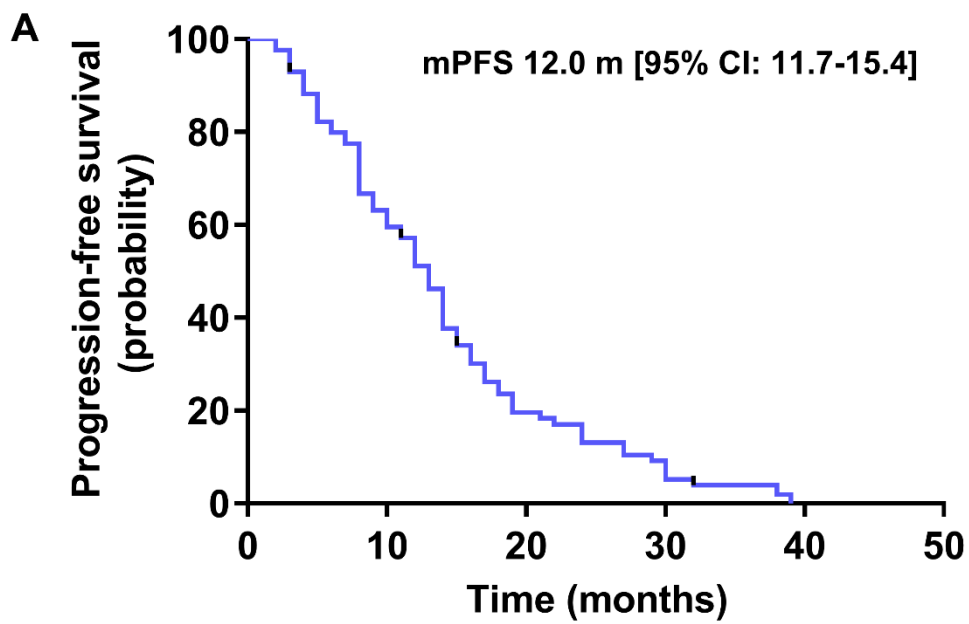
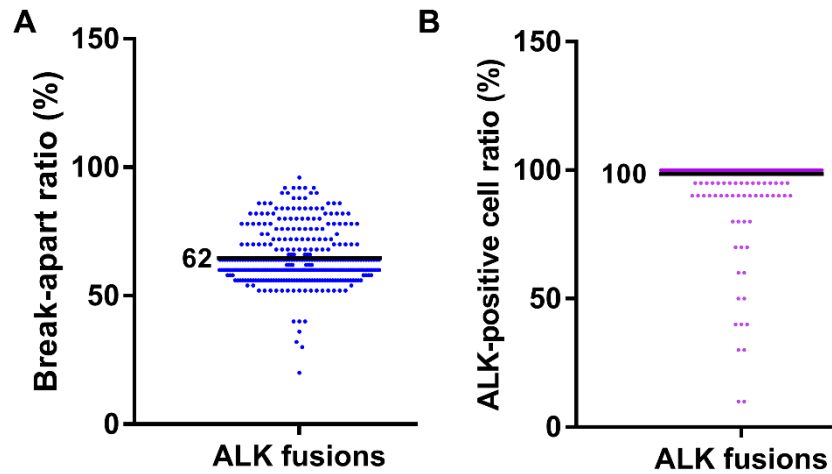


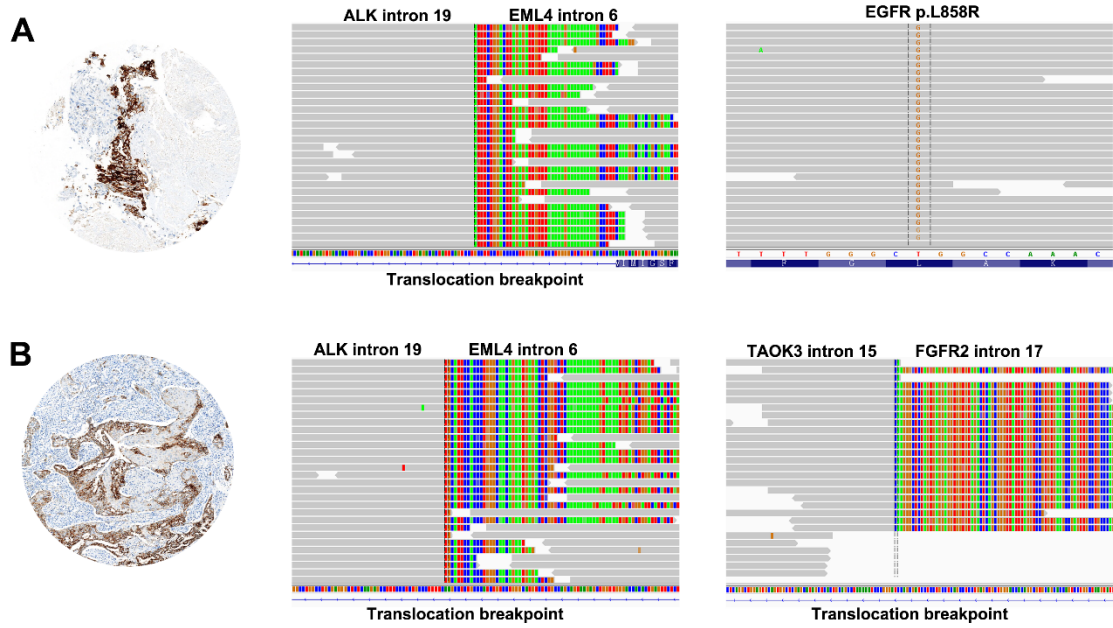
Supplementary Figure 1. Distribution of VAF of *ALK* fusions. Black line represents median VAF, with the value of 19.6%.



Supplementary Figure 2. Kaplan-Meier curve for 85 *ALK*-positive NSCLC patients who received first-line crizotinib therapy.



Supplementary Figure 3. *ALK* subclonality determined by FISH or IHC. (A) Distribution of *ALK* break-apart ratio determined by FISH. Black line represents median ratio, with the value of 62. (B) Distribution of IHC-positively stained cell ratio determined by IHC. Black line represents median ratio, with the value of 100.



Supplementary Figure 4. Molecular characteristic of two patients with concurrent *ALK* fusion and other actionable alterations. (A) Concurrent *ALK* fusion and *EGFR* p.L858R mutation were identified by IHC and NGS. (B) Concurrent *ALK* and *FGFR2* fusions were identified by IHC and NGS.

Supplementary Table 1. Inconsistent results of *ALK* fusions detected by NGS, FISH and IHC.

Case	Sex	Age	Diagnosis	NGS	FISH	IHC
P1	Female	57	ADC	Negative	Positive	Negative
P2	Female	65	ADC	Negative	Positive	Negative
P3	Male	63	ADC	Negative	Negative	Positive
P4	Male	67	ADC	Negative	Negative	Positive
P5	Female	48	ADC	EML4-ALK	Negative	Positive
P6	Female	41	ADC	EML4-ALK	Negative	Positive
P7	Female	48	ADC	EML4-ALK	Negative	Positive
P8	Male	63	ADC	Negative	Positive	Negative
P9	Female	65	ADC	Negative	Positive	Negative
P10	Male	62	ADC	Negative	Positive	Negative
P11	Female	42	ADC	EML4-ALK	Positive	Negative
P12	Female	49	ADC	EML4-ALK	Positive	Negative
P13	Male	59	SCC	Negative	Negative	Positive, heterogeneous staining
P14	Female	55	SCC	Negative	Negative	Positive, heterogeneous staining
P15	Male	61	SCC	Negative	Negative	Positive, heterogeneous staining
P16	Male	61	SCC	Negative	Negative	Positive, heterogeneous staining

ADC, adenocarcinoma; SCC, squamous cell carcinoma.

Supplementary Table 2. Clinical response to crizotinib treatment for *ALK* NGS-positive cases.

Response	First-line crizotinib (<i>n</i>=85)
CR (%)	0
PR (%)	60 (70.6)
SD (%)	18 (21.2)
PD (%)	7 (8.2)
ORR% [95% CI]	70.6 (60.2-79.2)
DCR% [95% CI]	91.8 (84.0-96.0)

Supplementary Table 3. Univariate and multivariate analyses of factors associated with PFS in the 85 *ALK*-positive NSCLC patients who received first-line crizotinib therapy.

Variable	Univariable Analysis		Multivariable Analysis	
	Hazard Ratio (95% CI)	<i>P</i>	Hazard Ratio (95% CI)	<i>P</i>
Age (≥ 60 vs < 60 years)	1.38 (0.83-2.27)	0.212	1.47 (0.88-2.45)	0.145
Sex (female vs male)	0.83 (0.53-1.29)	0.409	0.97 (0.60-1.56)	0.897
Smoking history (yes vs no)	0.93 (0.57-1.51)	0.777	-	
<i>TP53</i> (mutated vs wild-type)	1.87 (1.16-3.02)	0.010	1.95 (1.17-3.23)	0.010
Brain metastases at baseline (yes vs no)	0.91 (0.53-1.56)	0.724	-	

Supplementary Table 4. NGS revealing the molecular characteristics of ALK IHC-positive area (a) and IHC-negative area (b) in 5 NSCLC cases with heterogeneous ALK-IHC staining.

Case	Isolated area: (a)		Isolated area: (b)	
	Variant	VAF%	Variant	VAF%
1	<i>EML4-ALK</i>	9.7	<i>GSTM5-ROS1</i>	6.6
2	<i>EML4-ALK</i>	10.9	<i>EGFR</i> p.K823Q	14.3
3	<i>EML4-ALK</i>	21.4	<i>KRAS</i> p.G12C	3.8
4	<i>EML4-ALK</i>	16.4	Wild type	-
5	<i>EML4-ALK</i>	25.3	Wild type	-