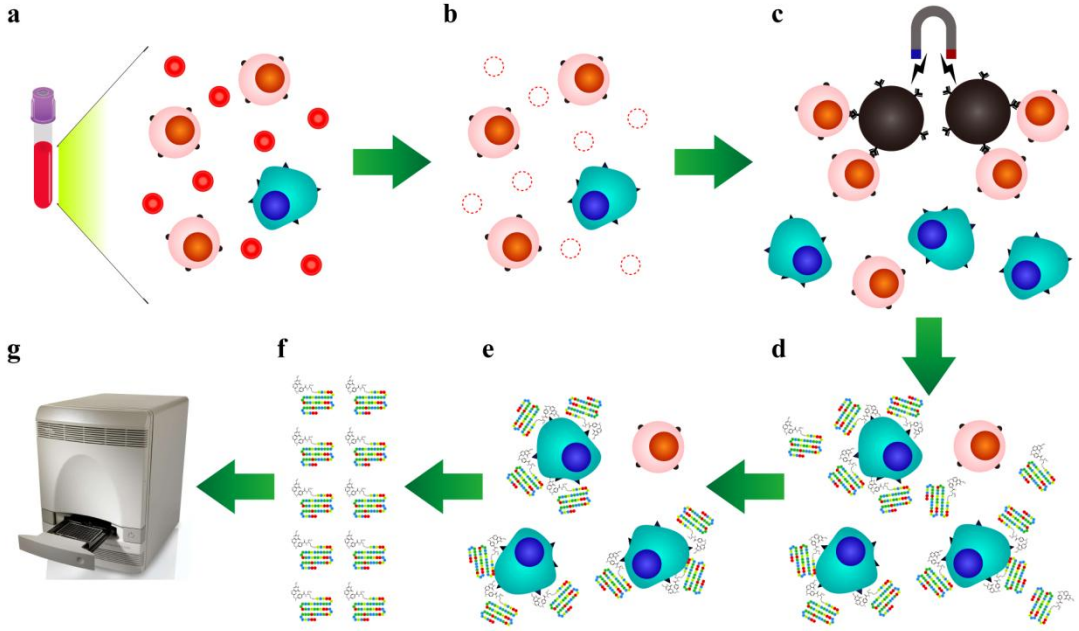
1 **Supplementary Figure 1: The schematic diagram of FR$^+$-CTC detection workflow.** (**a**) Collection of 3 mL of peripheral blood with an EDTA-containing anti-coagulant tube. (**b**) Lysis of erythrocytes. (**c**) Negative depletion of leukocytes with anti-CD45 immuno-magnetic beads. (**d**) Labelling of FR$^+$-CTCs with detection probes (a conjugate of tumour-specific ligand folic acid and oligonucleotide). (**e**) Washing away unbound detection probes. (**f**) Stripping of bound detection probes from CTCs. (**g**) quantitative PCR analysis.

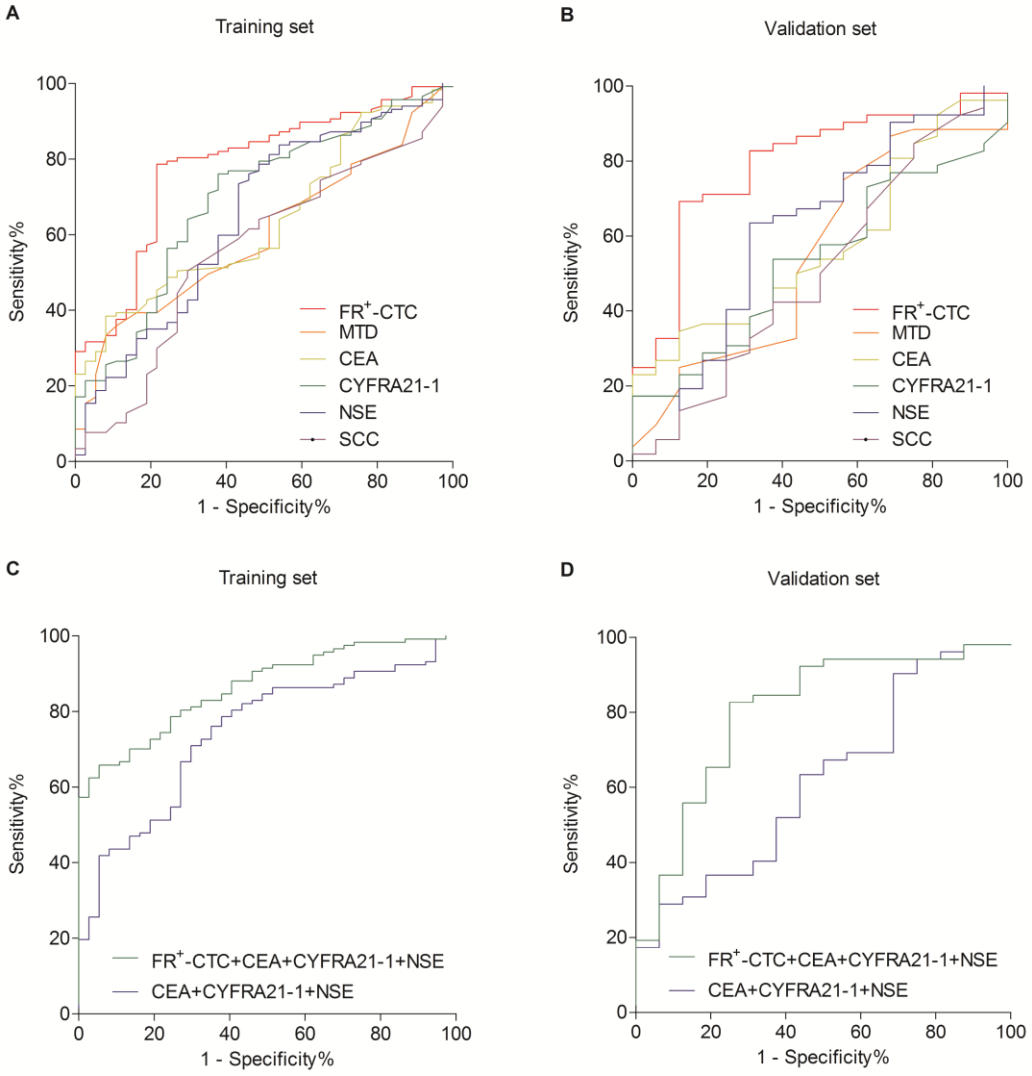


2 Supplementary Table 1: Percentage of FRα-positive cells in the immunohistochemistry experiment

Sample type	Sample ID	Percentage of FRα-positive cells	Average percentage of FRα-positive cells
AIS	1	8 44%	12 91%
AIS	2	N/A	
AIS	3	19 70%	
AIS	4	10 58%	
MIA	5	5 47%	11 07%
MIA	6	10 43%	
MIA	7	9 19%	
MIA	8	19 19%	
IPA	9	N/A	14 74%
IPA	10	7 69%	
IPA	11	N/A	
IPA	12	21 78%	
Benign lung disease	13	0%	4 19%
Benign lung disease	14	7 14%	
Benign lung disease	15	N/A	
Benign lung disease	16	5 44%	
Serous ovarian cancer	17	3 00%	8 17%
Serous ovarian cancer	18	4 65%	
Serous ovarian cancer	19	19 43%	
Serous ovarian cancer	20	5 60%	

FFPE samples from 4 individuals were collected for immunohistochemistry experiment in each group of pathological subtype. Serous ovarian cancer samples served as a positive control. N/A indicates sample not examined due to tissue section falling off slides.

3 **Supplementary Figure 2: The ROC curves (Malignant versus Benign).** ROC analysis of FR^+-CTC, MTD, and serum biomarkers in (**a**) training set and (**b**) validation set. ROC analysis of FR^+-CTC combined with serum biomarkers in (**c**) training set and (**d**) validation set. Only patients who were randomly selected to receive serum biomarker tests (117 lung cancer and 37 benign lung disease patients in the training set; 52 lung cancer and 16 benign lung disease patients in the validation set) were included in this ROC analysis. Serum biomarkers include CEA, CYFRA21-1, NSE, and SCC.



4 Supplementary Table 2: Clinical characteristics of patients with small-sized lung adenocarcinoma

	Training set				Validation set			
	AIS (n=30)	**MIA (n=41)**	**IPA (n=62)**	***P***	**AIS (n=21)**	**MIA (n=14)**	**IPA (n=34)**	***P***
Age, median (range), y	52 5 (25-72)	59 (21-76)	57 5 (37-78)	04	48 (27-68)	55 (31-65)	59 (43-79)	001
Sex				·10				·70
Male	12 (40)	10 (24)	28 (45)		8 (38)	5 (36)	16 (47)	
Female	18 (60)	31 (76)	34 (55)		13 (62)	9 (64)	18 (53)	
MTD, mean (SD), cm	0 87±0 41	0 96±0 27	1 46±0 38	< 001	0 74±0 24	1 01±0 36	1 53±0 38	< 001
Serum biomarkers, mean (SD)[a]								
CEA, ng/mL	1 22±0 60	1 70±1 25	3 45±7 73	·14	1 ·14±0 ·44	1 ·73±1 ·15	4 07±8 23	·11
CYFRA21-1, ng/mL	1 93±1 00	1 58±1 34	2 23±1 64	·10	1 54±0 64	1 89±1 85	1 76±1 28	88
NSE, ng/mL	13 92±4 55	13 28±6 54	12 82±7 01	·40	14 03±2 22	12 ·73±3 ·75	12 ·2±4 ·77	·42
SCC, ng/mL	0 87±0 28	1 09±0 92	0 88±0 22	86	0 92±0 31	0 91±0 ·18	1 03±0 86	81
Clinical stage								
0 (Tis)	30 (100)	0	0		21 (100)	0	0	
I	0	41 (100)	58 (94)		0	14 (100)	32 (94)	
II	0	0	1 (2)		0	0	2 (6)	
III	0	0	3 (5)		0	0	0	
Uncertain	0	0	0		0	0	0	

[a] In the training set, 17, 22, and 44 patients were randomly selected to receive serum biomarker tests in the AIS, MIA, and IPA group, respectively. In the validation set, 7, 12, and 22 patients were randomly selected to receive serum biomarker tests in the AIS, MIA, and IPA group, respectively.

5 Supplementary Table 3: FR^+-CTC count in patients with small-sized adenocarcinoma

	Training set	Validation set
FR^+-CTC count, median (range)		
AIS	8 6 (1 4-17 3)	9 0 (1 9-15 5)
MIA	9 6 (4 0-18 4)	10 0 (2 6-13 0)
IPA	10 3 (4 1-32 1)	10 3 (6 3-26 9)
***P* value**		
AIS versus MIA	12	61
MIA versus IPA	26	44
AIS versus IPA	001	15
AIS versus MIA+IPA	004	20
AIS+MIA versus IPA	01	16

The FR^+-CTC count of the subgroup of patients with lung adenocarcinoma ≤2 cm. In the training set, 30, 41, and 62 patients had AIS, MIA, and IPA, respectively. In the validation set, 21, 14, and 34 patients had AIS, MIA, and IPA, respectively.

6 **Supplementary Figure 3: The ROC curves (AIS versus MIA versus IPA).** ROC analysis of FR^+-CTC combined with MTD in "AIS versus IPA" in (**a**) training set and (**b**) validation set. ROC analysis of FR^+-CTC combined with MTD in "AIS versus MIA+IPA" in (**c**) training set and (**d**) validation set. ROC analysis of FR^+-CTC combined with MTD in "AIS+MIA versus IPA" in (**e**) training set and (**f**) validation set. Only patients who were randomly selected to receive serum biomarker tests (17 AIS, 22 MIA, and 44 IPA patients in the training set; 7 AIS, 12 MIA, and 22 IPA patients in the validation set) were included in this ROC analysis.