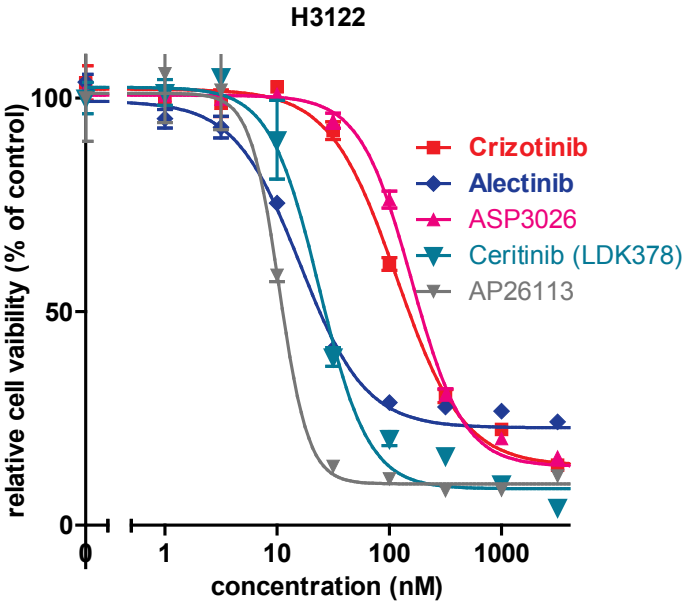
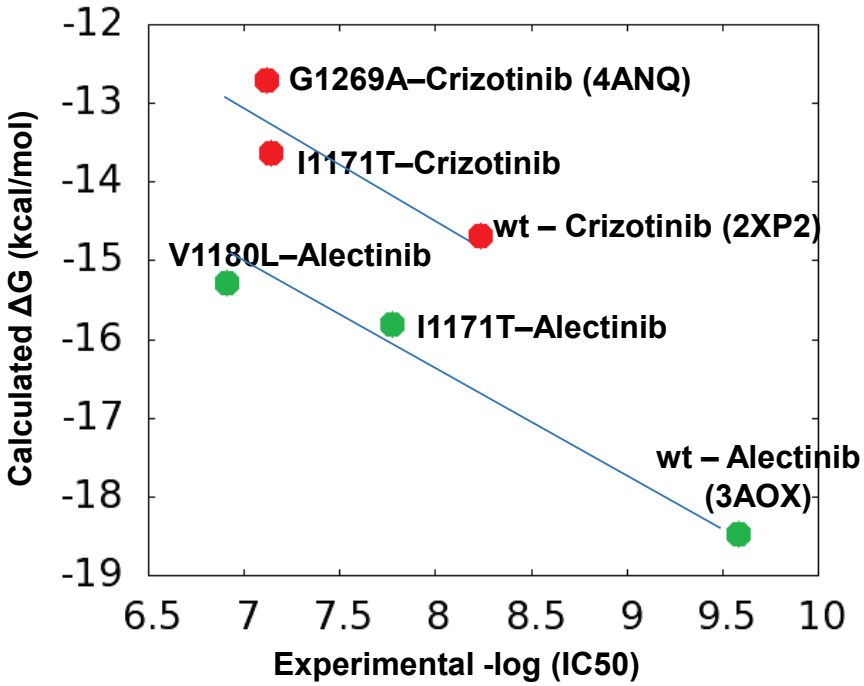
Supplementary Figure S1



Supplementary Figure S1: EML4-ALK harboring H3122 cells are sensitive to 2nd generation ALK inhibitors.
Cells were seeded in 96-well black plates and treated with increasing concentration of crizotinib, alectinib, ASP3026, ceritinib (LDK378), and AP26113 for 72 hr. Cell survival was analyzed using the CellTiter-Glo assay.

Supplementary Figure S2



Supplementary Figure S2: Comparison of experimental IC50 values and calculated free energy values.

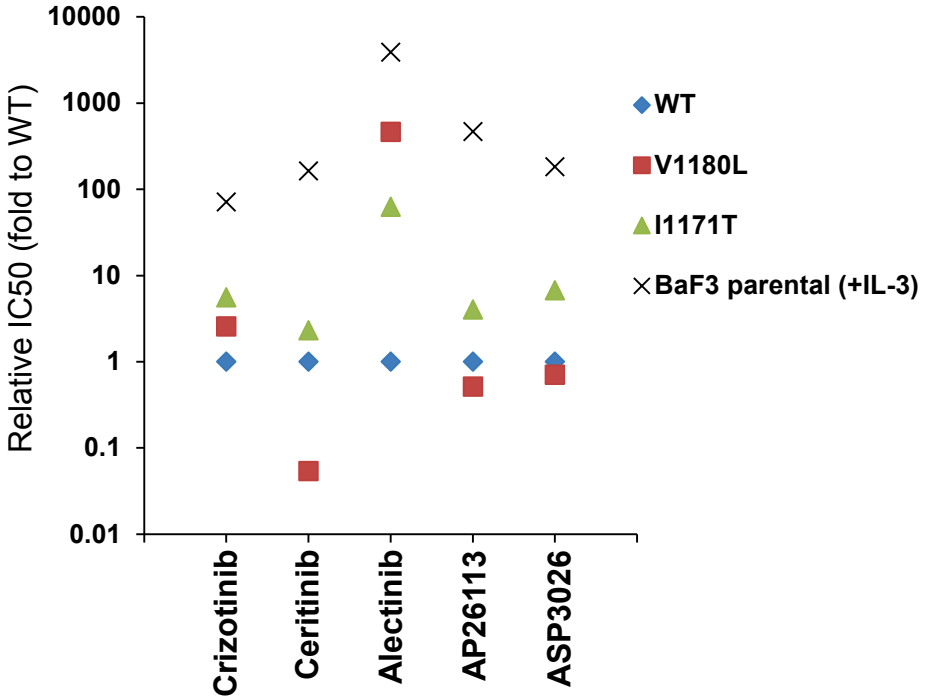
The IC_{50} value of WT or each resistant mutation harboring Ba/F3 cells to crizotinib or alectinib (CH5424802), and the corresponding calculated free energy values of WT or each resistant mutation with alectinib or crizotinib were plotted on the graph. The approximation straight line of each inhibitor was depicted on the graph.

Supplementary Figure S3

A

IC50 (nM) (fold > WT)	EML4-ALK WT	V1180L	I1171T	BaF3 parental (+ IL-3)
Crizotinib	6.05 (1)	15.5 (2.6)	33.7 (5.6)	429.0 (71)
Ceritinib (LDK378)	2.58 (1)	0.14 (0.054)	5.94 (2.3)	420.8 (163)
Alectinib	0.27 (1)	123.0 (463)	16.6 (62.5)	1027 (3870)
AP26113	0.95 (1)	0.49 (0.51)	3.82 (4.0)	441.7 (466)
ASP3026	27.31 (1)	19.3 (0.71)	184.1 (6.7)	4955 (181)

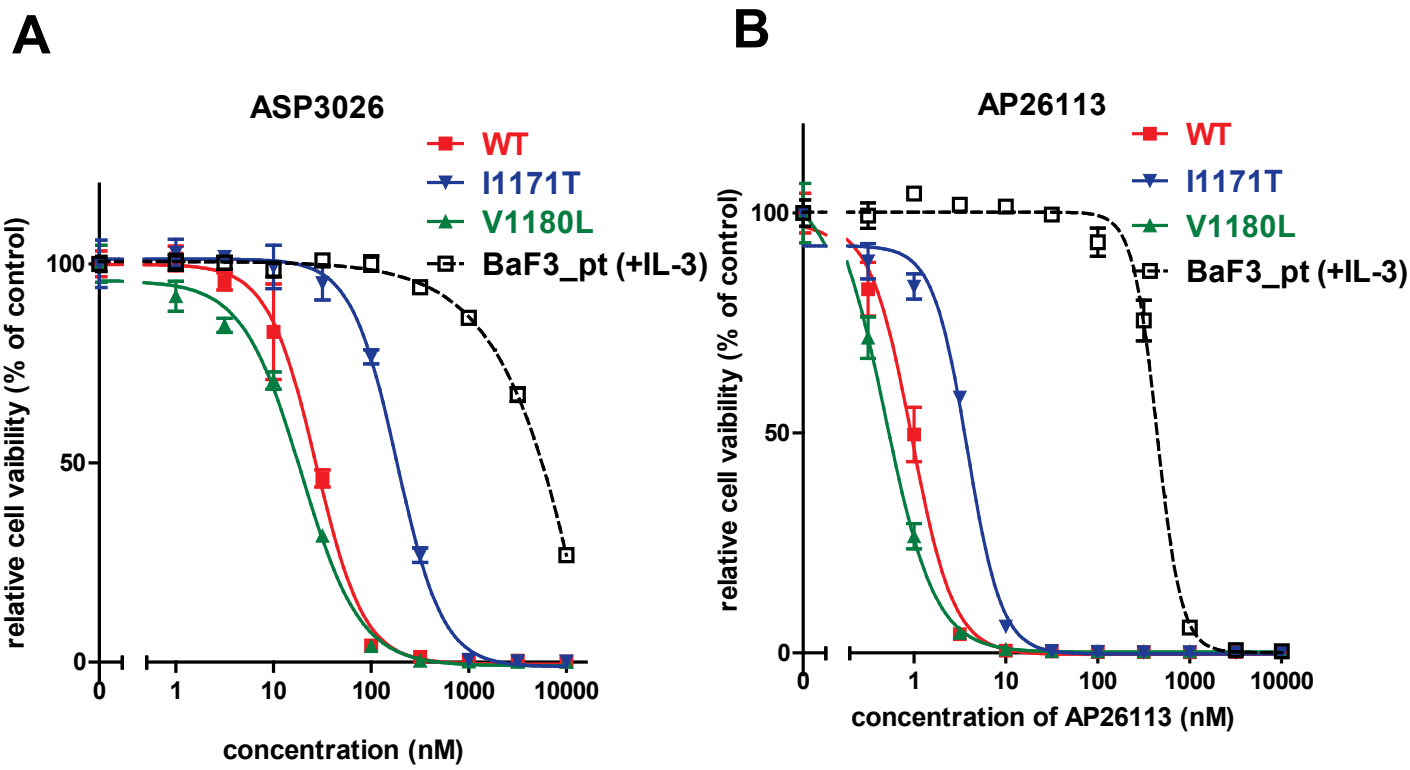
B



Supplementary Figure. S3. Sensitivity of secondary resistance mutations within the ALK TK domain to various ALK inhibitors.

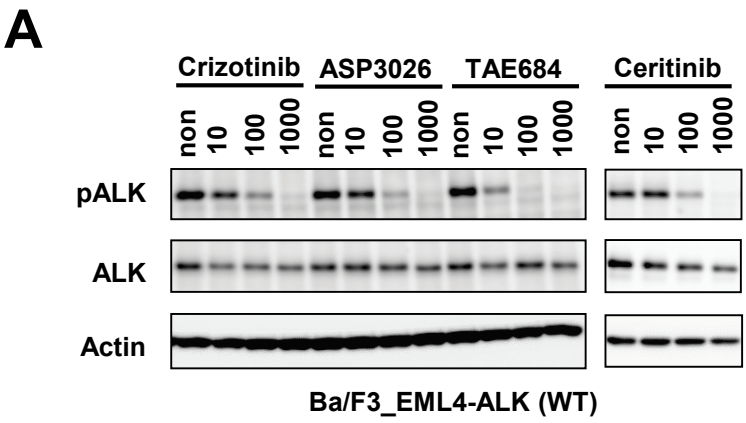
(A) The average IC50s of each drug across 4 different Ba/F3 cell lines, including parental, IL-3 dependent Ba/F3 cells as well as transformed Ba/F3 cells expressing the indicated EML4-ALK constructs (WT, V1180L or I1171T). For each drug tested, the number in parentheses represents the ratio of the IC50 value shown relative to the IC50 in wild-type EML4-ALK-expressing Ba/F3 cells. (B) The relative IC50 of each drug across 4 different Ba/F3 cell lines is shown.

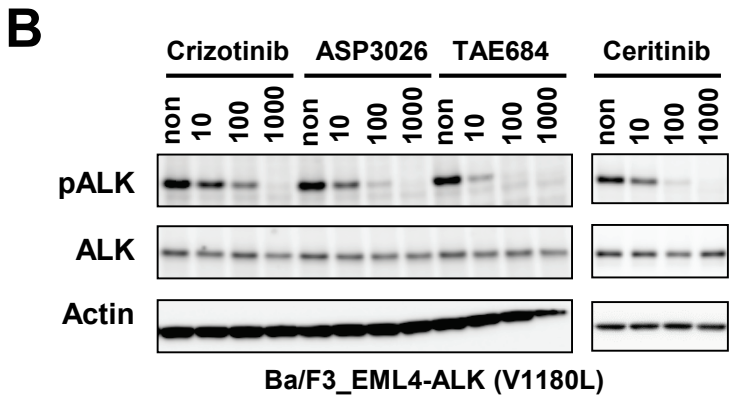
Supplementary Figure S4

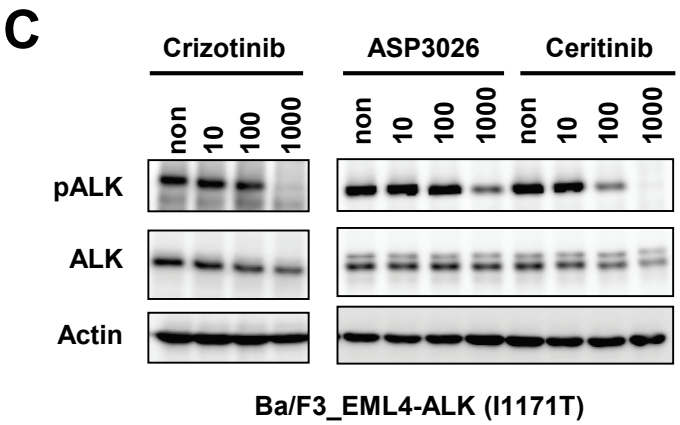


Supplementary Figure S4: Sensitivity of wt and alectinib-resistant mutant EML4-ALK expressing Ba/F3 cells to ASP3026 or AP26113.
(A, B) WT, I1171T or V1180L mutated EML4-ALK expressing Ba/F3 cells were seeded in 96 well-black plates and treated with increasing concentration of ASP3026 (A) or AP26113 (B) for 72 hr. Cell survival was analyzed using the CellTiter-Glo assay.

Supplementary Figure S5

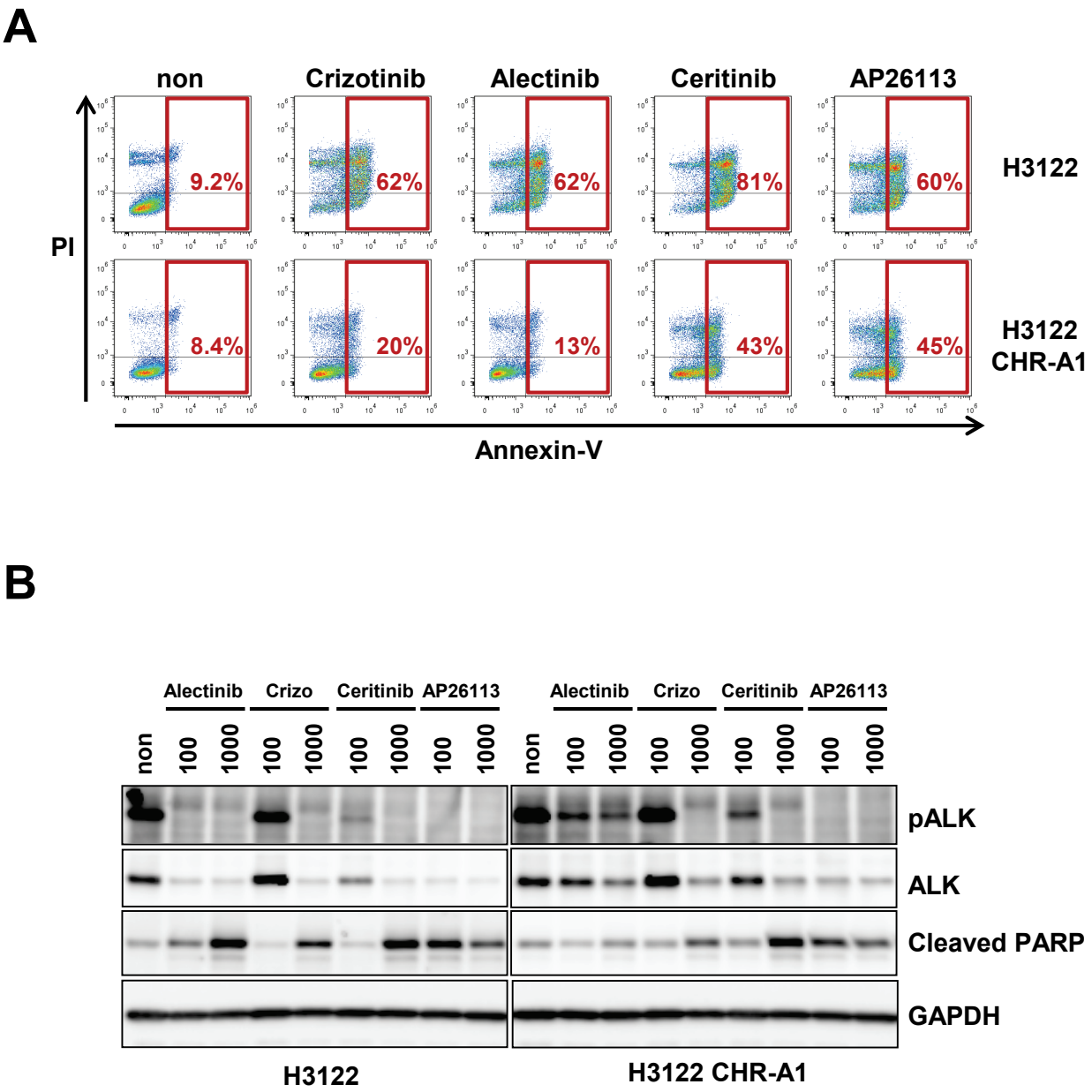






Supplementary Figure S5: Inhibition of phospho-ALK by the second generation ALK inhibitors in wt or mutant EML4-ALK expressing BaF3 cells.
(A-C) WT or mutated EML4-ALK overexpressing BaF3 cells were exposed to increasing concentrations of crizotinib, ASP3026, ceritinib (LDK378) or TAE684 for 2 hr. Cell lysates were immunoblotted to detect the indicated proteins.

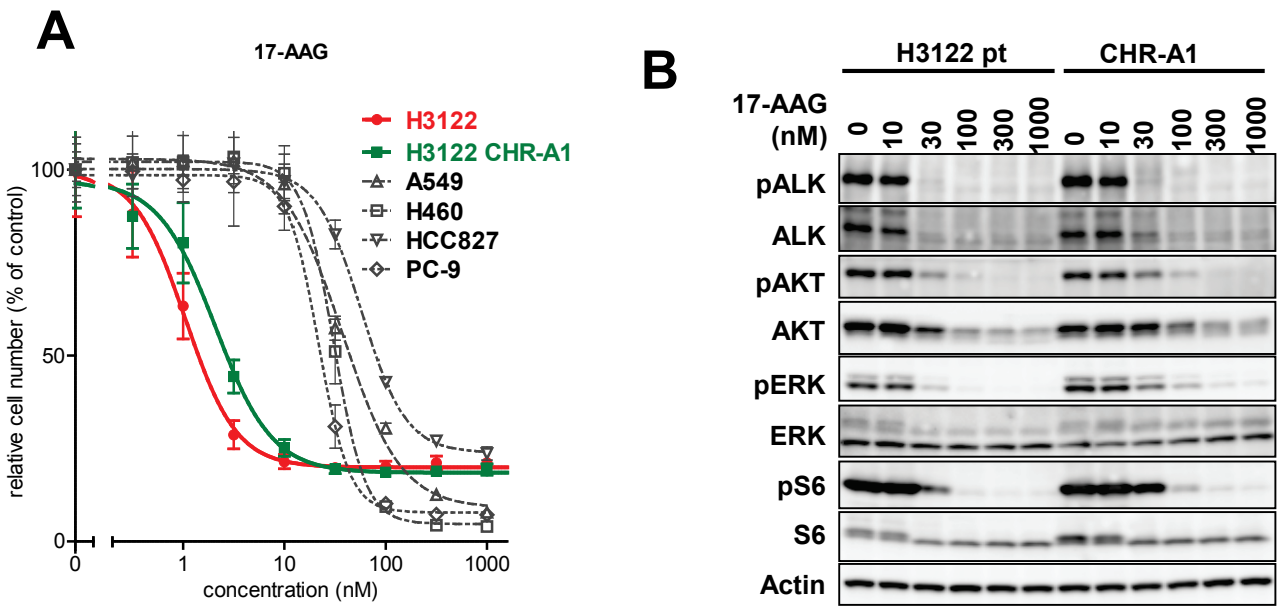
Supplementary Figure S6



Supplementary Figure. S6. Ceritinib and AP26113, but not crizotinib, induce apoptosis to alectinib-resistant H3122CHR-A1 cells

(A) H3122 parental and resistant (CHR-A1) cells were treated with the indicated concentrations of crizotinib (Crizo), alectinib, ceritinib (LDK378), or AP26113. After 72 hr, cells were stained with Alexa-633 labeled Annexin-V and PI, and analyzed by flow cytometry. The percent of cell undergoing apoptosis is shown in red. (B) Cells were treated as in (A) for 24 hrs. Cell lysates were immunoblotted with an anti-PARP antibody to detect PARP cleavage.

Supplementary Figure S7



Supplementary Figure. S7. The Hsp90 inhibitor 17-AAG can overcome alectinib resistance in H3122 CHR-A1 cells

(A) Cell lines were seeded in 96 well plates and treated with increasing concentration of Hsp90 inhibitor, 17-AAG for 72 hr. Cell viability was measured with the CellTiter-Glo assay kit. Both parental H3122 cells (red line) and H3122 CHR-A1 cells (green line) were sensitive to 17-AAG than non-ALK rearranged cell lines (A549, H460, HCC827 and PC-9 cells). (B) Induction of protein degradation of EML4-ALK fusion protein and suppression of ALK signaling by 17-AAG in both parental and alectinib resistant H3122 cells. H3122 parental and alectinib-resistant H3122 CHR-A1 cells were exposed to increasing concentrations of 17-AAG for 24 hr. Cell lysates were immunoblotted to detect the indicated proteins.