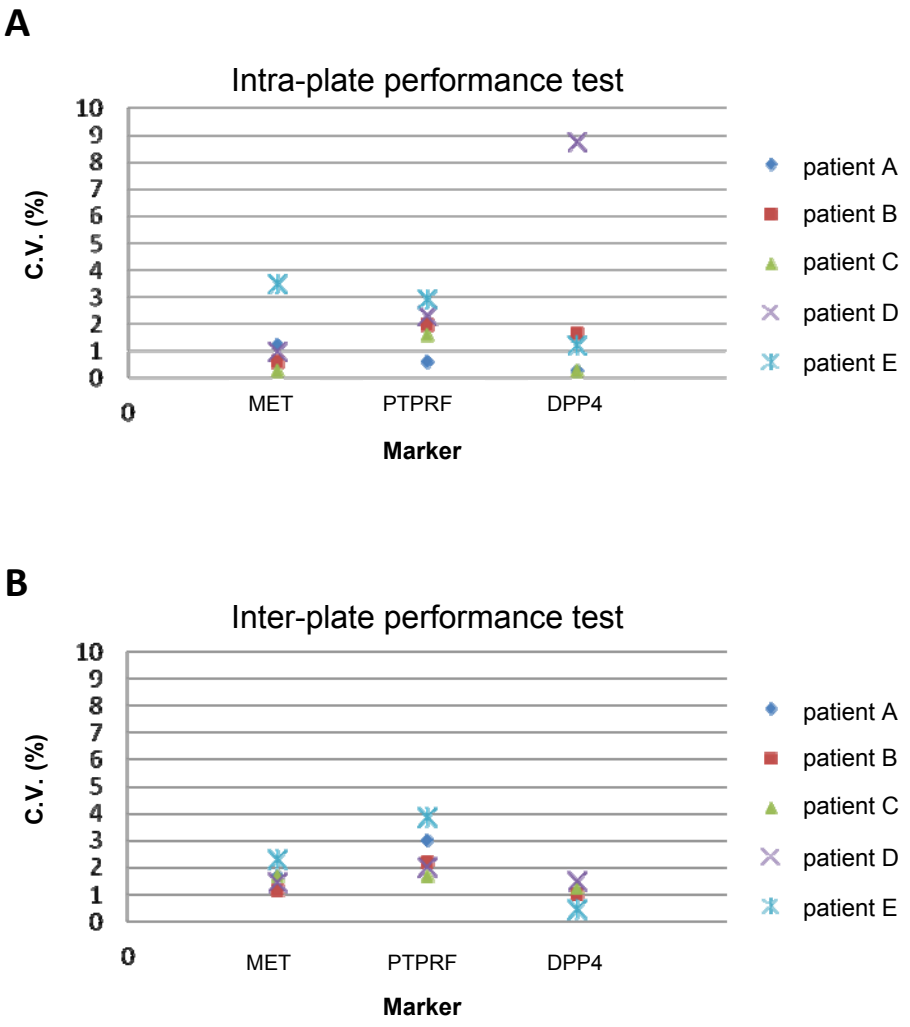
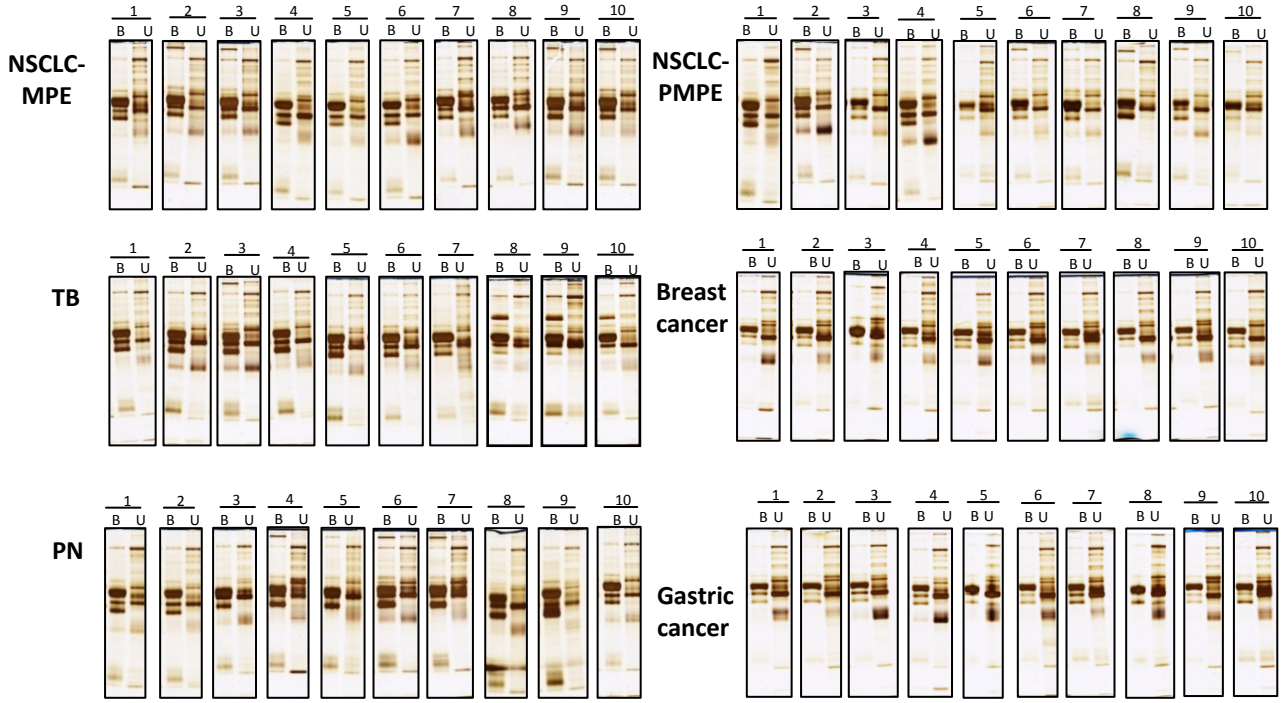
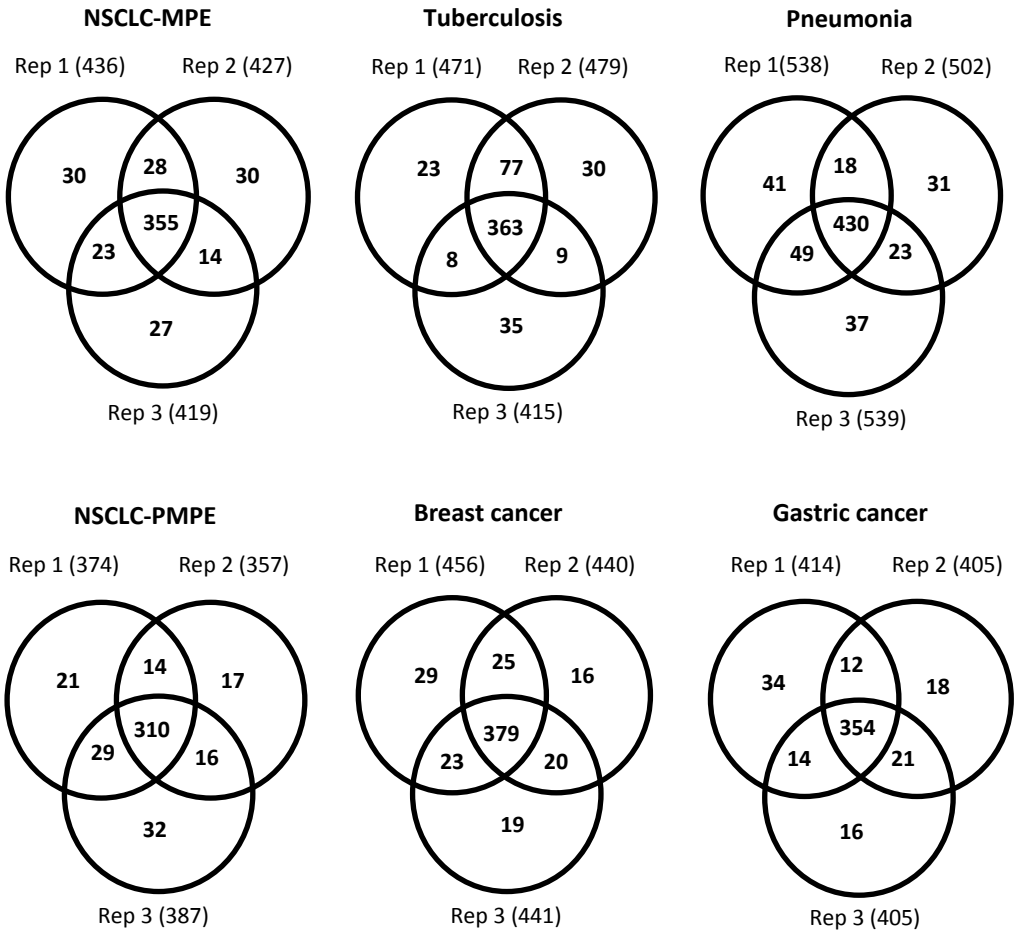


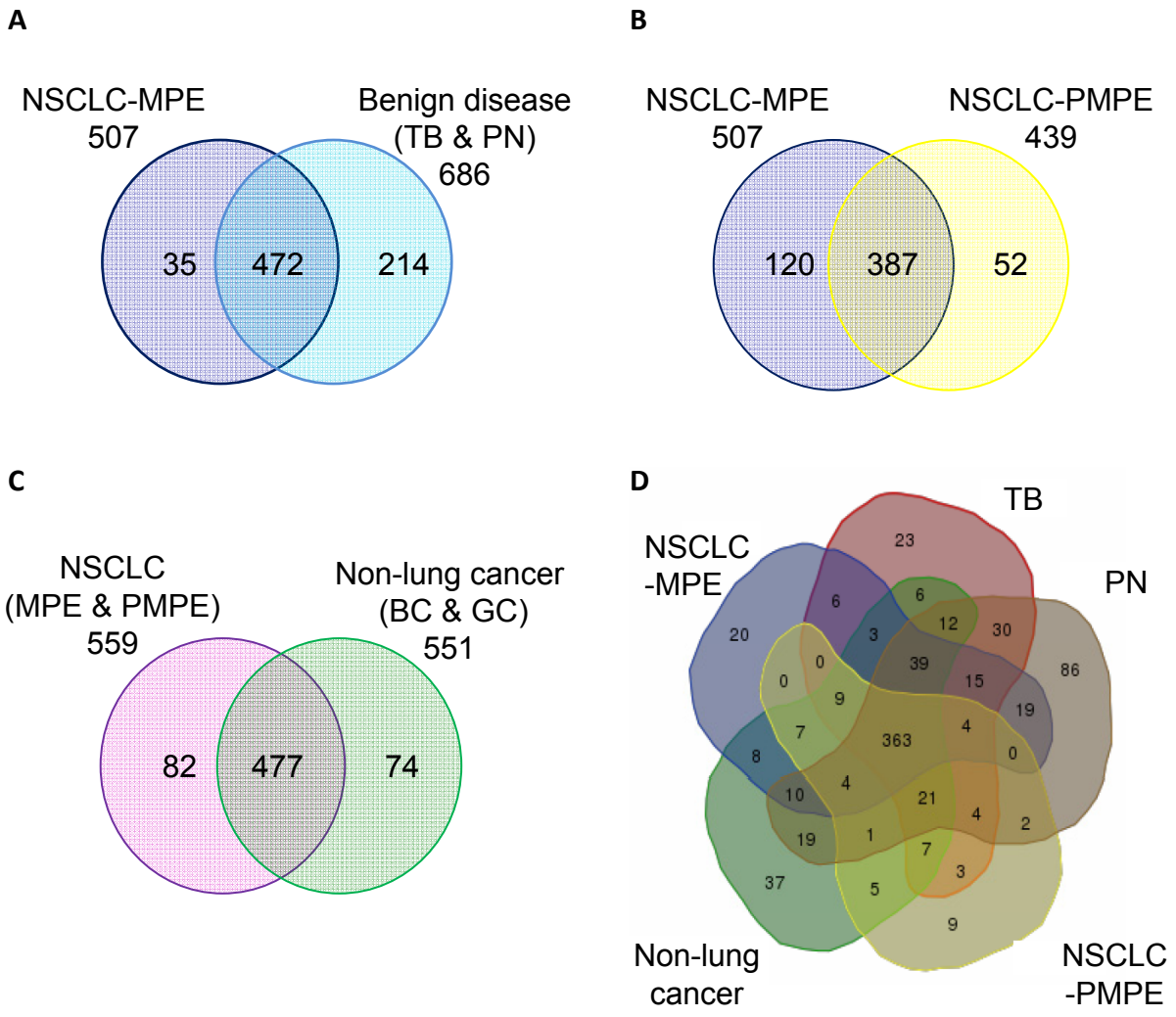
Supplemental Figure S1. The performance test of ELISA assay. To determine the PE levels of three potential PE biomarkers, the pleural effusion samples obtained from five individuals (patient A-E) were subjected to duplicate ELISA as indicated. (A) The average CV% values derived from single ELISA plate for each marker present the intra-plate performance of ELISA assay. (B) The average CV% values obtained from different batches of ELISA assays (8 plates for each potential biomarker) present the inter-plate performance of ELISA assay.



Supplemental Figure S2. Validation of depletion efficacy and homogeneity of fractionated PE samples. Three micrograms of proteins obtained from six types of PEs (10 individuals for each type of PE) were separated by 10% SDS-PAGE followed by silver staining. B, crude PE samples prepared before depletion of high-abundance proteins. U, unbound fractions obtained from high-abundance proteins removal system.



Supplemental Figure S3. The overlap of proteins identified from three independent LC-MS/MS experiments for each type of PE. The overlap of identified proteins between any two of three independent experiments (Rep 1-3) for NSCLC-MPE, tuberculosis, pneumonia, NSCLC-PMPE, breast cancer, and gastric cancer is 82.8% (420/507), 83.9% (457/545), 82.7% (520/629), 84.1% (369/439), 87.5% (447/511) and 85.5% (401/469), respectively.



Supplemental Figure S4. The overlap and the intersection of proteins identified in different types of PEs (A) The overlap between NSCLC-MPE and benign disease (TB and PN) is 65.5% (472/721). (B) The overlap between NSCLC-MPE and NSCLC-PMPE is 69.2% (387/559). (C) The overlap between NSCLC (MPE and PMPE) and non-lung cancer (breast cancer and gastric cancer) is 73.4% (477/633). (D) The proteins identified in different types of PE samples (NSCLC-MPE, TB, PN, NSCLC-PMPE and non-lung cancer) were analyzed for the overlap using a free program developed by Bioinformatics and System Biology of Gent (http://bioinformatics.psb.ugent.be/webtools/Venn/). Of the 772 unique proteins identified in the present study, 363 (47.0%) were common to all types of PEs.

A NSCLC-MPE

correlation (r)	Rep1	Rep2	Rep3
Rep1	1	0.987*	0.989*
Rep2		1	0.998*
Rep3			1

B Tuberculosis

correlation (r)	Rep1	Rep2	Rep3
Rep1	1	0.998*	0.991*
Rep2		1	0.991*
Rep3			1

C Pneumonia

correlation (r)	Rep1	Rep2	Rep3
Rep1	1	0.995*	0.995*
Rep2		1	0.996*
Rep3			1

D NSCLC-PMPE

correlation (r)	Rep1	Rep2	Rep3
Rep1	1	0.996*	0.996*
Rep2		1	0.994*
Rep3			1

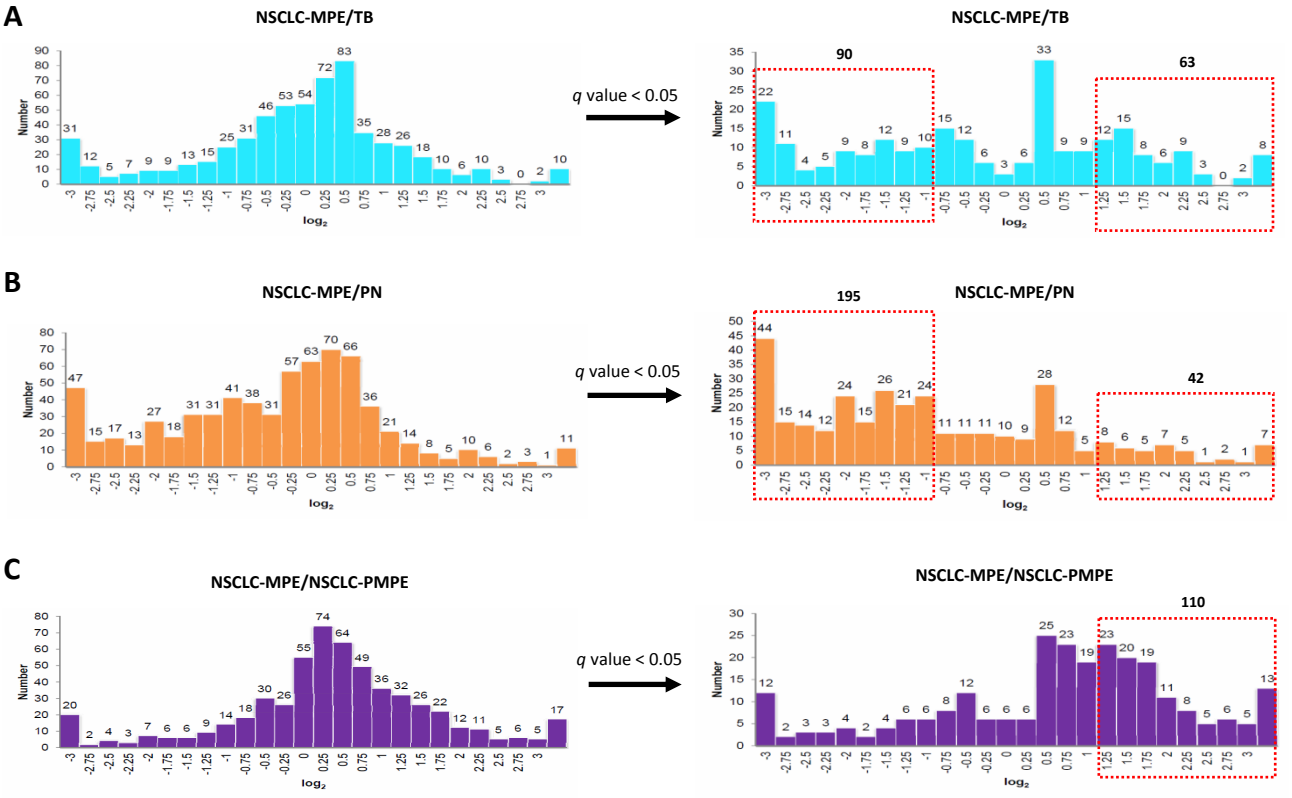
E Breast cancer

correlation (r)	Rep1	Rep2	Rep3
Rep1	1	0.998*	0.996*
Rep2		1	0.995*
Rep3			1

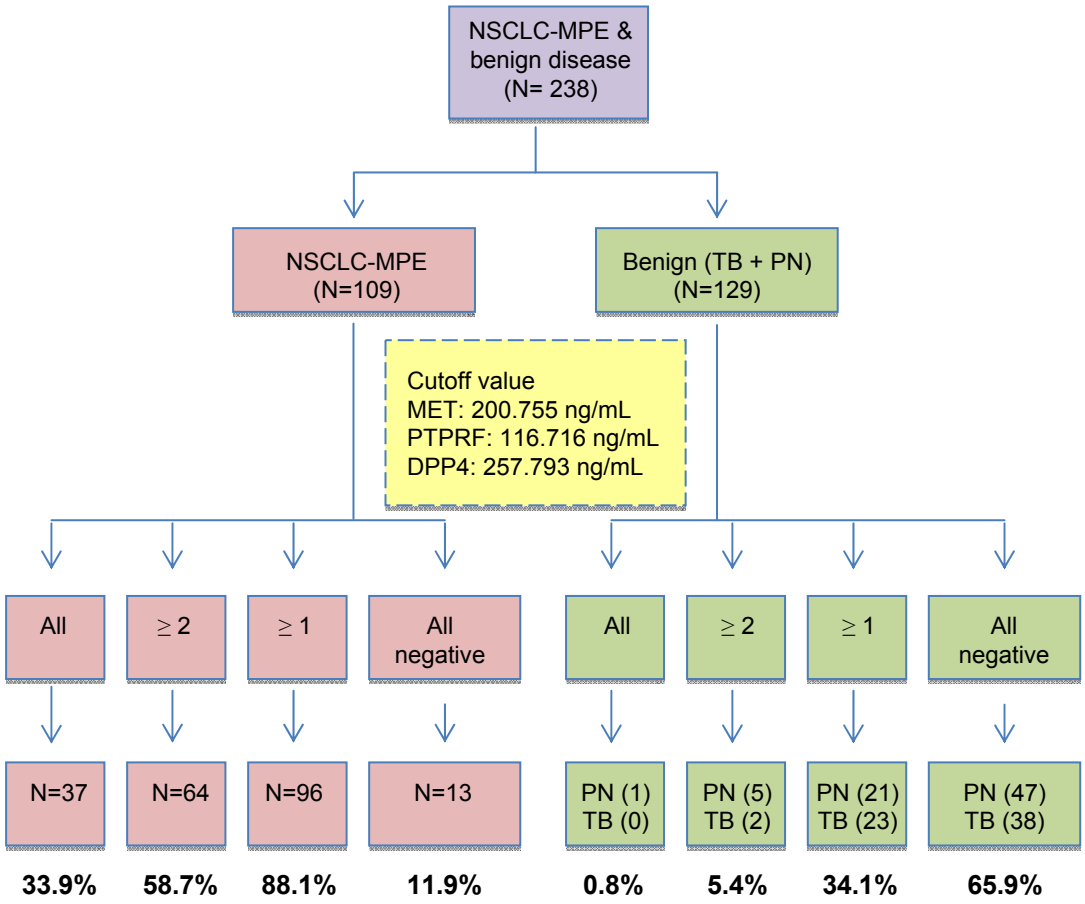
F Gastric cancer

correlation (r)	Rep1	Rep2	Rep3
Rep1	1	0.995*	0.994*
Rep2		1	0.998*
Rep3			1

Supplemental Figure S5. The correlation analysis among the three independent GeLC-MS/MS experiments for each type of PE proteomes. The identified proteins in each type of PE were semi-quantified based on spectral counts, as described in Experimental Procedures. The correlation between any two of three independent experiments (Rep 1-3) for (A) NSCLC-MPE, (B) tuberculosis, (C) pneumonia, (D) NSCLC-PMPE, (E) breast cancer, and (F) gastric cancer was determined by Pearson correlation coefficient calculator. *, a *p* value of less than 0.01 indicates statistical significance.



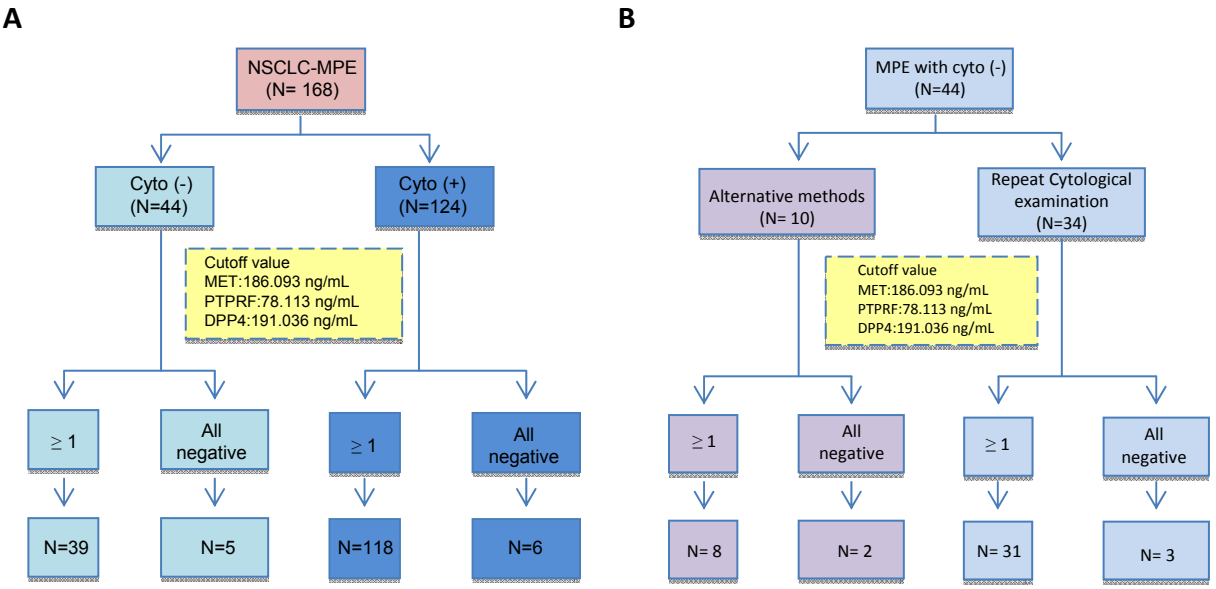
Supplemental Figure S6. The $\log_2$ ratio distributions of identified/quantified PE proteomes. The PE proteins were quantified based on spectrum counting as described in Materials and Methods. The proteins were defined as significantly changed only in those with a *q* value of less than 0.05**.** (A) There were 613 proteins identified in NSCLC-MPE and TB proteomes, including 153 differentially expressed proteins with two-fold changes between NSCLC-MPE and TB. (B) There were 682 proteins identified in NSCLC-MPE and PN proteomes, including 237 differentially expressed proteins with two-fold changes between NSCLC-MPE and PN. (C) There are 559 proteins identified in NSCLC-MPE and NSCLC-PMPE proteomes, including 110 differentially expressed proteins that increased two-fold in NSCLC-MPE compared with NSCLC-PMPE.



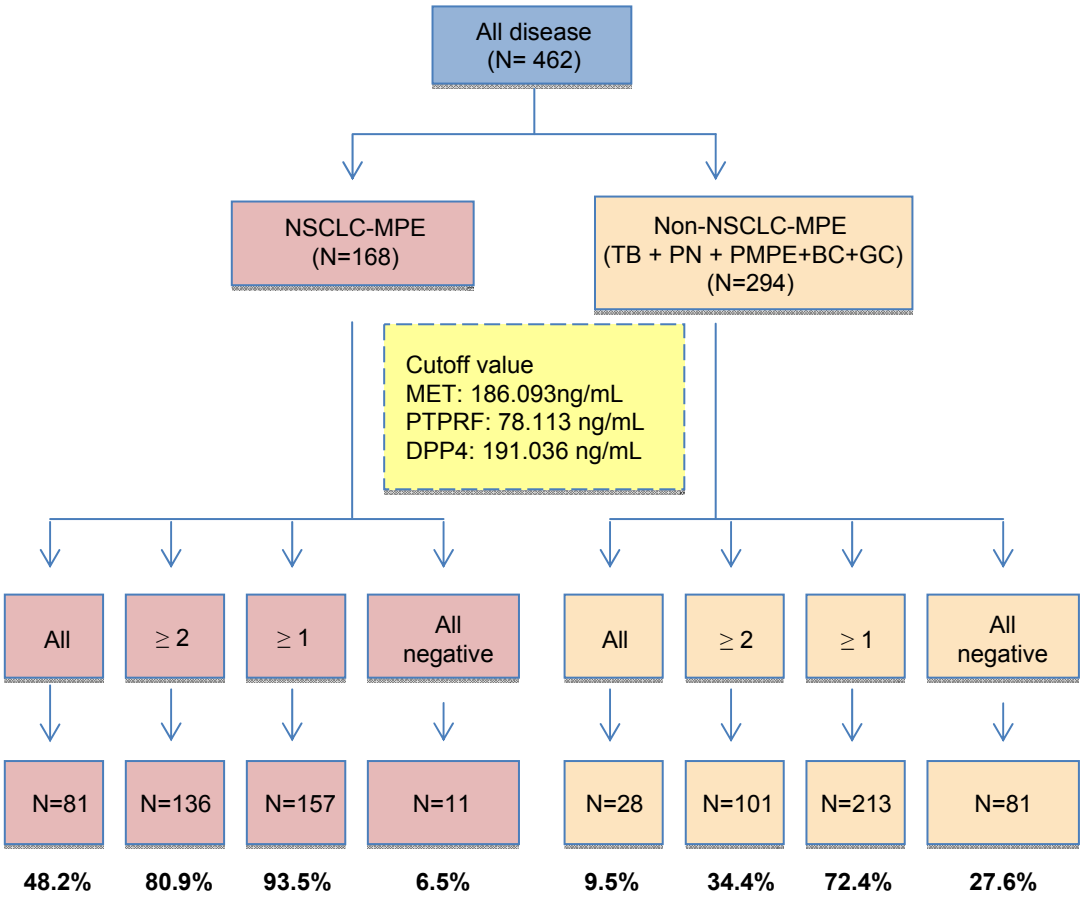
	Lung disease (109+129)	
	NSCLC-MPE	TB+PN
≥ 1 Marker	96 (a)	44 (b)
All negative	13 (c)	85 (d)

Sensitivity: a/(a+c) × 100% = 88.07%
Specificity: d/(b+d) × 100% = 65.89%
PPV: a/(a+b) ×100% = 68.57%
NPV: d/(d+c) × 100% = 86.73%

Supplemental Figure S7. The proposed application of three potential biomarkers in differential diagnosis of NSCLC-MPE and benign diseases. When any one (≥ 1) of three markers (MET, PTPRF and DPP4) was applied with a given cut-off value to discriminate NSCLC-MPE from benign diseases (TB and PN), the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 88.07%, 65.89%, 68.57% and 86.73%, respectively. ≥1, at least one of three biomarkers' levels is higher than the given cutoff value; ≥2, at least two of three biomarkers' levels are higher than the given cutoff values. All, all of the three biomarkers' levels are higher than the given cutoff values.



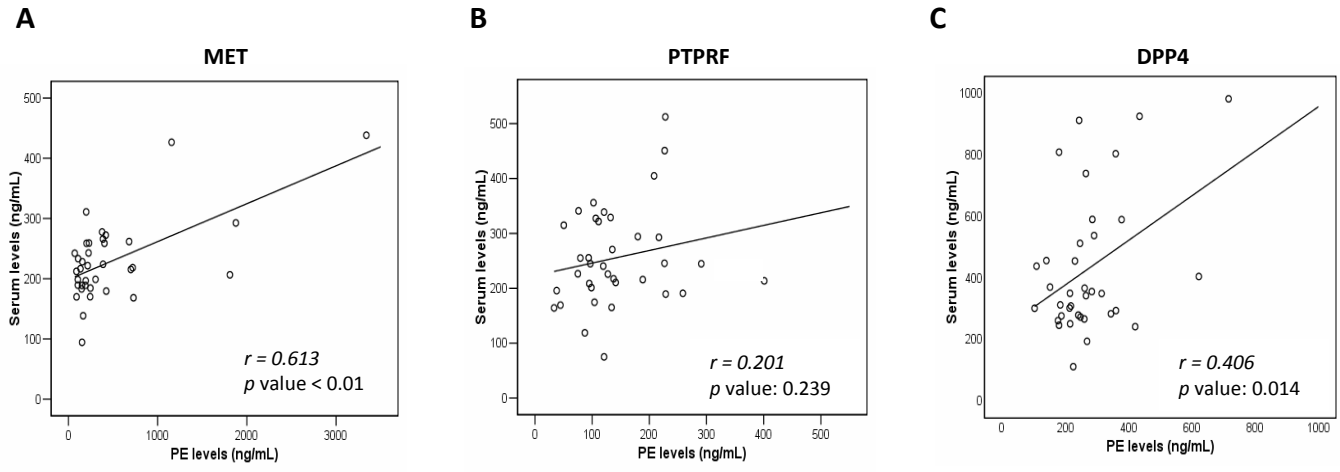
Supplemental Figure S8. The proposed application of three potential biomarkers used to rescue the false negative of cytological examination for NSCLC-MPE diagnosis. (A) The sensitivity of cytological examination for NSCLC-MPE diagnosis was 73.80%, in which only 124 of 168 NSCLC-MPE were examined as positive cytological samples. When any one of three biomarkers with a given cut-off value was applied for NSCLS-MPE diagnosis, the sensitivity was 93.45% (157/168). ≥1, at least one of three biomarkers' levels is higher than the given cutoff value. All negative, none of three biomarkers' levels is higher than the given cutoff value. (B) The 44 NSCLC-MPE patients with negative cytology were diagnosed as MPE by three alternative methods, including repeated cytological examination (34 patients) and other alternative methods (5 pleural biopsy and 5 pleural seeding nodules detected via computed tomography). The sensitivity of repeated cytological examination, alternative methods, and PE biomarkers was 77.3% (34/44), 22.7% (10/44), and 88.6% (39/44), respectively.



	All disease (168+294)	
	NSCLC-MPE	Non-malignancy
≥ 1 Marker	157 (a)	213 (b)
All negative	11 (c)	81 (d)

Sensitivity: a/(a+c) × 100% = 93.45%
Specificity: d/(b+d) × 100% = 27.55%
PPV: a/(a+b) ×100% = 42.43%
NPV: d/(d+c) × 100% = 88.04%

Supplemental Figure S9. The proposed application of three potential biomarkers in differential diagnosis of NSCLC-MPE and all other tested pleural types (TB, PN, NSCLC-PMPE, BC and GC). When any one (≥ 1) of three markers (MET, PTPRF and DPP4) with a given cut-off value was applied to differentiate NSCLC-MPE from NSCLC-PMPE, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 93.45%, 27.55%, 42.43% and 88.04%, respectively. ≥1, at least one of three biomarkers' levels is higher than the given cutoff value. ≥2, at least two of three biomarkers' levels are higher than the given cutoff values. All, all of the three biomarkers' levels are higher than the given cutoff values.



Supplemental Figure S10. The correlation between PE and serum levels of three potential biomarkers. The PE and serum levels of (A) MET, (B) PTPRF, and (C) DPP4 were determined by duplicate ELISA as described in Experimental Procedures. The paired PE and serum samples obtained from 36 lung cancer patients were included in this analysis. A *p* value of less than 0.05 indicates statistical significance using the Pearson correlation coefficient calculator.