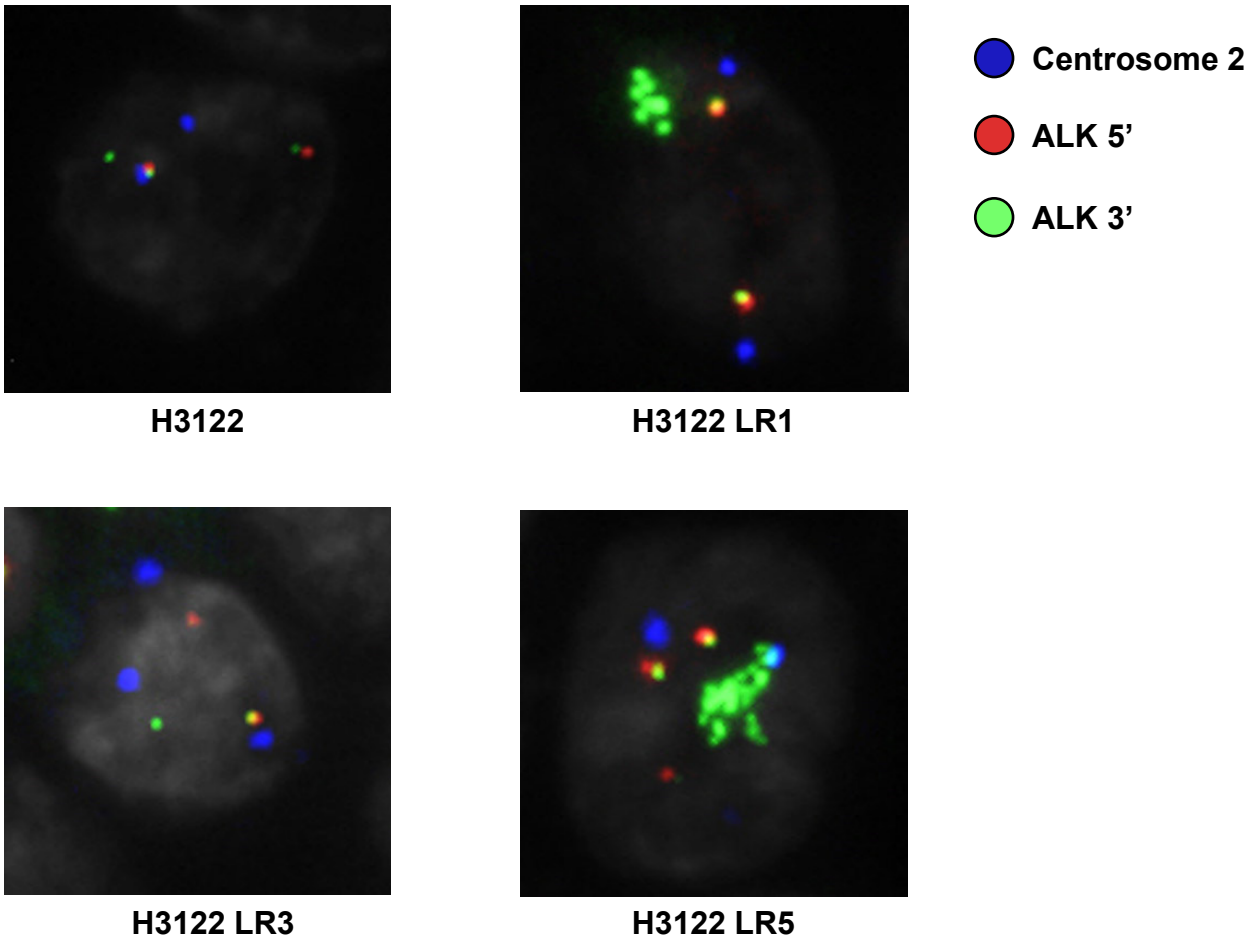
Supplementary Figure S1

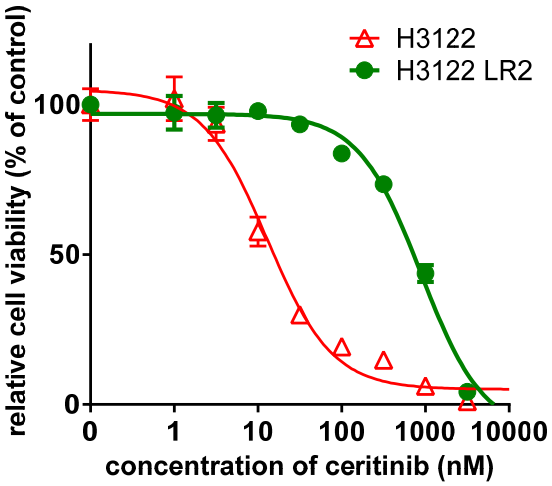


Supplementary Figure S1. FISH analysis revealed increased ALK split gene signals in H3122 LR1 and H3122 LR5 cells

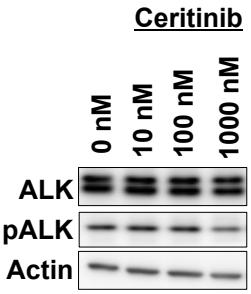
FISH analysis was performed on H3122, H3122 LR1, H3122 LR3 and H3122 LR5 cells. 5'ALK was indicated in red, 3'ALK in green, CEP2 in blue. CEP2 is located near the centromeric region of chromosome 2 and used to evaluate amplification of chromosome 2. More green signals (ALK split) were observed in H3122 LR1 and H3122 LR5 cells than in H3122 and H3122 LR3 cells.

Supplementary Figure S2

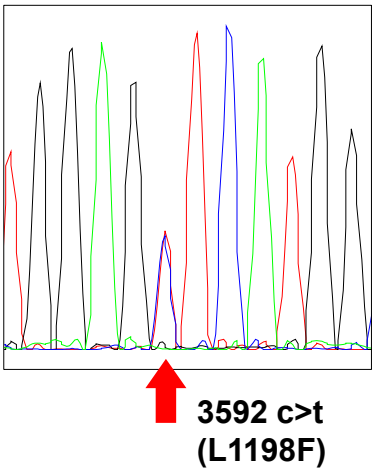
(A)



(B)



(C)

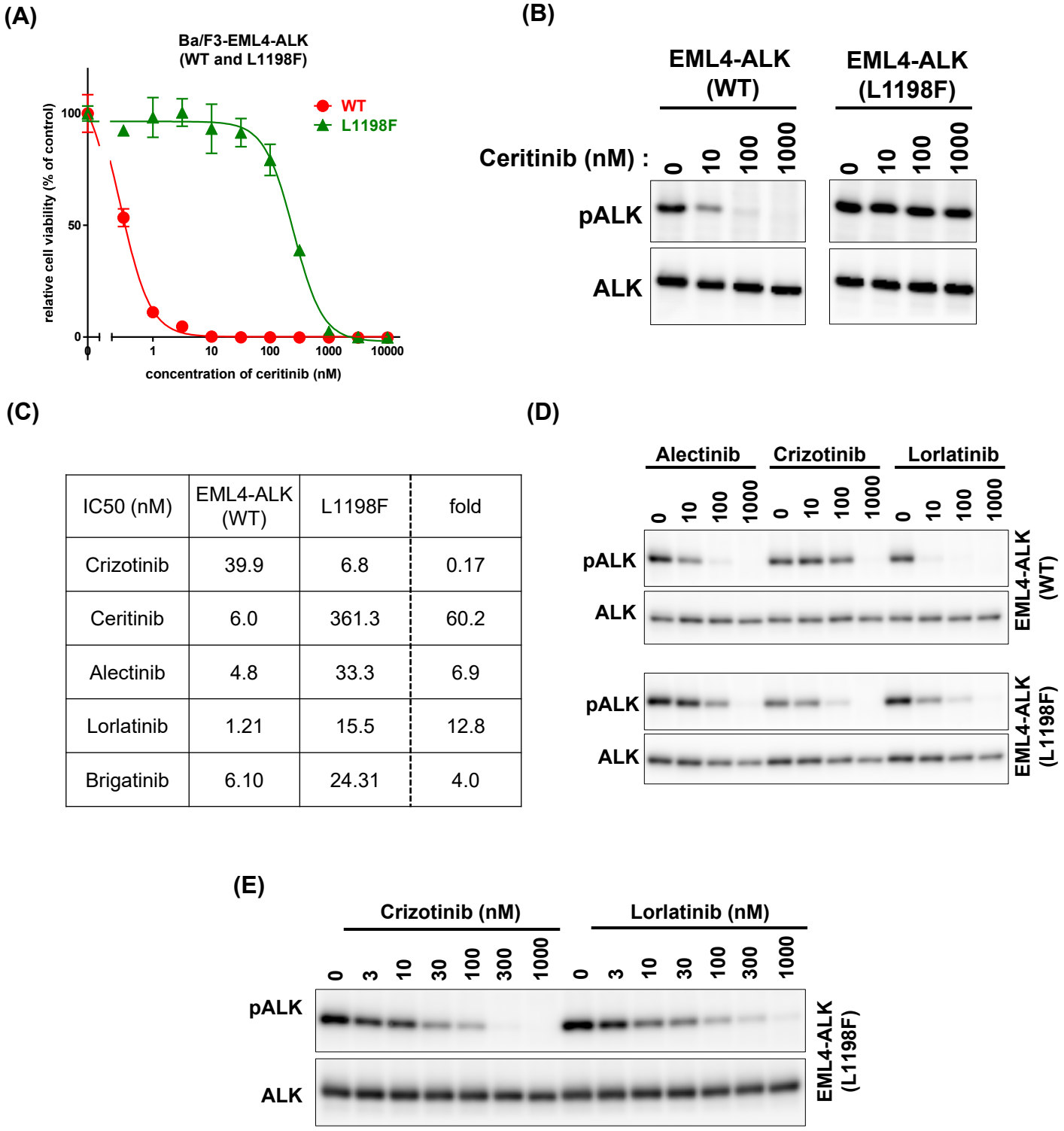


(D)

IC50 (nM)	H3122	H3122LR2 (L1198F)
Crizotinib	124.5	13.6
Lorlatinib	3.57	53.1
Alectinib	6.35	107.5
Ceritinib	2.08	1120

Supplementary Figure S2. Crizotinib potently inhibit L1198F mutated EML4-ALK
(A, D) The H3122 cells and H3122 LR2 cells were treated with the indicated concentrations of ALK inhibitors for 72 h. Cell viability was measured using a CellTiter-Glo assay. Calculated IC50 values are shown in **(D)**. **(B)** The H3122 LR2 cells were treated with the indicated concentrations of each ALK inhibitor for 6 hr. After incubation, the cells were lysed and analyzed by immunoblotting. **(C)** Sanger sequencing of the ALK domain was analyzed using cDNA from the H3122 LR2 cells.

Supplementary Figure S3

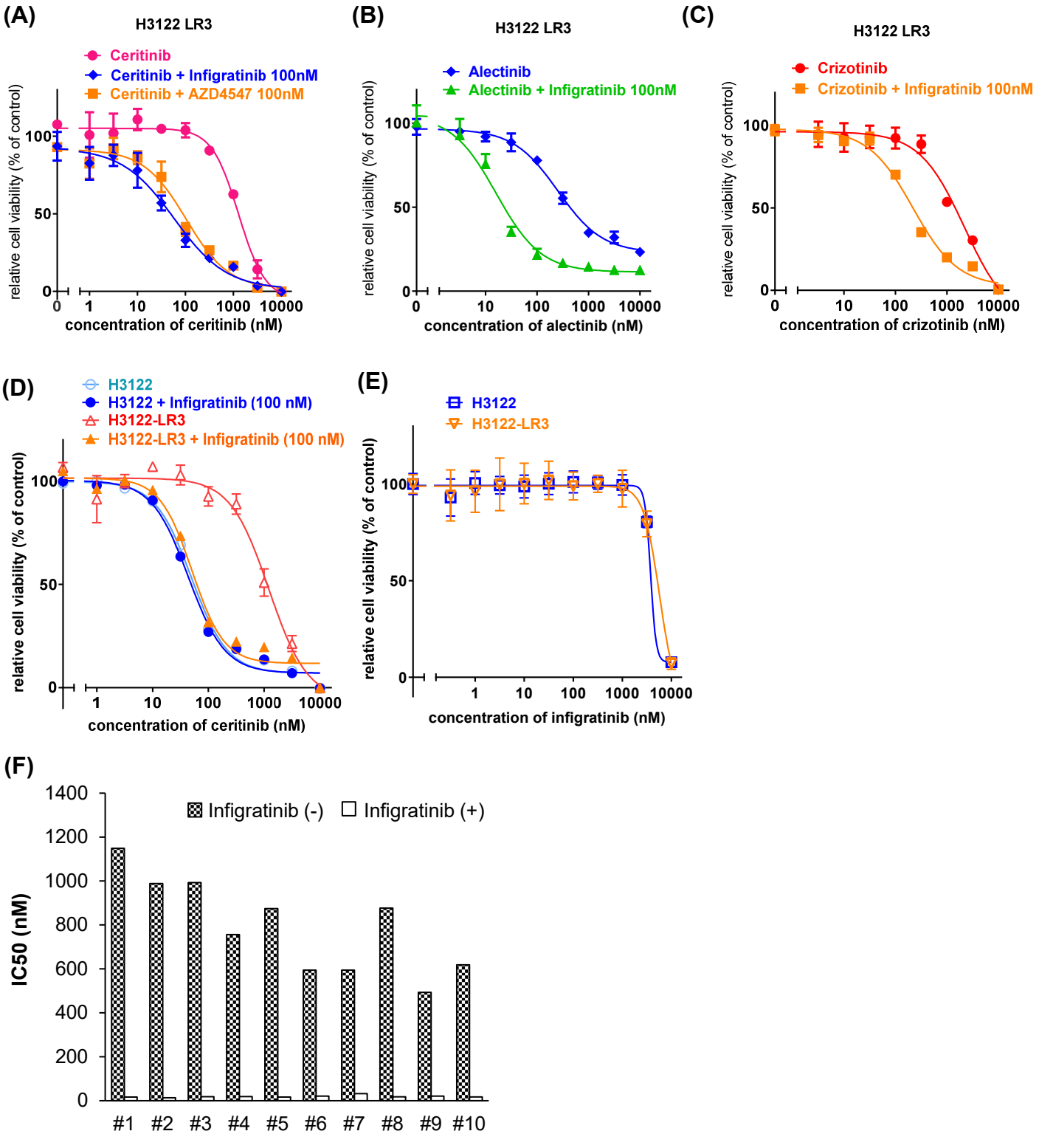


IC50 (nM)	EML4-ALK (WT)	L1198F	fold
Crizotinib	39.9	6.8	0.17
Ceritinib	6.0	361.3	60.2
Alectinib	4.8	33.3	6.9
Lorlatinib	1.21	15.5	12.8
Brigatinib	6.10	24.31	4.0

Supplementary Figure S3. Crizotinib was the most effective for the Ba/F3 expressing EML4-ALK-L1198F

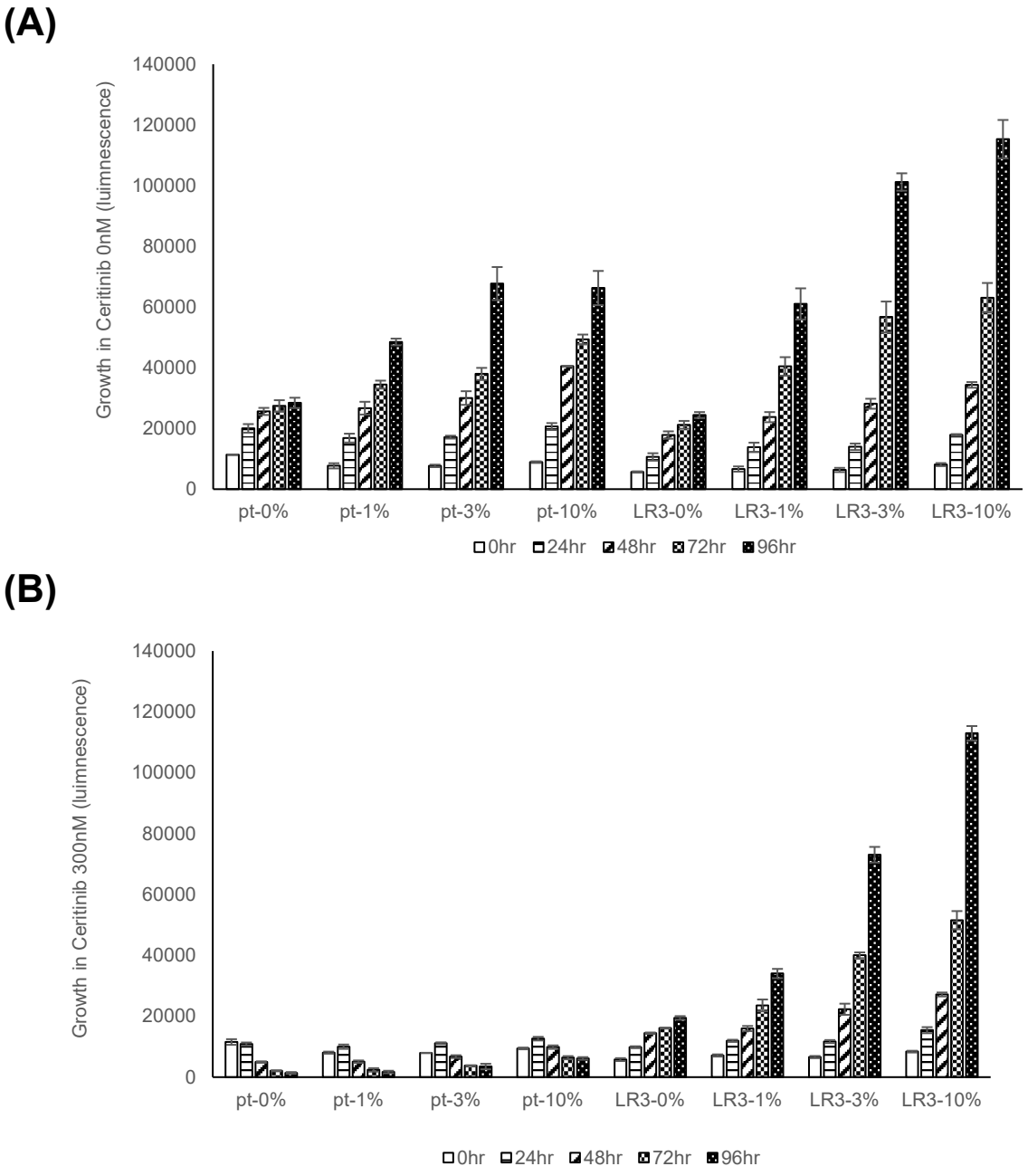
(A, C) The Ba/F3-EML4-ALK-WT and -L1198F cells were treated with the indicated concentrations of each ALK inhibitor for 72 h. Cell viability was measured using a CellTiter-Glo assay. Calculated IC50 values are shown in **(C)**. **(B, D, E)** The Ba/F3 expressing EML4-ALK-WT or -L1198F cells were treated with the indicated concentrations of each ALK inhibitor for 4 hr. After incubation, the cells were lysed and analyzed by immunoblotting.

Supplementary Figure S4



Supplementary Figure S4. FGFR3 activation caused high ceritinib resistance
(A-D) The H3122 or H3122 LR3 cells were treated with the indicated concentrations of ALK inhibitors (crizotinib, ceritinib or alectinib) with or without FGFR inhibitors (AZD4547 or infigratinib) for 72 hr. Cell viability was measured using a Cell-titer Glo assay. **(E)** The H3122 and H3122 LR3 cells were treated with the indicated concentrations of infigratinib for 72 hr. Cell viability was measured using a Cell-titer Glo assay. (F) 10 single clones established from the H3122 LR3 cells were treated with each concentration of ceritinib with or without infigratinib for 72 hr, and each IC50 was calculated.

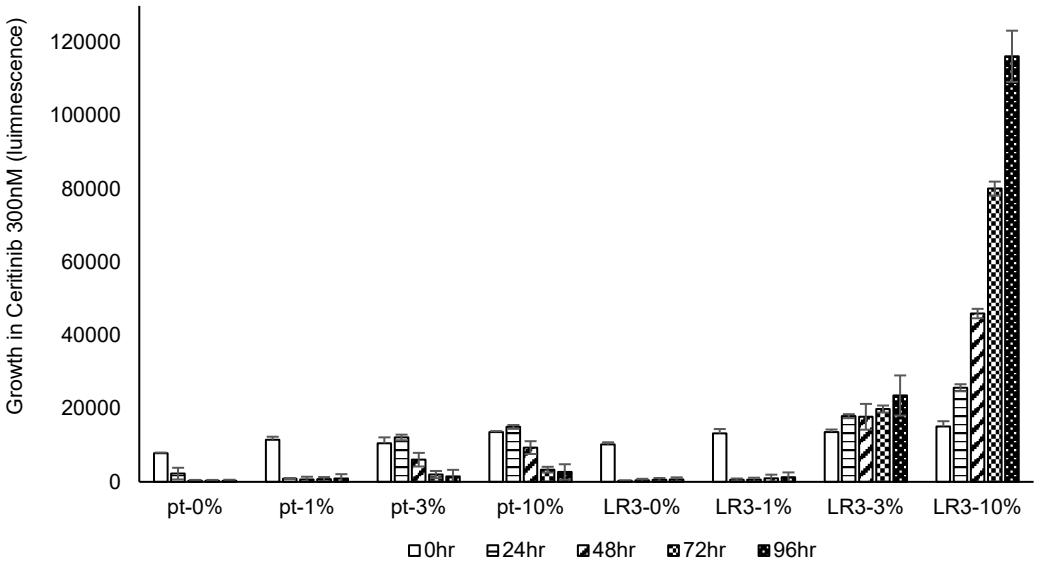
Supplementary Figure S5



Supplementary Figure S5. The induction of ceritinib resistance in the H3122 LR3 cells was dependent on FBS concentrations

The H3122 or H3122 LR3 were plated in RPMI-1640 containing the indicated concentrations of FBS. From the next day, they were cultured without **(A)** or with **(B)** 300 nM ceritinib for 0-96 hr. Cell viability was measured using a Cell-titer Glo assay.

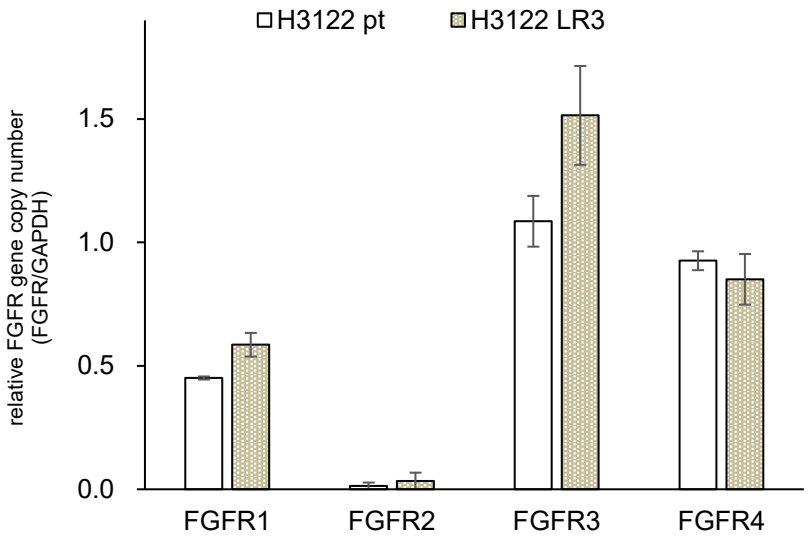
Supplementary Figure S6



Supplementary Figure S6. The H3122 LR3 cells showed ceritinib resistance in the medium containing human serum

The H3122 or H3122 LR3 were plated in RPMI-1640 containing the indicated concentrations of Human AB Serum (Mediatech). From the next day, they were cultured with 300 nM ceritinib for 0-96 hr. Cell viability was measured using a Cell-titer Glo assay.

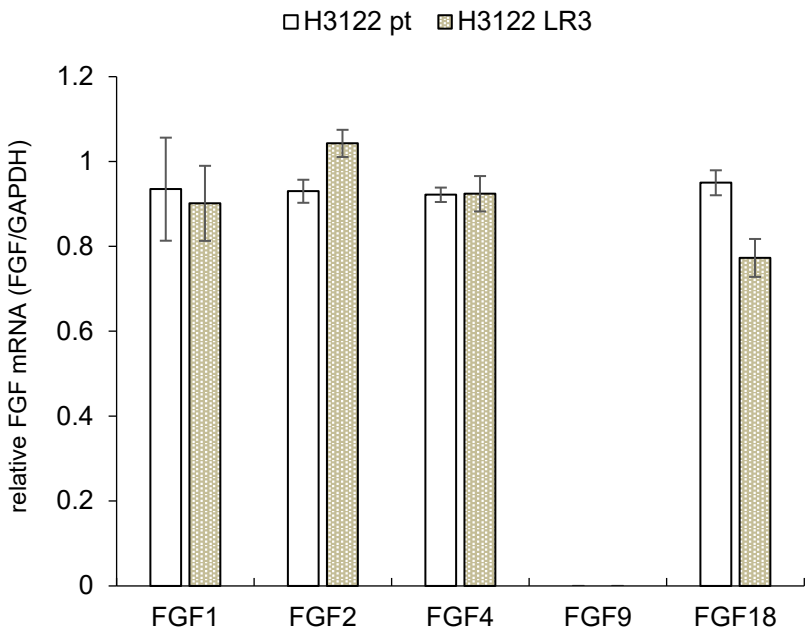
Supplementary Figure S7



Supplementary Figure S7. Gene amplification was not observed in FGFR family genes in the H3122 LR3 cells

Genomic DNA of the H3122 cells and H3122 LR3 cells was extracted and relative FGFR gene copy number was analyzed by qPCR.

Supplementary Figure S8

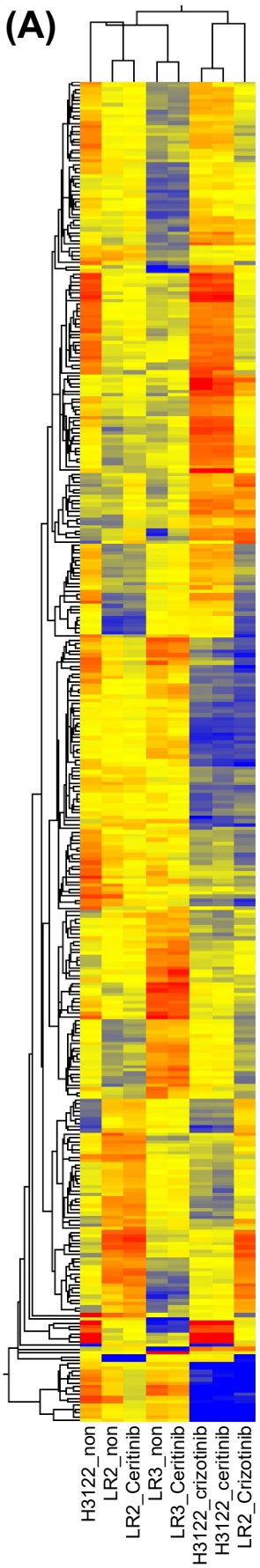


Supplementary Figure S8. No difference of FGFR3 ligand mRNA expression was observed

Quantitative real-time PCR was performed to measure FGFR3 ligands' mRNA levels in H3122 and H3122 LR3 cells. Each relative mRNA expression level (FGFs/GAPDH) is shown in the bar graph.

Supplementary Figure S9

(A)



(B)

Increased gene expression in H3122-LR2 (fold increase to H3122)

Rank	Gene	Ratio (H3122-LR2 /H3122)
1, 5	AKR1C2	7.01, 4.10
2	ITLN1	4.38
3	KCNS3	4.31
4	CALML5	4.16
6	NELL1	4.04

Decreased gene expression in H3122-LR2 (fold increase to H3122)

Rank	Gene	Ratio (H3122/ H3122-LR2)
1, 4	PAEP	9.66, 6.46
2	CADM1	7.56
3	S100A9	6.66
5	THBS1	5.92
6	IGFL1	5.88

(C)

Increased gene expression in H3122-LR3 (fold increase to H3122)

Rank	Gene	Ratio (H3122-LR3 /H3122)
1	FGFR3	6.95
2	AKR1C2	5.78
3, 4	IFI35	4.80, 4.13
5	AKR1C1	4.03
18	ABCG2	2.79

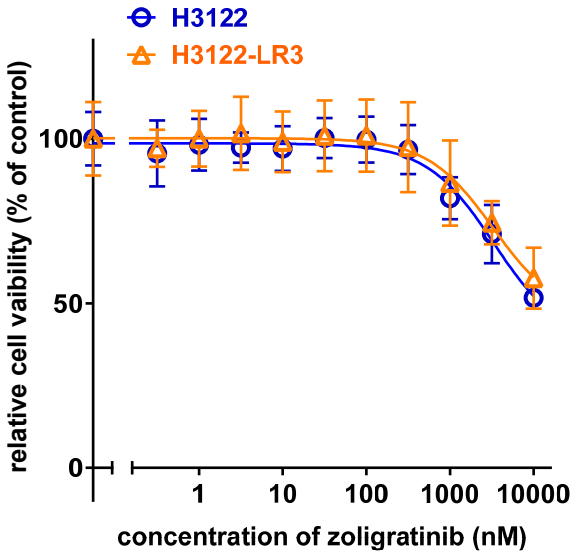
Decreased gene expression in H3122-LR3 (fold increase to H3122)

Rank	Gene	Ratio (H3122/ H3122-LR3)
1	S100A9	30.6
2	BPIFA1	11.1
3	IGFL1	10.5
4	GPC6	10.1
5, 6	CADM1	8.37, 8.29

Supplementary Figure S9. Gene expression analysis of the H3122, H3122-LR2 and H3122-LR3 ceritinib resistant cells by cDNA microarray

(A) Heat map of clustering analysis (B, C) TOP 5 list of upregulated or downregulated in the H3122-LR2 (B) or H3122-LR3 (C) cells.

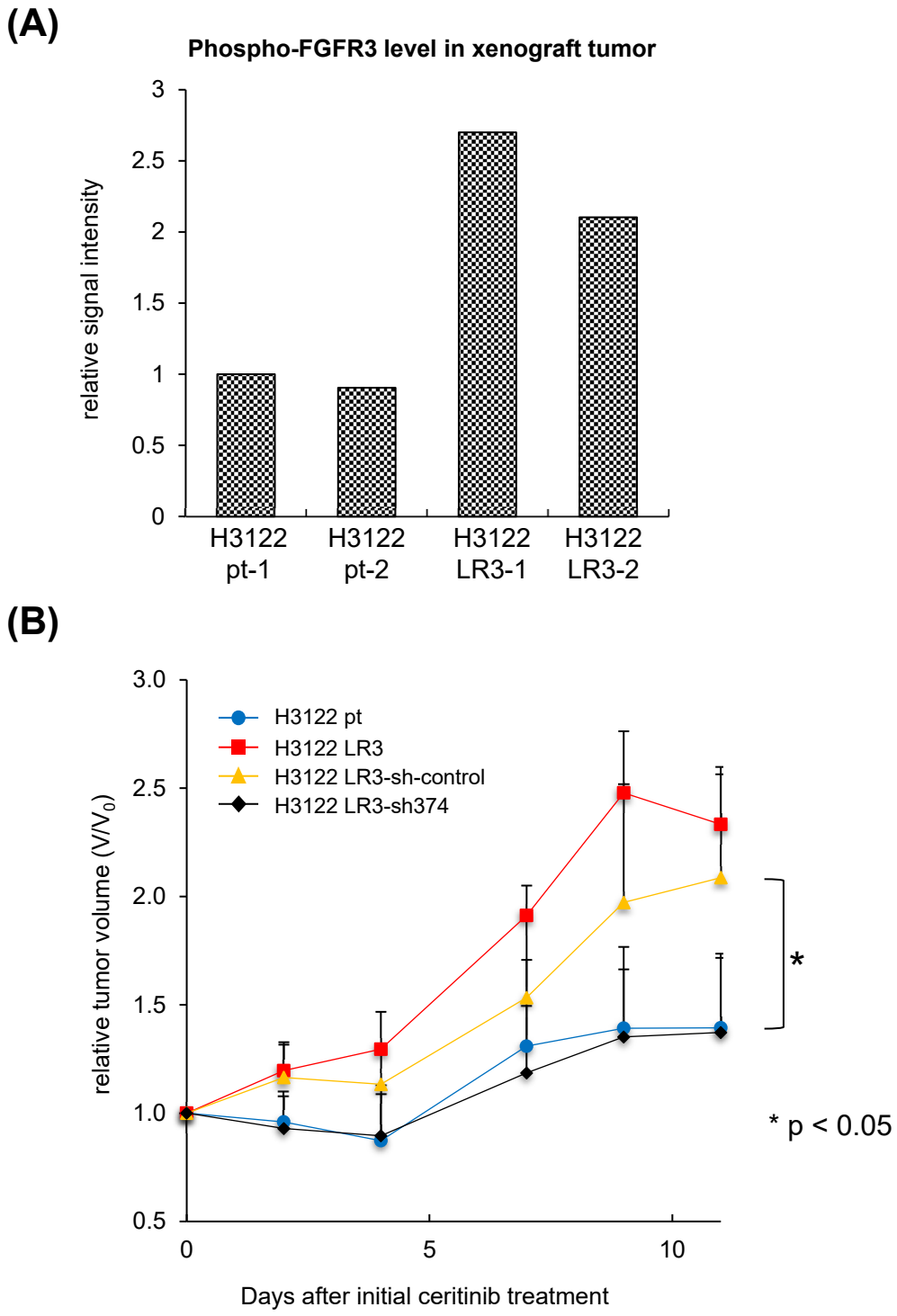
Supplementary Figure S10



Supplementary Figure S10. Zoligratinib didn't affect the growth of the H3122 LR3 cells

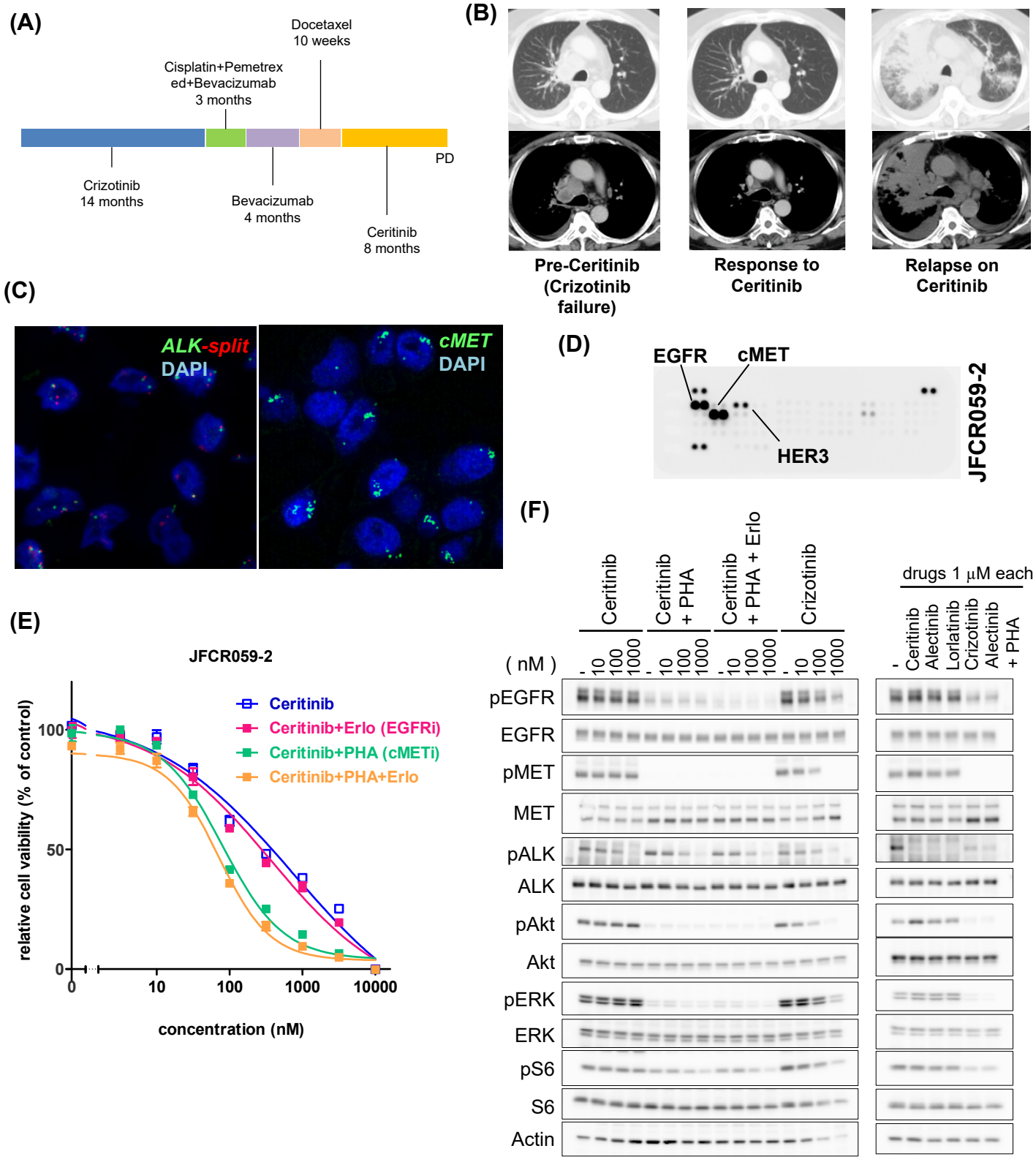
The H3122 LR3 cells were treated with the indicated concentrations of zoligratinib for 72 hr. Cell viability was measured using a Cell-titer Glo assay.

Supplementary Figure S11



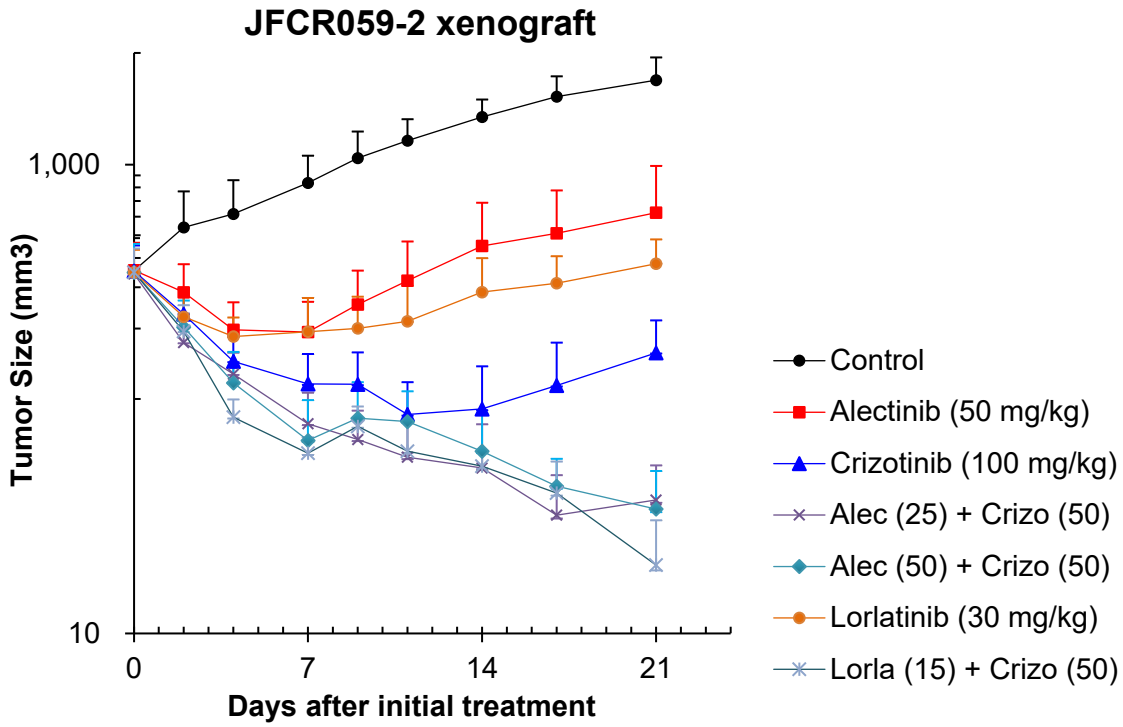
Supplementary Figure S11. Ceritinib treatment was effective to an shFGFR3-induced H3122 LR3 tumor
(A) Tumor xenografts were lysed and analyzed by phosphor-RTK array. Average signal intensity of phospho-FGFR3 divided by positive control signal was standardized by the value from the H3122 parental cell's xenograft.
(B) Indicated cell lines were subcutaneously injected into nude mice. After the tumors reached sizes of approximately 100-150mm^3, ceritinib treatment (50 mg/kg) was initiated and given daily for 12 days.

Supplementary Figure S12



Supplementary Figure S12. Bypass-pathway activation mediated by cMET amplification induced ceritinib resistance in patient JFCR-059
(**A**) Treatment history of JFCR-059. (**B**) Axial computed tomography scan images of the patient's chest showing the tumor burden before ceritinib treatment (left), after responding to ceritinib treatment (center), and at the time of disease progression while on ceritinib (right). (**C**) In-house break-apart FISH analysis with a 5′-anaplastic lymphoma kinase (ALK) (red), a 3′-ALK (green) (left), and a 3′-cMET (green) probe (right) of the FFPE specimen of JFCR-059-2. ALK rearrangement (left) and cMET amplification (right) were identified. (**D**) The phosphorylation levels of 49 RTKs in the JFCR-059-2 cells were measured using an RTK array. (**E**) The JFCR-059-2 cells were treated with varying concentrations of ceritinib with or without erlotinib (1 μM) and PHA665752 (1 μM) for 72 h. (**F**) The JFCR-059-2 cells were treated with the indicated concentration of ALK-TKIs (ceritinib, alectinib, lorlatinib, or crizotinib) with or without PHA665752 (1 μM) and/or erlotinib (1 μM) for 6 h. After incubation, the cells were lysed and analyzed by immunoblotting.

Supplementary Figure S13



Supplementary Figure S13. Half doses of alectinib or lorlatinib with crizotinib can overcome cMET amplification mediated resistance.

Ceritinib resistant patient derived JFCR-059-2 cell lines were subcutaneously injected into nude mice. After the tumors reached sizes of approximately over 100 mm^3, indicated doses of ALK inhibitors treatment (crizotinib, alectinib or lorlatinib) was initiated and given daily for 21 days (5 days drug treatment and 2 days off schedule).

Supplementary Table S1

Patient ID	ALK-TKI treatment			Ceritinib resistance mechanism
JFCR-021	Crizotinib (5 month)	Ceritinib (0.7 month)	Alectinib (1 month)	F1174V / G1202R mutation in ALK (after Alectinib, only G1202R mutation observed)
JFCR-013	Ceritinib (6 month)	Crizotinib (1 month)		P-glycoprotein overexpression
JFCR-025	Alectinib (15 month)	Crizotinib (1 month)	Ceritinib (1.5 month)	P-glycoprotein overexpression (L1196M in ALK)
JFCR-041	Alectinib (13 month)	Ceritinib (12 month)		G1202R mutation in ALK
JFCR-059	Crizotinib (15 month)	Ceritinib (8 month)		cMET gene amplification
JFCR-060	Ceritinib (3 month)	Alectinib		mechanisms unidentified (no mutation in ALK)
JFCR-072	Alectinib (13 month)	Ceritinib (12 month)		mechanisms unidentified (no mutation in ALK)
JFCR-093	Alectinib	Ceritinib		mechanism un identified (no mutation in ALK)
MGH011	Crizotinib	Ceritinib		S1206Y mutation (after crizotinib) and G1202R mutation (after ceritinib) in ALK
MGH023	Crizotinib	Ceritinib		F1174C mutation in ALK
MGH051	Crizotinib	Ceritinib		G1202R mutation in ALK
MGH015	Crizotinib	Ceritinib		cKIT amplification (after crizotinib) and P-glycoprotein overexpression

Supplementary Table S1. Ceritinib resistance mechanisms in clinically developed ceritinib-resistant specimens.

Table shows the summarized ceritinib resistance mechanisms among 12 cases we have been published in the previous paper (Friboulet L et al, Cancer Discov 2014, Katayama R et al, EBioMedicine 2016, Yanagitani N et al, Cancer Science 2020, Shimizu Y et al, npj Precis Oncol 2022). Treatment history and potential resistance mechanisms were shown in the table.