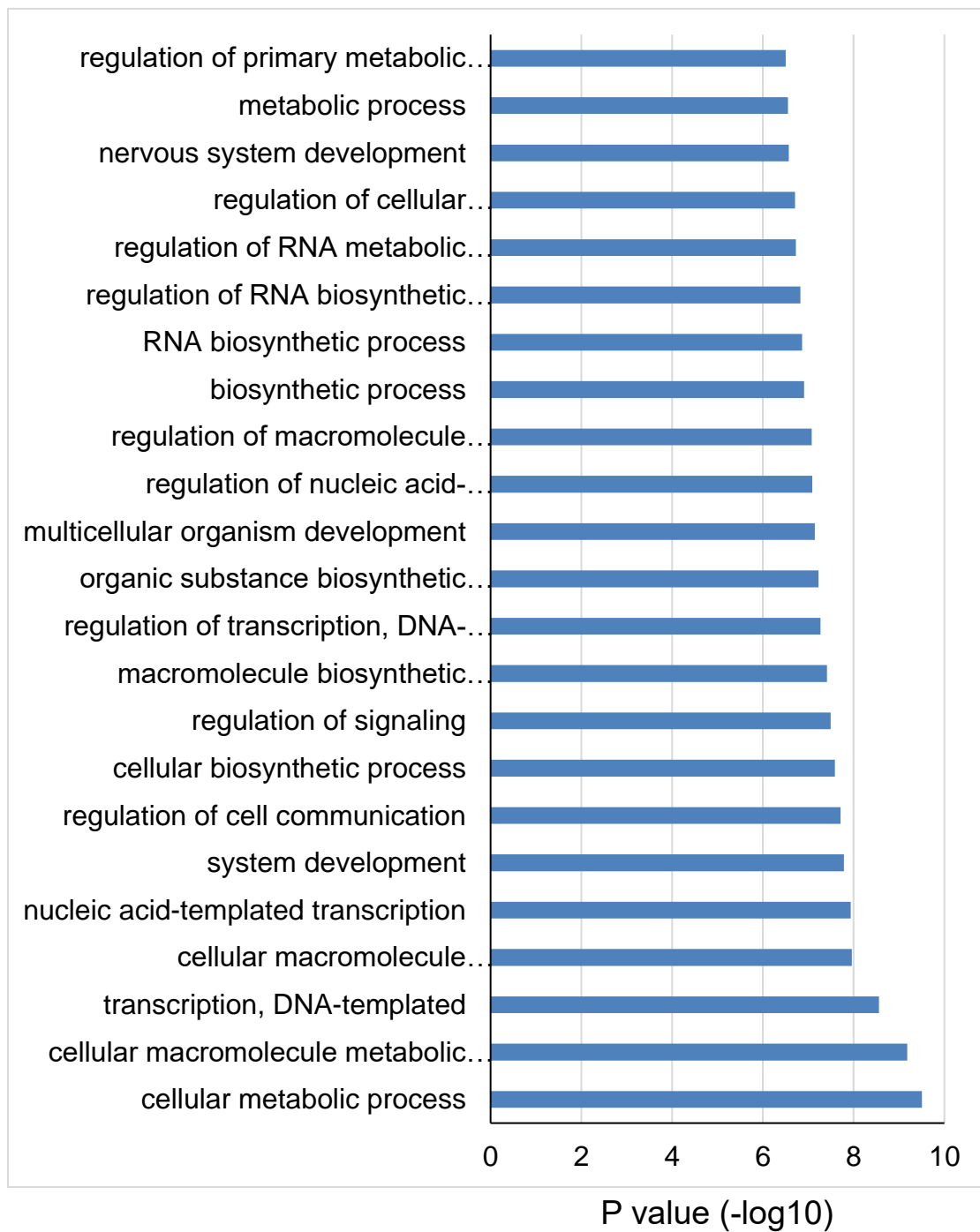
**Figure S8.** Receiver-operator characteristic (ROC) curve of serum 4-miRNA panel in distinguishing NSCLC (n=128) from COPD (n=17) patients with AUC of 0.92, 95% confidence interval (95% CI) of 0.855-0.988, accuracy of 91.72%, sensitivity of 94.53% and specificity of 70.19% when optimal cut-off value was set by Youden's index.

### GO BP term



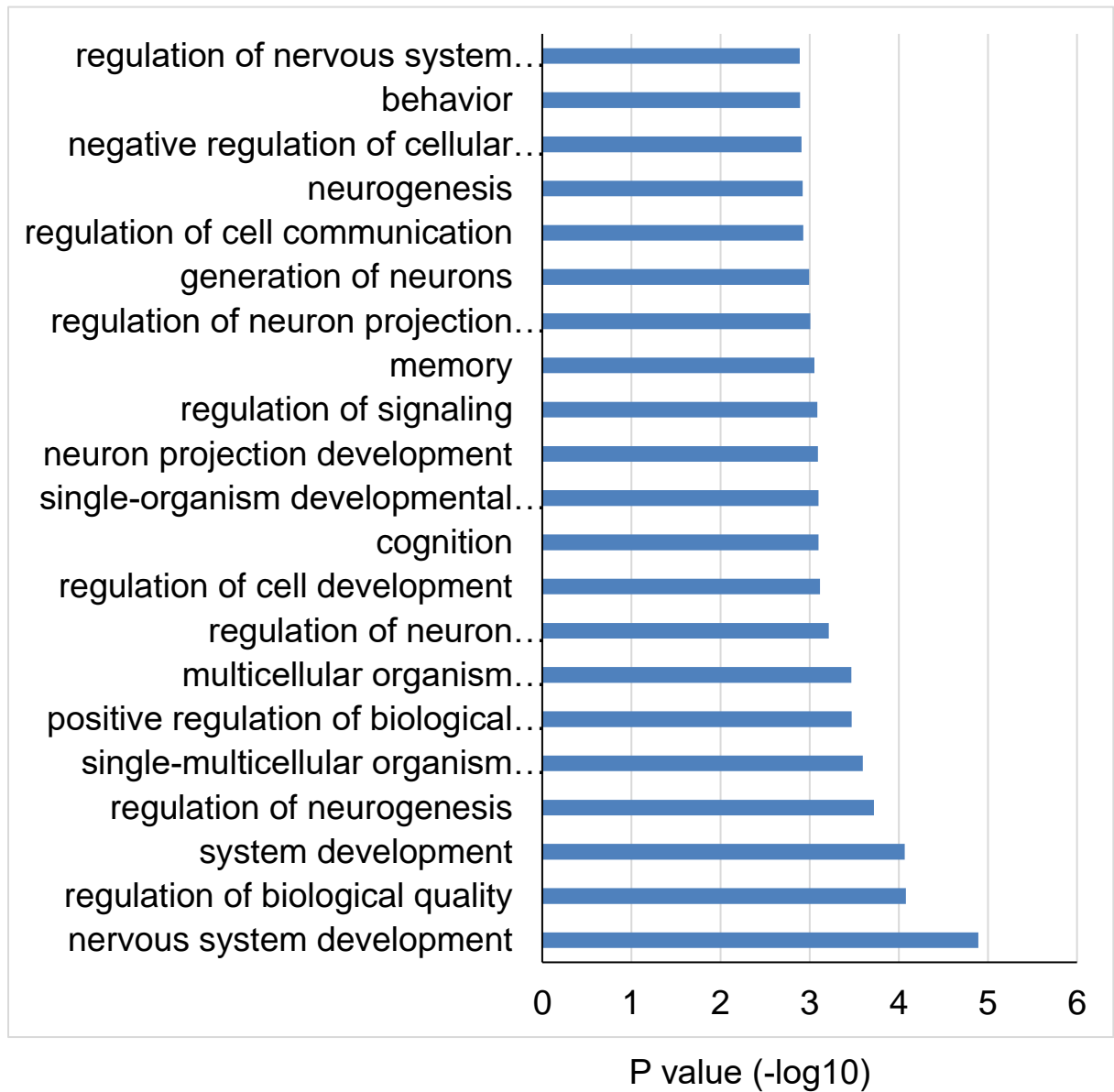
**Figure S9.** DAVID Gene Ontology analysis of miR-205 target genes

## GO BP term



**Figure S10.** DAVID Gene Ontology analysis of miR-30b target genes

### GO BP term



**Figure S11.** DAVID Gene Ontology analysis of miR-29C target genes

**Table S1 Reported miRNAs changed in cancer**

miRNA	Deregulated in cancers	References
miR-146b	Up	[1-4]
miR-205	Up	[5-8]
miR-29c	Up	[9-11]
miR-30b	Up	[12-14]
miR-31	Up	[15-19]
miR-337	Up	[20-22]
miR-411	Down	[23-27]
miR-708	Down	[28-32]

**Reference:**

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**Table S2: miRNAs differentially expressed in the training or testing set, or the two sets combined, together with the relative ROC analysis**

	miRNA	AUC	95% CI	Sensitivity (%)	Specificity (%)
Training set (TR)	miR-146b	0.87	0.7826 to 0.9548	84.62	86.67
	miR-205	0.88	0.8074 to 0.9582	70.31	93.33
	miR-29c	0.94	0.8951 to 0.9917	88.89	93.33
	miR-30b	0.72	0.617 to 0.8316	63.49	86.67
	miR-337	0.71	0.5812 to 0.8453	61.9	80
Testing set (TS)	miR-146b	0.85	0.7718 to 0.9306	72.31	87.5
	miR-205	0.92	0.8709 to 0.9748	81.82	93.75
	miR-29c	0.89	0.8255 to 0.9552	84.62	84.38
	miR-30b	0.76	0.6646 to 0.8551	73.85	62.5
	miR-337	0.69	0.5811 to 0.7939	63.08	68.75
Two sets combined (TR+TS)	miR-146b	0.85	0.7942 to 0.9154	73.85	87.23
	miR-205	0.91	0.8655 to 0.9501	81.54	87.23
	miR-29c	0.91	0.862 to 0.9516	83.59	87.23
	miR-30b	0.74	0.6683 to 0.8147	64.16	82.98
	miR-337	0.69	0.6053 to 0.7705	63.28	72.34

**Table S3: ROC analysis of 4 individual miRNAs in adenocarcinoma (AC) and squamous cell carcinoma (SCC) subgroup patients.**

	miRNA	AUC	95% CI	Sensitivity (%)	Specificity (%)
AC (n=66) vs. cancer-free controls (n=47)	miR-146b	0.93	0.8884 to 0.9794	87.23	89.39
	miR-205	0.93	0.8844 to 0.9837	97.87	84.85
	miR-29c	0.98	0.953 to 0.9973	91.49	92.42
	miR-30b	0.87	0.8054 to 0.9344	85.11	80.3
SCC (n=62) vs. cancer-free controls (n=47)	miR-146b	0.77	0.6828 to 0.8601	78.72	70.97
	miR-205	0.88	0.8134 to 0.9402	80.85	80.65
	miR-29c	0.83	0.7578 to 0.9103	87.23	70.97
	miR-30b	0.60	0.4996 to 0.7101	82.98	40.32



**Table S4: Clinicopathological characteristics of the 128 NSCLC patients, 17 COPD patients and 30 healthy subjects recruited in this study**

Variables	NSCLC patients	COPD patients	Healthy individuals
	n (%)	n (%)	n (%)
<b>Number</b>	128	17	30
<b>Age (years)</b>			
Mean $\pm$ SD	67.6 $\pm$ 9.5	65.0 $\pm$ 9.4	63.6 $\pm$ 9.1
<b>Gender</b>			
Male	68 (53%)	10 (59%)	15 (50%)
Female	60 (47%)	7 (41%)	15 (50%)
<b>Smoking status</b>			
Nonsmoker	10 (8%)	4 (24%)	11 (37%)
Smoker	118 (92%)	13 (76%)	19 (63%)
>50p/y	53 (44%)	6 (35%)	7 (23%)
<50p/y	65 ((56%)	11 (65%)	23 (77%)
<b>Histological type</b>			
Adenocarcinoma (AC)	66 (52%)	N/A	N/A
Squamous cell carcinoma	62 (48%)	N/A	N/A
<b>TNM stage</b>			
I	75 (59%)	N/A	N/A
II	33 (26%)	N/A	N/A
III	20(15%)	N/A	N/A
<b>Histological grade</b>			
Well	16 (12%)	N/A	N/A
Moderate	53 (42%)	N/A	N/A
Poor	44 (34%)	N/A	N/A
Unknown	15 (12%)	N/A	N/A

**Table S5: Univariate logistic regression analysis on the training and testing set for 5 individual miRNAs**

miRNA	Training set (n=78)			Testing set (n=97)		
	Regression coefficient ( $\beta$ )	95% CI	p-value	Regression coefficient ( $\beta$ )	95% CI	p-value
<b>miR-146b</b>	0.63	0.53-0.74	5.89E-06	0.49	0.39-0.58	3.82152E-10
<b>miR-205</b>	0.55	0.44-0.67	1.05E-07	0.37	0.28-0.46	4.47107E-16
<b>miR-29c</b>	0.53	0.42-0.64	5.13E-09	0.44	0.33-0.54	1.15544E-10
<b>miR-30b</b>	0.77	0.68-0.87	0.0293	0.62	0.53-0.71	3.89786E-05
<b>miR-337</b>	0.79	0.7-0.88	0.0457	0.63	0.54-0.73	0.0039