Figure S1

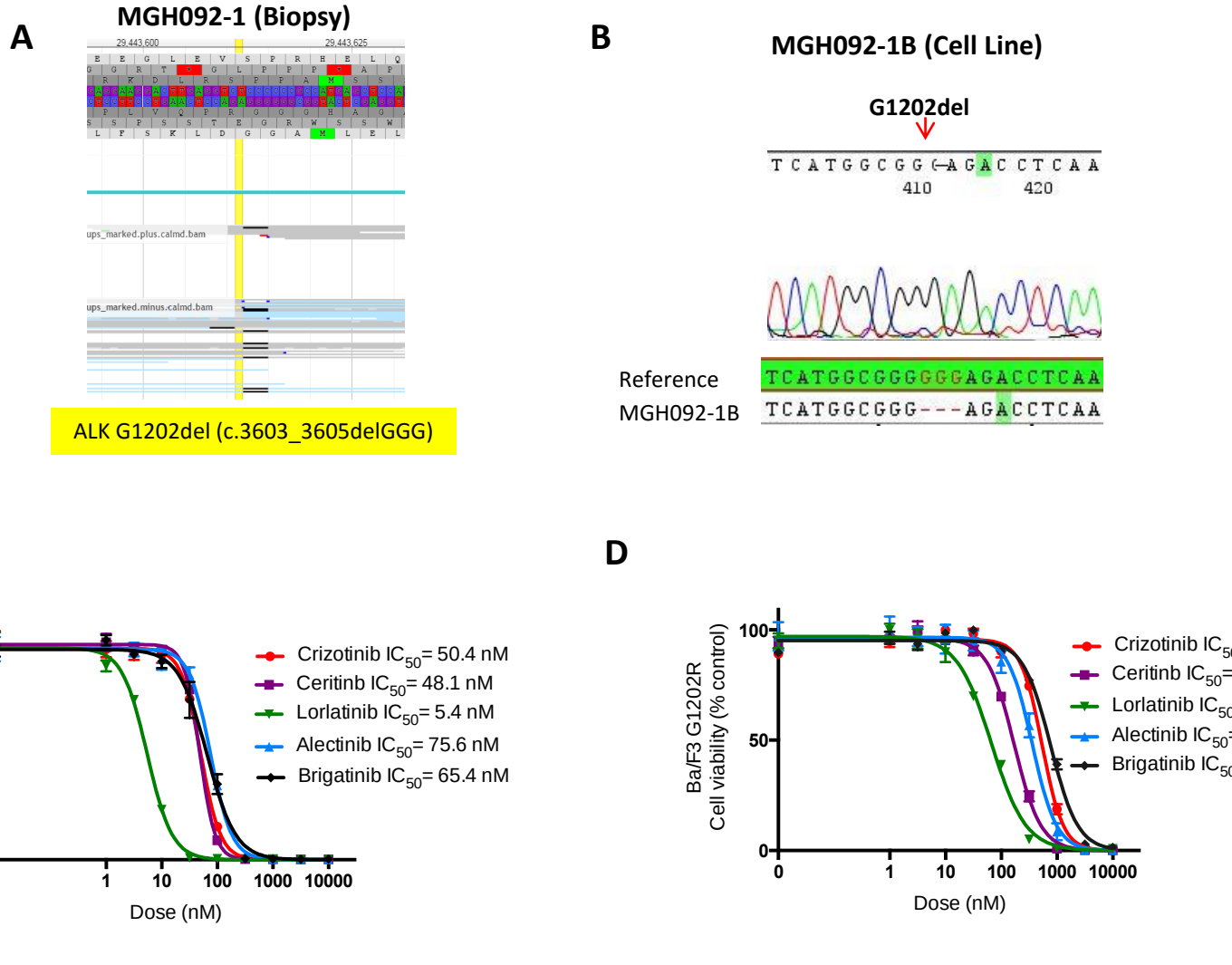


Fig. S1: *ALK* G1202del is a novel resistance mutation in ALK-rearranged lung cancer: A. Pile-up of the ALK G1202del identified by next-generation sequencing in a lung biopsy specimen from a patient progressing on ceritinib. **B.** Confirmation of an *ALK* G1202del mutation in a corresponding patient-derived cell line (MGH092-1B) using Sanger sequencing of complementary DNA. **C-D.** Cell viability of Ba/F3 cells harboring *EML4-ALK* G1202del and G1202R, respectively, treated with first-, second- and third-generation ALK inhibitors.

Figure S2

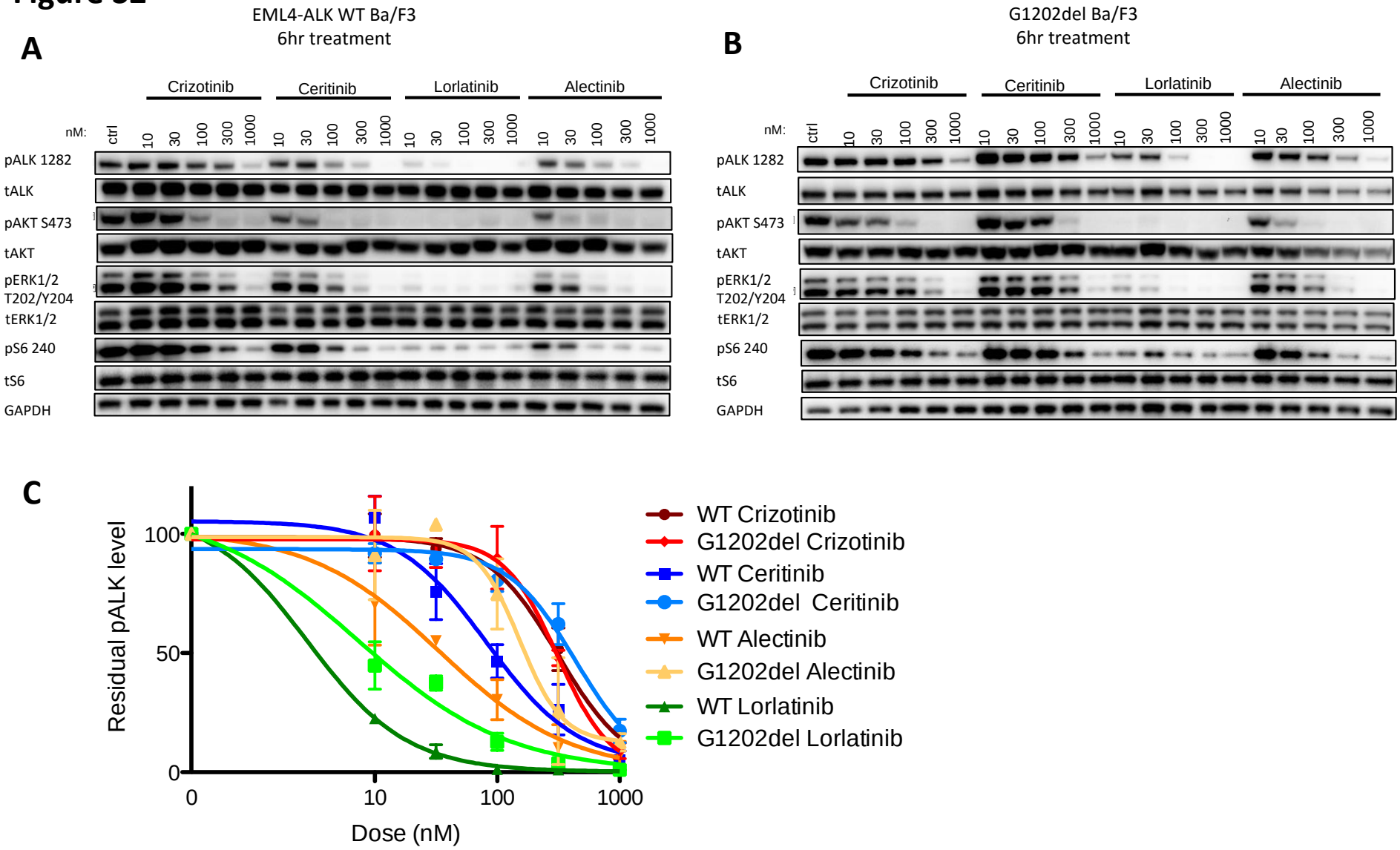


Fig. S2: *ALK* G1202del in ALK-rearranged lung cancer: A-B. Ba/F3 cells transformed by wildtype or G1202del *EML4-ALK*, respectively, were treated with the indicated concentrations of crizotinib, ceritinib, alectinib and lorlatinib for 6 hours. Lysates were probed with antibodies directed against the specified proteins. **C.** Dose response western blot curves show ALK phosphorylation levels in *EML4-ALK* WT and G1202del Ba/F3 cells treated with indicated doses of crizotinib, ceritinib, alectinib and lorlatinib for 6 hours. Error bars indicate SEMs of three independent experiments.

Figure S3

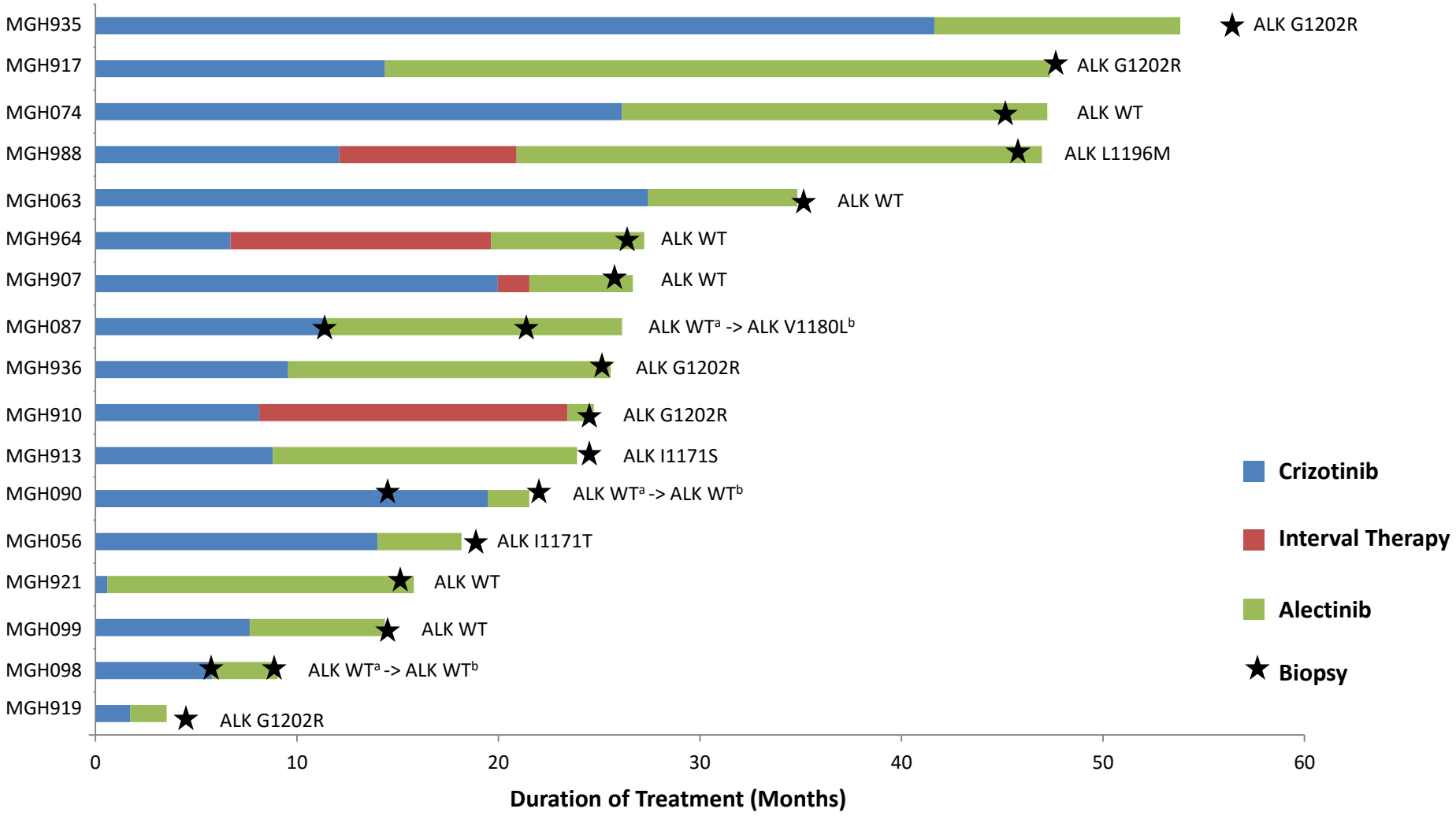


Fig. S3: Swimmer's plots depicting ALK inhibitor treatment courses and biopsy time-points for patients with alectinib-resistant biopsies. Therapies received prior to crizotinib are not depicted. Intervening therapies were received by four patients and included ceritinib (MGH907, MGH910, and MGH964) and pemetrexed/crizotinib (MGH988). ALK WT refers to absence of an ALK resistance mutation. Among patients with two separate biopsies, (a) indicates findings in a post-crizotinib biopsy and (b) indicates findings in a post-alectinib biopsy.

Figure S4

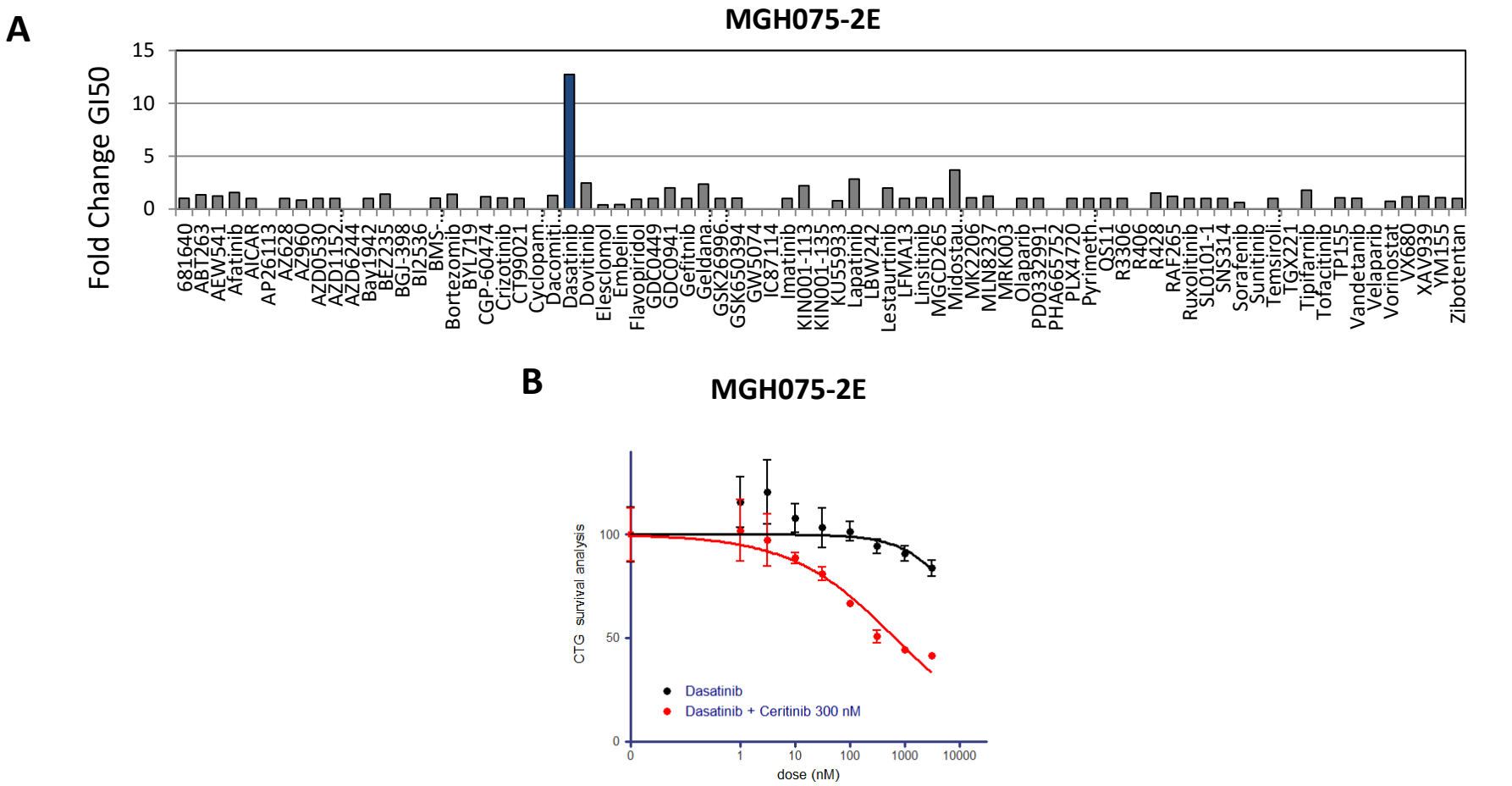


Fig. S4: Evaluation of MGH075-2E using a combination drug screen. A. The combination of dasatinib and ceritinib was observed as the only hit in a combination drug screen in the patient-derived cell line MGH075-2E. **B.** Dasatinib, a multi-kinase inhibitor that targets SRC, was more potent in combination with 300 nM ceritinib (red) than as a single agent (black).

Figure S5

MGH067-1
Ceritinib-Resistant, Soft Tissue Biopsy

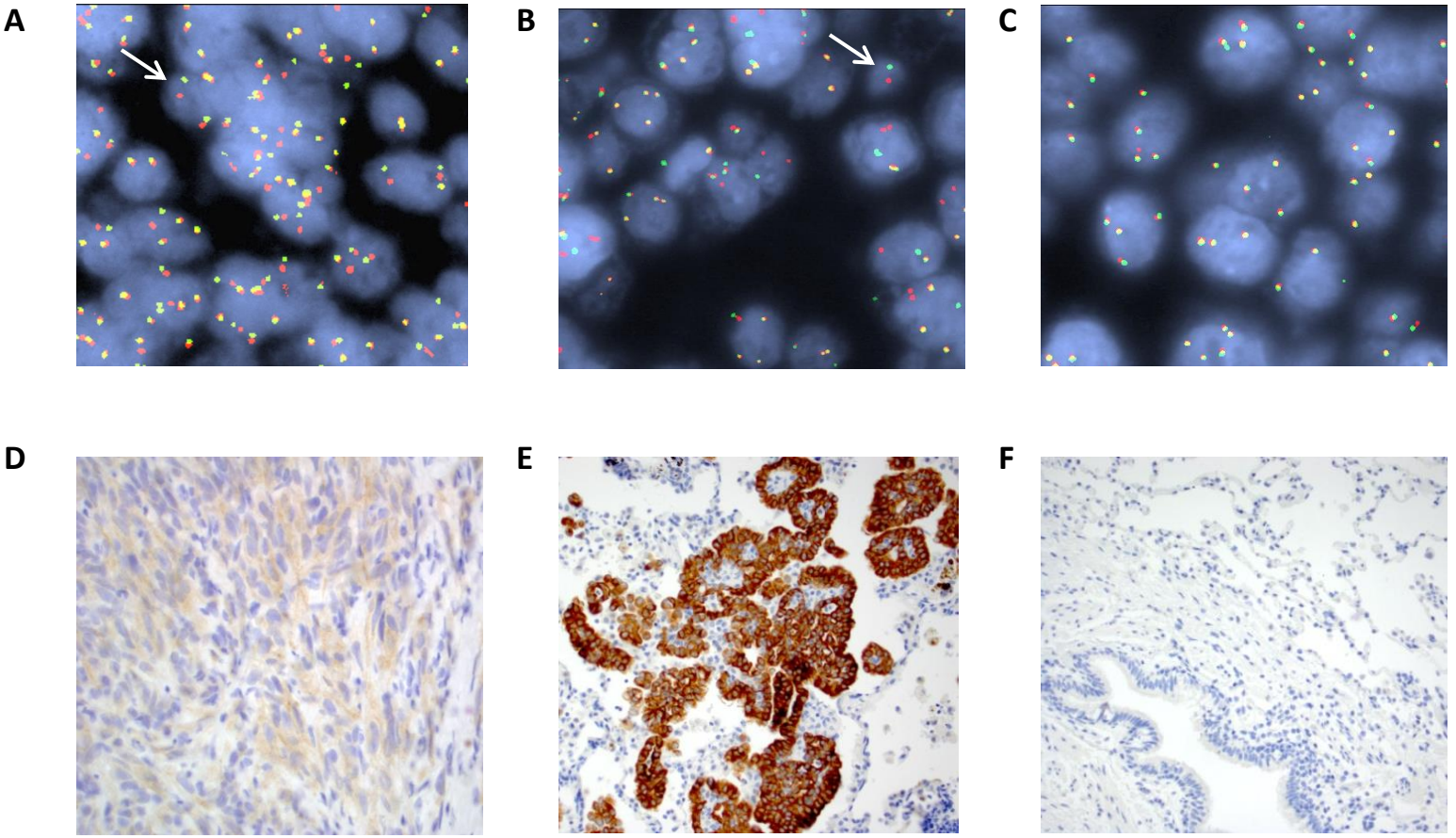


Fig. S5: MGH067-1 harbors an *ALK* rearrangement. A. MGH067-1 was obtained from a soft tissue lesion of a patient progressing on ceritinib. The standard, break-apart ALK fluorescence in situ hybridization (FISH) assay demonstrated split signals (white arrow) in 42% of cells, consistent with an *ALK* rearrangement. **B.** A positive control consisting of an independently validated lung tumor confirmed by FISH to harbor an ALK rearrangement. **C.** A negative control consisting of an independently confirmed FISH negative lung cancer. **D.** Immunohistochemical staining for ALK revealed mild membranous staining in MGH067-1. **E.** Positive control consisting of an independently confirmed ALK-positive lung cancer assessed by immunohistochemistry. **F.** Negative control of an independently confirmed ALK-negative lung cancer based upon immunohistochemistry.

Figure S6

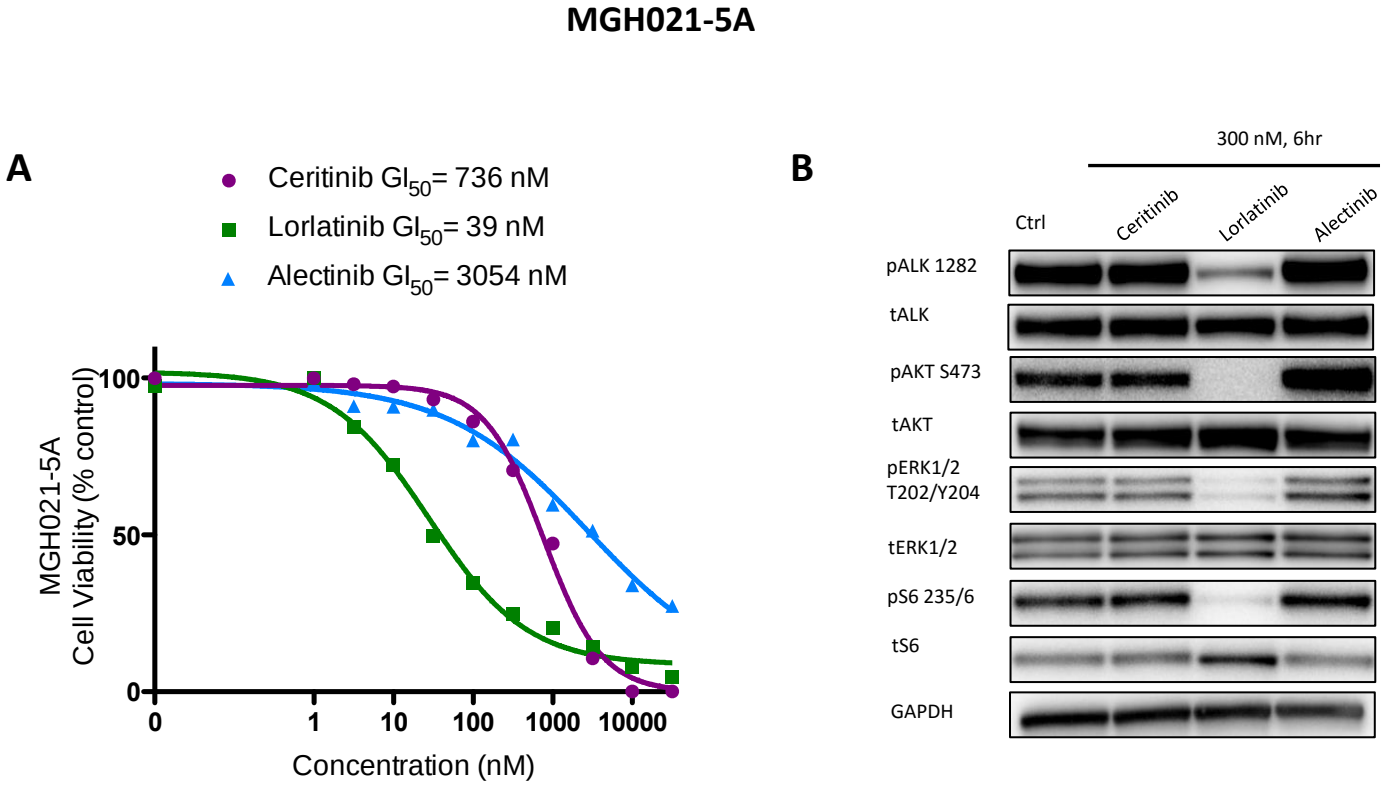
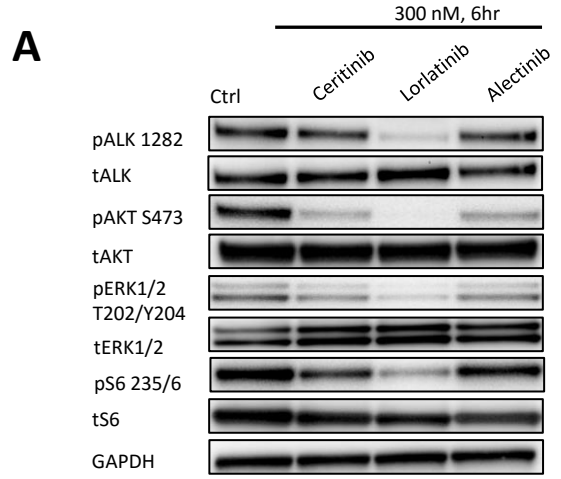
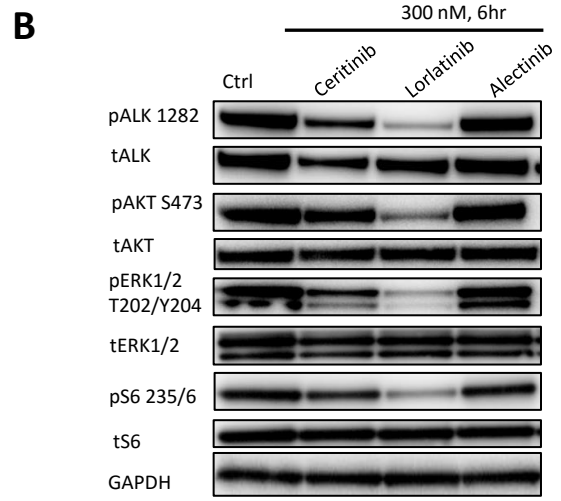
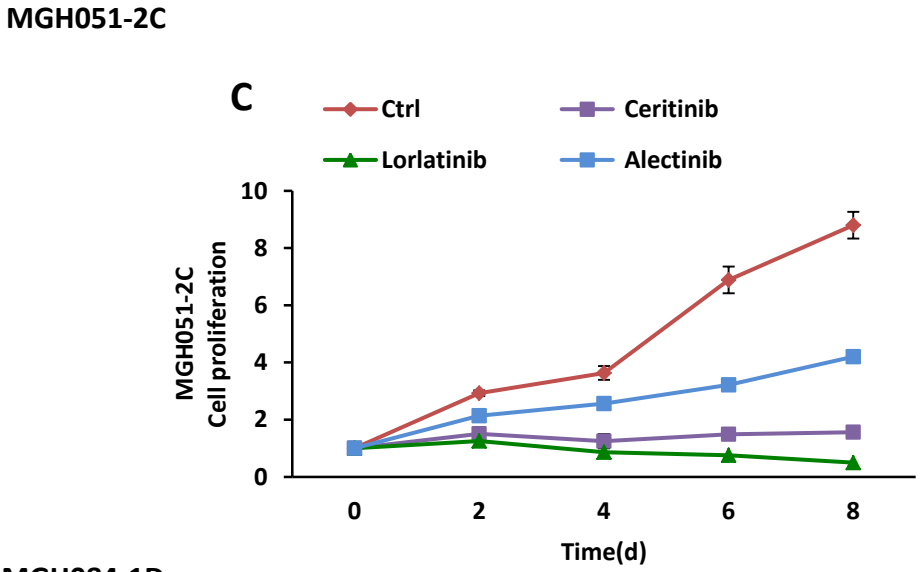


Fig. S6: *ALK* resistance mutations predict for sensitivity to lorlatinib in patient-derived cell line models of acquired resistance to ceritinib. A. Cell viability assays of the ceritinib-resistant, patient-derived cell line MGH021-5A (*SQATM1-ALK*^{G1202R}) treated with ceritinib, alectinib and lorlatinib. The number of cells seeded and the duration of treatment were adjusted in order to have a consistent proliferation index (3.5 to 5) at the end of treatment. Values are presented as means (N=3). **B.** Western blot analysis of MGH021-5A. Cells were treated with vehicle, ceritinib (300 nM), lorlatinib (300 nM), or alectinib (300 nM) for 6 hours. Lysates were analyzed with antibodies to the indicated proteins.

Figure S7







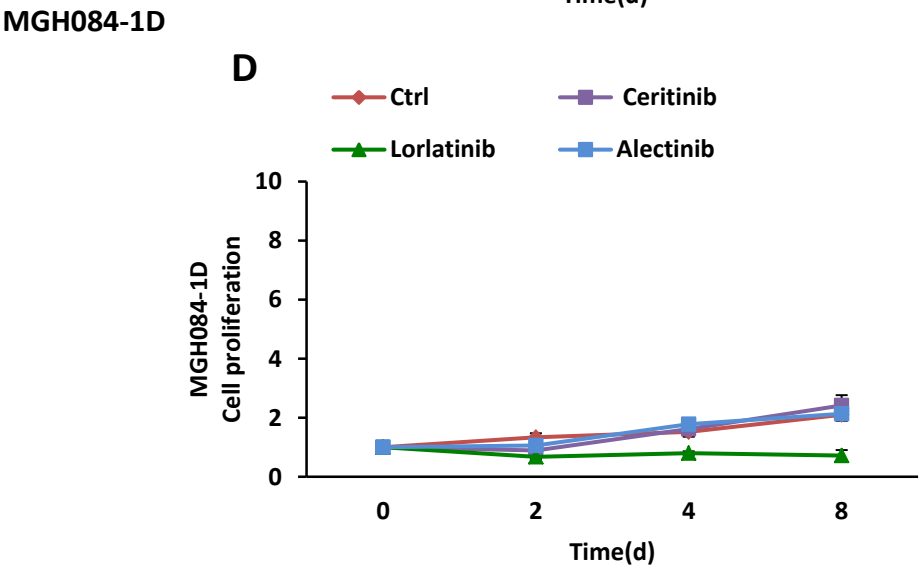


Fig. S7: *ALK* resistance mutations predict for sensitivity to lorlatinib in patient-derived cell line models of acquired resistance to ceritinib. A-B . Western blot analysis of MGH051-2C (*EML4-ALK*^{G1202R}) and MGH084-1D (*EML4-ALK*^{I1171N,C1156Y}), respectively. Cells were treated with vehicle, ceritinib (300 nM), lorlatinib (300 nM), or alectinib (300 nM) for 6 hours. Lysates were analyzed with antibodies to the indicated proteins. **C-D.** Cellular proliferation of MGH051-2C and MGH084-1D cells, respectively, treated with control, ceritinib, alectinib or lorlatinib. The number of cells seeded and the duration of treatment were adjusted for each cell line in order to have a consistent proliferation index (3.5 to 5) at the end of treatment.

Figure S8

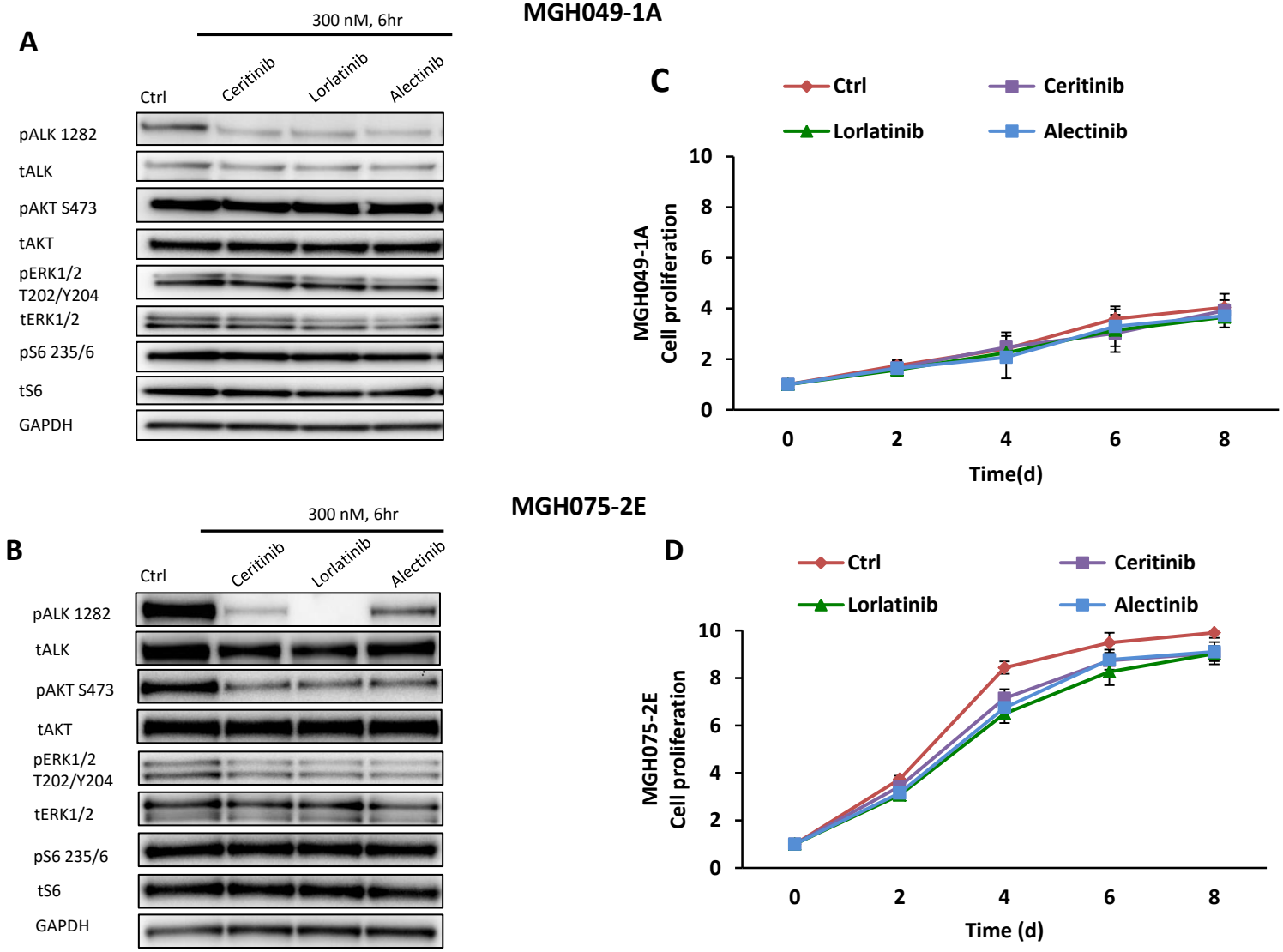


Fig. S8: Patient-derived cell line models of acquired resistance to ceritinib that lack ALK resistance mutations are insensitive to lorlatinib. A-B . Western blot analysis of MGH049-1A (*EML4-ALK*^{WT}) and MGH075-2E (*EML4-ALK*^{WT}), respectively. Cells were treated with vehicle, ceritinib (300 nM), lorlatinib (300 nM), or alectinib (300 nM) for 6 hours. Lysates were analyzed with antibodies to the indicated proteins. **C-D.** Cellular proliferation of MGH049-1A and MGH075-2E cells treated with control, ceritinib, alectinib or lorlatinib. The number of cells seeded and the duration of treatment were adjusted for each cell line in order to have a consistent proliferation index (3.5 to 5) at the end of treatment.

Figure S9

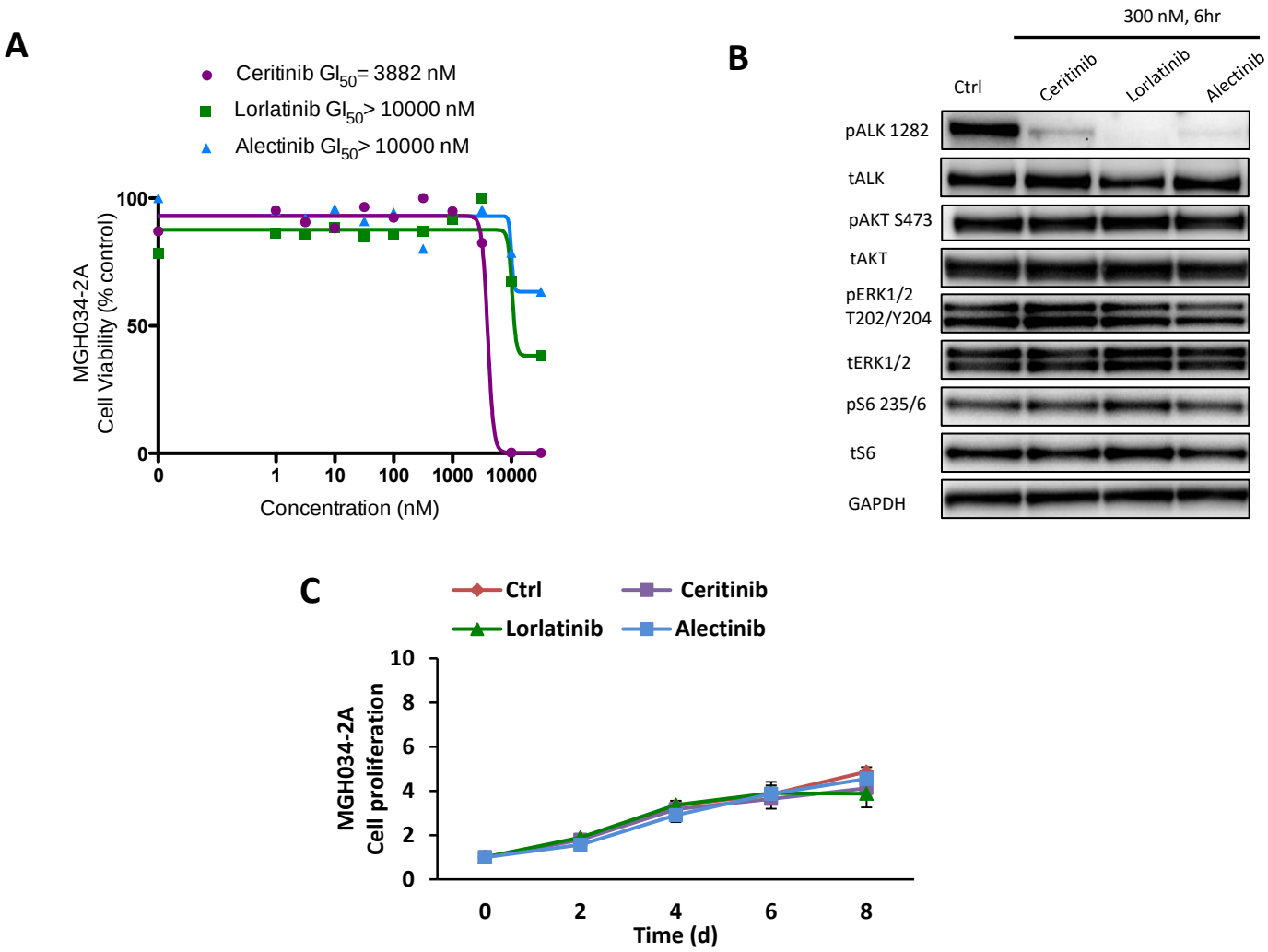


Fig. S9: Patient-derived cell line models of acquired resistance to ceritinib that lack *ALK* resistance mutations are insensitive to lorlatinib. A. Cell viability assays of the ceritinib-resistant, patient-derived cell line MGH034-2A (*EML4-ALK*^{WT}) treated with ceritinib, alectinib and lorlatinib. Values are presented as means (N=3). **B.** Western blot analysis of MGH034-2A. Cells were treated with vehicle, ceritinib (300 nM), lorlatinib (300 nM), or alectinib (300 nM) for 6 hours. Lysates were analyzed with antibodies to the indicated proteins. **C.** Cellular proliferation of MGH034-2A cells treated with control, ceritinib, alectinib or lorlatinib. The number of cells seeded and the duration of treatment were adjusted in order to have a consistent proliferation index (3.5 to 5) at the end of treatment.