Supplementary Information for

Development of a Serum miRNA Panel for Detection of Early Stage Non-Small Cell Lung Cancer

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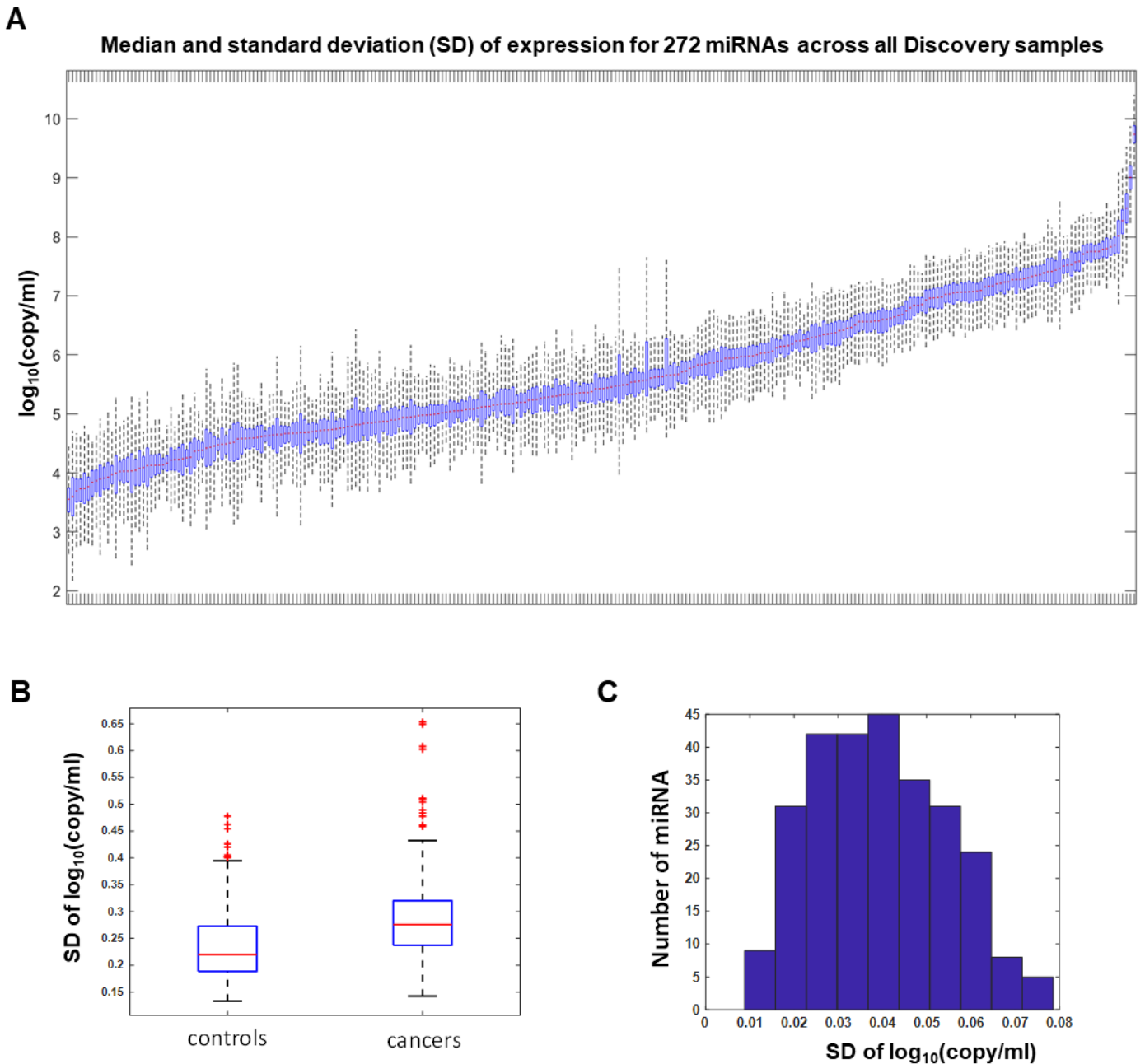
This PDF file includes:
Figures S1 to S6
Table S1

Figure S1. Variability and measurement precision of miRNA expression. (A) Median and standard deviation of expression of 272 miRNAs measured to have >500 copies/ml by RT-qPCR across all Discovery Cohort samples. Each miRNA was measured in duplicate for each sample. **(B)** Boxplots of standard deviation of expression of 272 miRNAs in control and cancer samples. **(C)** Histogram of standard deviation of expression from 272 miRNAs measured in six reference serum samples showing consistency of repeated measurements from the same sample.
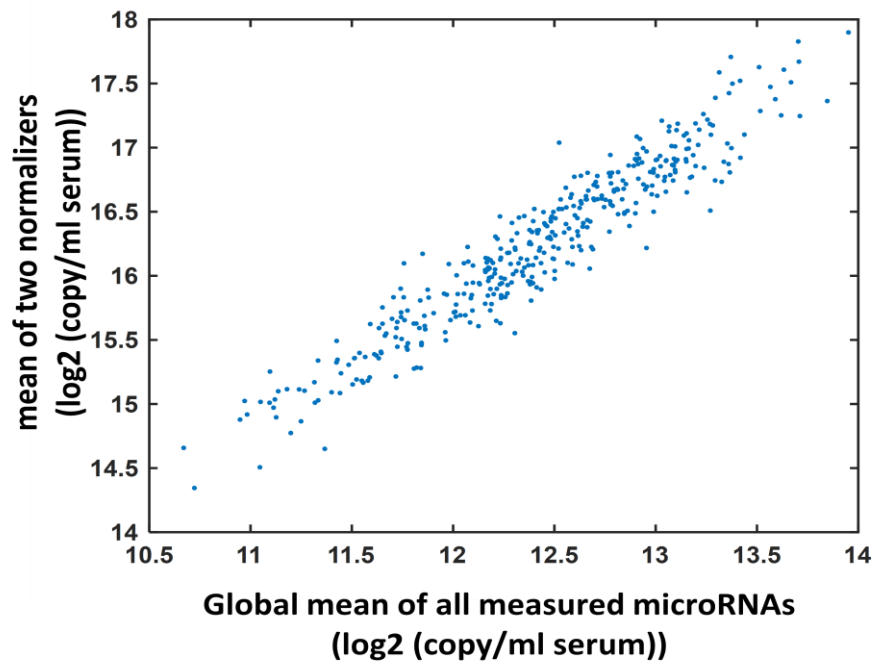
Fig. S2. Stable expression of endogenous miRNA controls. Correlation of mean expression of two selected

normalizers (miR-361-5p and miR-425-5p) against global mean expression of all measured miRNAs.
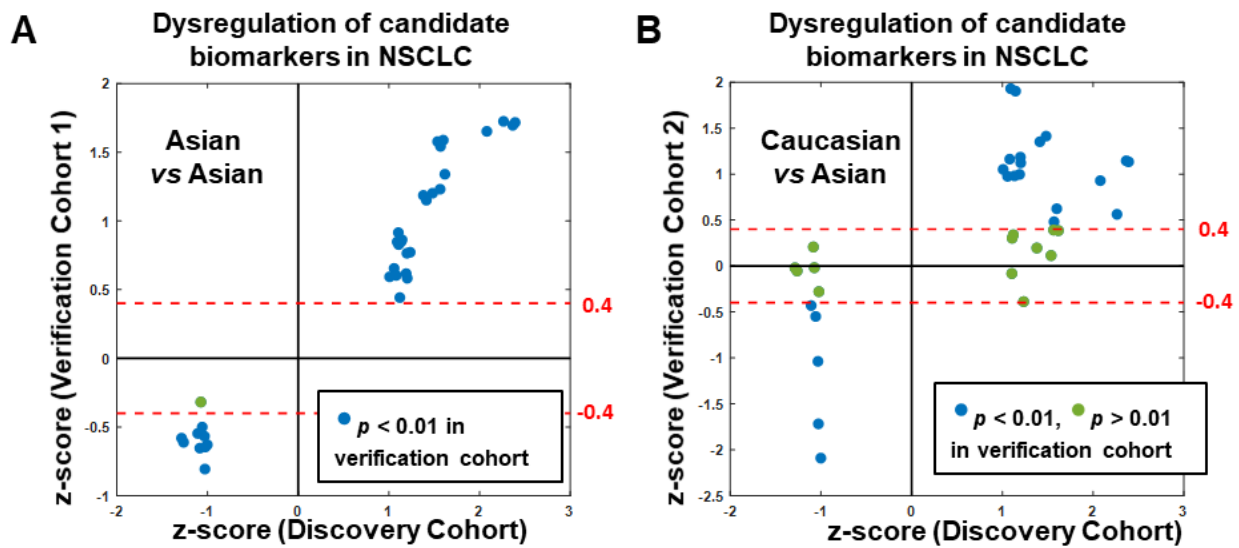
Fig. S3. Validation of candidate NSCLC miRNA biomarkers. Comparison of candidate biomarker z-scores between

the Discovery Cohort and Verification Cohort 1 and 2. *P*-values were determined by the Student's t-test between
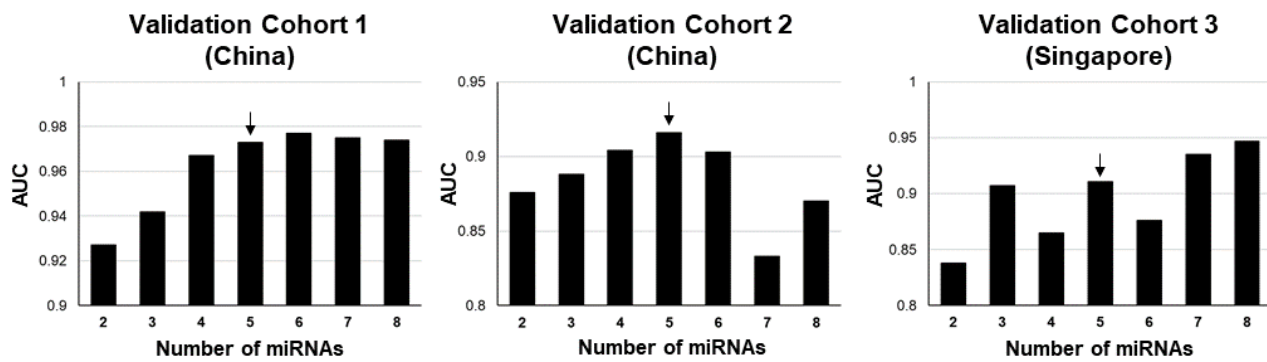
NSCLC cases and controls.

Fig. S4. Optimal panel for NSCLC detection. Comparison of AUC values for NSCLC detection by biomarker panels with two to eight miRNAs in Validation Cohorts 1-3.
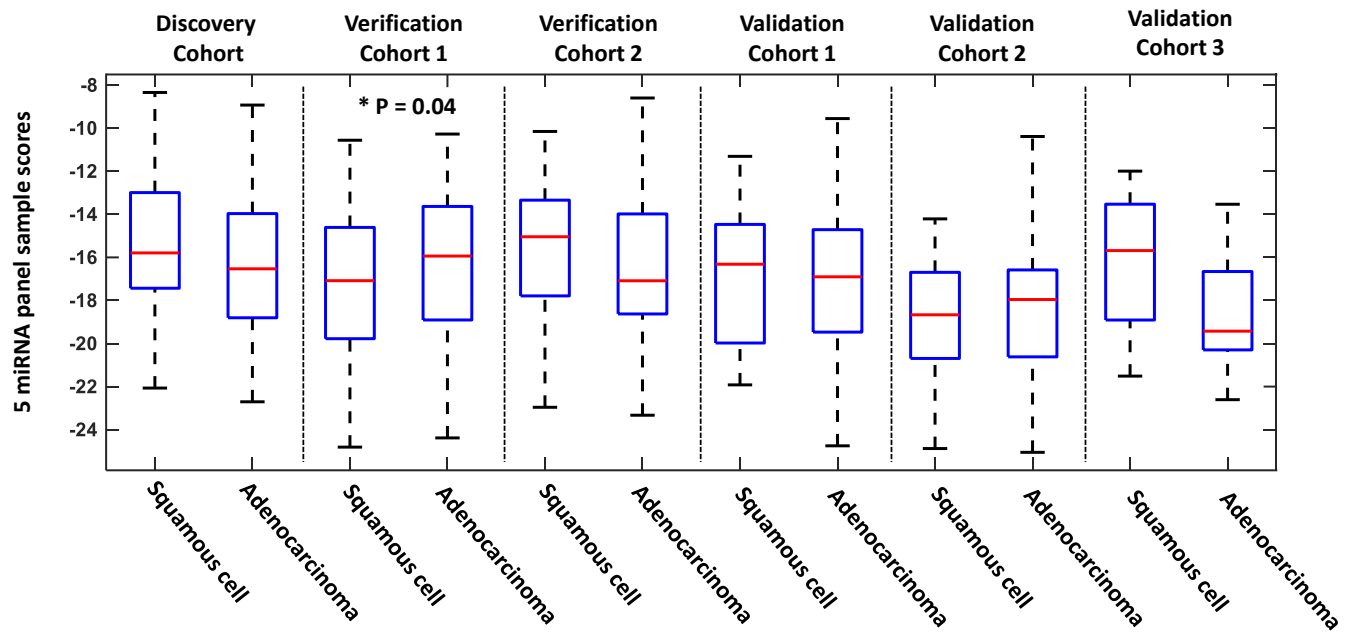
Fig. S5. Comparison of five-miR biomarker panel scores between NSCLC subtypes in each cohort. Boxplots of five-miR biomarker panel scores for NSCLC samples belonging to adenocarcinoma and squamous cell carcinoma subtypes in all six study cohorts. Statistical significance determined by Student's t-test *p*<0.05.
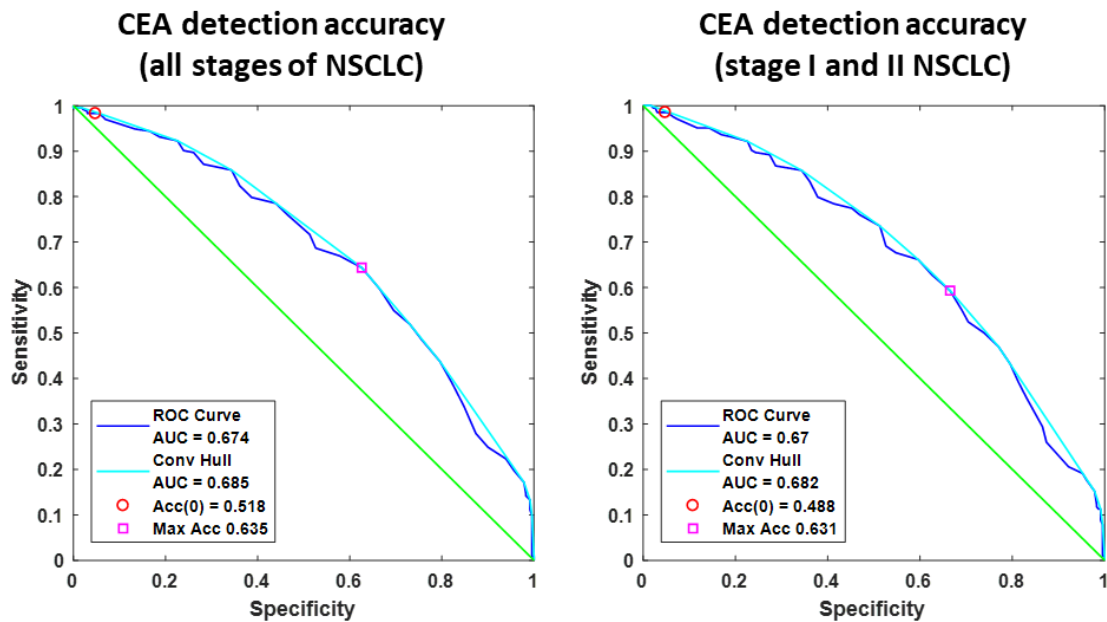
Fig. S6. CEA detection accuracy. AUC for NSCLC detection by CEA in a China (Zhejiang) cohort. CEA was

measured with an ELISA kit in the sera of 233 lung cancer patients (120 stage I, 84 stage II and 29 stage III) and 230

matched control subjects selected from the Discovery Cohort, Verification Cohort 1 and Validation Cohort 1.

Table S1. Comparison of published circulating miRNA biomarkers for NSCLC detection

Publication	Patient Cohorts	Biomarker(s)	Performance (Sensitivity, Specificity, and AUC where reported)
Fehlmann et al. (14)	606 cases vs 2240 controls	14-miRNA whole blood panel: miR-1285-3p, miR-205-5p, miR-1260a, miR-1260b, miR-3152-3p, miR-378b, miR-1202, miR-139-5p, miR-16-2-3p, miR-18a-3p, miR-23b-3p, miR-3907, miR-551b-3p, miR-93-3p	Sensitivity: 81% Specificity: 96.1% AUC: 0.965
Shen et al. (22)	Discovery: 28 cases vs 28 controls Validation: 58 cases vs 29 controls	4-miRNA plasma panel: miR-21, miR-126, miR-210, miR-486-5p	Sensitivity: 86.2% Specificity: 96.6%
Arab et al. (23)	Discovery: 34 cases vs Validation: 72 cases vs 50 controls	Serum miR-141	Sensitivity: 82.7% Specificity: 98%
Pan et al. (24)	Validation: 42 cases vs 42 controls	2-miRNA serum panel: miR-33a-5p and miR-128-3p	Sensitivity: 90.2% Specificity: 92.7%
Bagheri et al. (25)	Discovery & Validation: 30 cases vs 30 controls.	3-miRNA sputum panel: miR-145, miR-126, miR-7	Sensitivity: 90% Specificity: 90%
Geng et al. (26)	Validation: 126 cases vs 60 controls	5 individual miRNAs: miR-20a, miR-145, miR-21, miR-221, miR-223 (best performance)	Sensitivity: 87% Specificity: 86% AUC = 0.94
Peng et al. (27)	Discovery: 36 cases vs 30 controls Validation: 120 cases vs 71 controls	3-miRNA + 1 lncRNA serum panel: miR-1254, miR-485-5p, miR-574-5p, MALAT1	AUC = 0.844
Chen et al. (28)	Discovery: 200 cases vs 110 controls Validation: 200 cases vs 110 controls	10-miRNA serum panel: miR-20a, miR-24, miR-25, miR-145, miR-152, miR-199a-5p, miR-221, miR-222, miR-223, miR-320	Sensitivity: 92.5% Specificity: 90% AUC = 0.972
Yang et al. (29)	Discovery: 63 cases vs 15 controls Validation: 65 cases vs 32 controls	4-miRNA serum panel: miR-146b, miR-205, miR-29c, miR-30b	Sensitivity: 95.4% Specificity: 78.1% AUC = 0.93
Abdollahi et al. (30)	Discovery & Validation: 43 cases vs 43 controls	4-miRNA whole blood panel: miR-21, miR-638, miR-148, miR-152	Sensitivity: 96.4% Specificity: 86.7%
Lu et al (31)	Discovery & Verification: 330 cases vs 253 controls Validation: 203 cases vs 203 controls	6-miRNA plasma panel: miR-17, miR-190b, miR-19a, miR-19b, miR-26b, miR-375	Sensitivity: 80% Specificity: 80% AUC = 0.868