**Supplementary Materials and Methods**

**Short Tandem Repeat Polymerase Chain Reaction Analysis**

The identities of all parental and resistant cells were confirmed by short tandem repeat polymerase chain reaction analysis using the Cell ID System (Promega) according to the manufacturer’s instructions.

**Sequencing of the ALK Kinase Domain**

cDNA was synthesized, and the exons within the ALK kinase domain were amplified by PCR. PCR products were processed using the BigDye Terminator Cycle sequencing kit (Applied Biosystems) according to the manufacturer’s protocol and analyzed in both the sense and antisense directions for the presence of mutations on the ABI 3100 sequencer (Applied Biosystems). PCR primers and conditions are available upon request.

**Supplementary Figure Legends**

**Supplementary Figure 1**

**Establishing a method for the alectinib-resistant cell lines and morphological observations**

A, Schema of the method for establishing the alectinib-resistant cell lines.

Parental cells were cultured with increasing concentrations of alectinib, starting with the IC50, to establish the alectinib-resistant lines. Doses were increased in a stepwise pattern when normal cell proliferation resumed. Fresh drug was added every 96 h. Resistant cells began to grow in 1 μM alectinib after 5 months of culture with the drug. Each clone was derived using a single-cell cloning method.

B and C, Microscopic images. Microscopic images were recorded 6 days after cell seeding (1 × 106 per flask). Scale bars, 100 μm.

**Supplementary Figure 2**

**Identification by STR analysis**

A, H2228 and H2228/CHR. B, ABC-11 and ABC-11/CHR.

**Supplementary Figure 3**

**Characteristics of the H2228 and H2228/CHR clones**

A, Alectinib and crizotinib sensitivity assays performed on the H2228 and H2228/CHR clones. Cells were seeded and treated with various concentrations of alectinib or crizotinib. Viable cells were assessed by MTT assay. Anti-proliferative effects are shown as IC50 values. Data are mean ± SE values from three independent experiments. B, Effects of alectinib on phosphorylated ALK levels and downstream signaling in H2228/CHR clones. Cells were incubated with alectinib (0 or 1 μM) for 4 h. Lysates were analyzed by Western blotting.

**Supplementary Figure 4**

**Characteristics of the ABC-11/CHR and ABC-11/CHR clones**

A, Alectinib and crizotinib sensitivity assays performed on the ABC-11 and ABC-11/CHR clones. Cells were seeded and treated with various concentrations of alectinib or crizotinib. Viable cells were assessed by MTT assay. Anti-proliferative effects are shown as IC50 values. Data are mean ± SE values from three independent experiments. B, Effects of alectinib on phosphorylated ALK levels and downstream signaling in the ABC-11/CHR clone. Cells were incubated with alectinib (0 or 1 μM) for 4 h. Lysates were analyzed by Western blotting.

**Supplementary Figure 5**

***ALK* kinase domain sequencing**

(A) ABC-11, (B) ABC-11/CHR1, (C) ABC-11/CHR2, (D) ABC-11/CHR3

**Supplementary Figure 6**

**External HGF and conditioned medium leads to alectinib resistance in the *EML4-ALK*-positive cell line.**

A, ABC-11 cells (2 × 105/well) were seeded on 6-well plates and cultured for 24 h. Thereafter, the culture medium was replaced with fresh medium, conditioned medium, or fresh medium containing 50 ng/mL HGF and cultured with 0 or 0.1 M alectinib for 72 h. Viable cells were trypsinized and counted. B, H2228 cells (2 × 105/well) were tested in the same manner as the ABC-11 cells. Data are expressed as the percentage of the control (without alectinib) and are represented as mean ± SE values from three independent experiments.

**Supplementary Figure 7**

**Characteristics of ABC-11/CHR-r**

A, HGF concentrations in the culture medium. Cells (ABC-11 and ABC-11/CHR-r clones) (1 × 105/well)were seeded in 6-well plates and incubated for 48 h in 2 mL culture medium. The culture supernatants were cleared by centrifugation, and the supernatants from viable cells were assessed by ELISA. Data represent the mean ± SE values from three independent experiments. The values for resistant cells were compared with those for parental cells by using the two-tailed paired Student’s *t-*test (\*\*\**P* < 0.001). B, Alectinib and crizotinib sensitivity assays. Cells were seeded in 96-well plates and treated with various concentrations of alectinib or crizotinib. Viable cells were assessed by MTT assay. Anti-proliferative effects are shown as the IC50. Data are mean ± SE from three independent experiments.

**Supplementary Figure 8**

**Long-term effects of crizotinib on tumor growth in xenograft models**

The ABC-11/CHR tumors were treated with 50 mg/kg/day crizotinib for 53 days. The vehicle group was treated for 32 days. Tumor volume are presented as mean + SE.