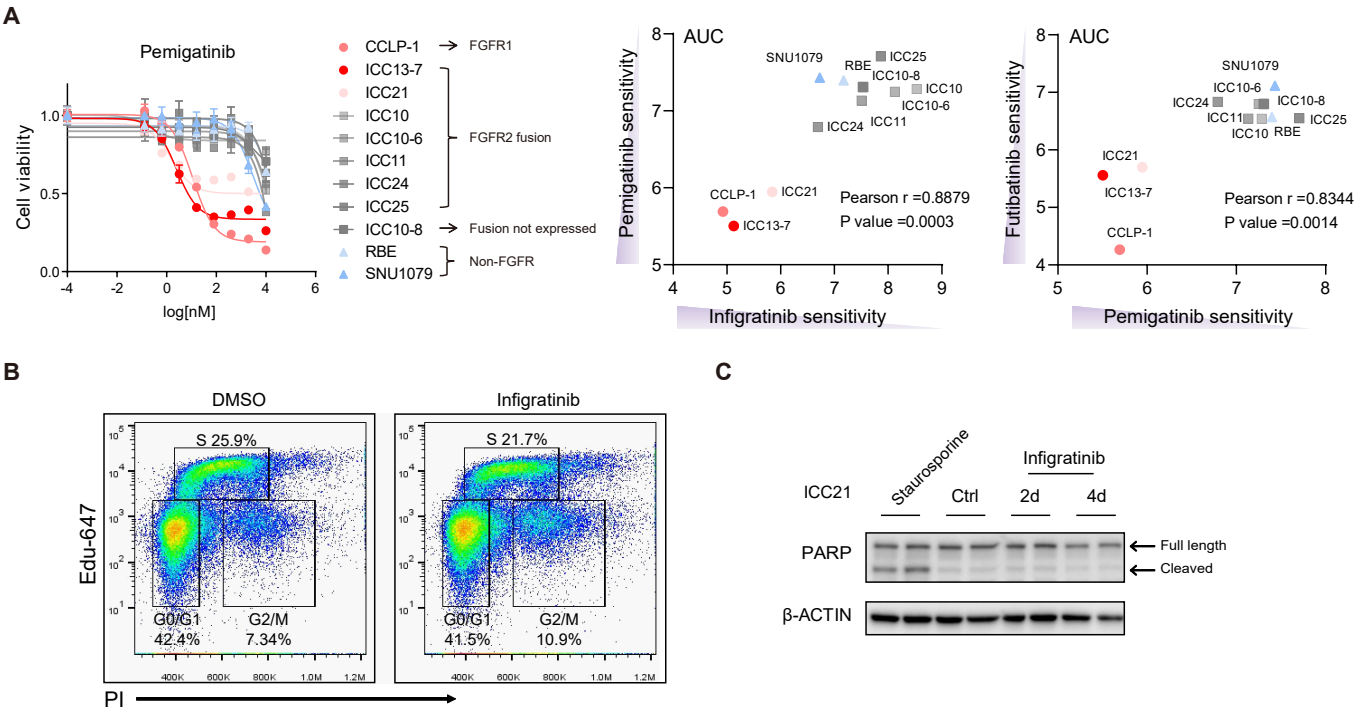
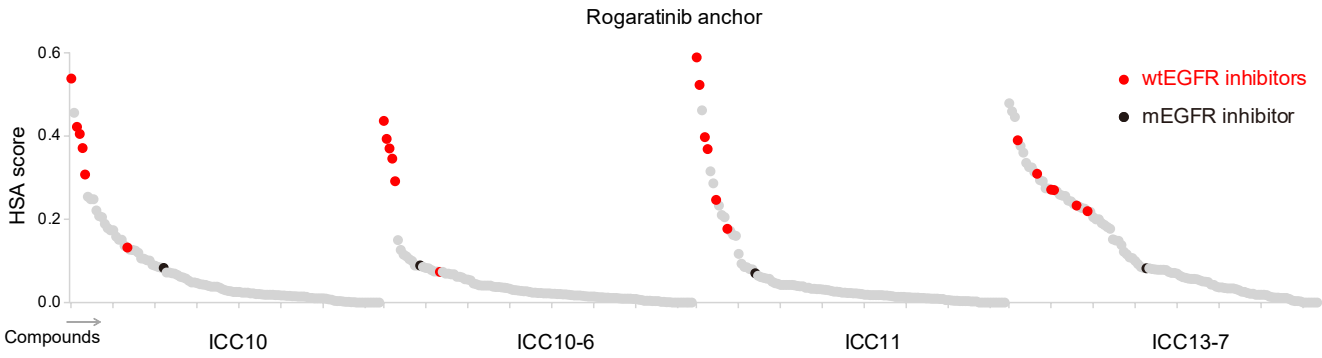
Supplementary Figure 1.



Supplementary Figure 1. Characterization of models derived from patients with FGFR2-fusion+ ICC. A. IC50 assay evaluating sensitivity of a series of ICC cell lines to pemigatinib. Each point on the dose response curves represents 2 technical replicates. The graphs show correlation between pemigatinib and infigratinib sensitivity (middle) and between pemigatinib and futibatinib sensitivity (right). Data are shown as Mean ± SD. AUC denotes area under curve calculated by GraphPad Prism 9. **B**. Cell cycle distributions in ICC13-7 cells under DMSO or 100 nM infigratinib treatment for 24 hours were detected by EdU incorporation and PI staining and analyzed by flow cytometry. **C**. Immunoblot analysis of PARP cleavage in ICC21 cells upon treatment with 100 nM infigratinib or vehicle for the indicated times. Staurosporine (1000 nM) served as the positive control.

Supplementary Figure 2.

A



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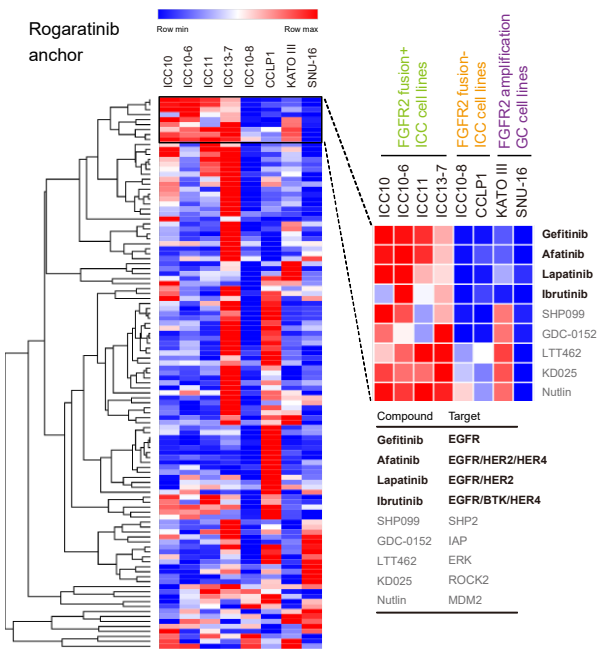
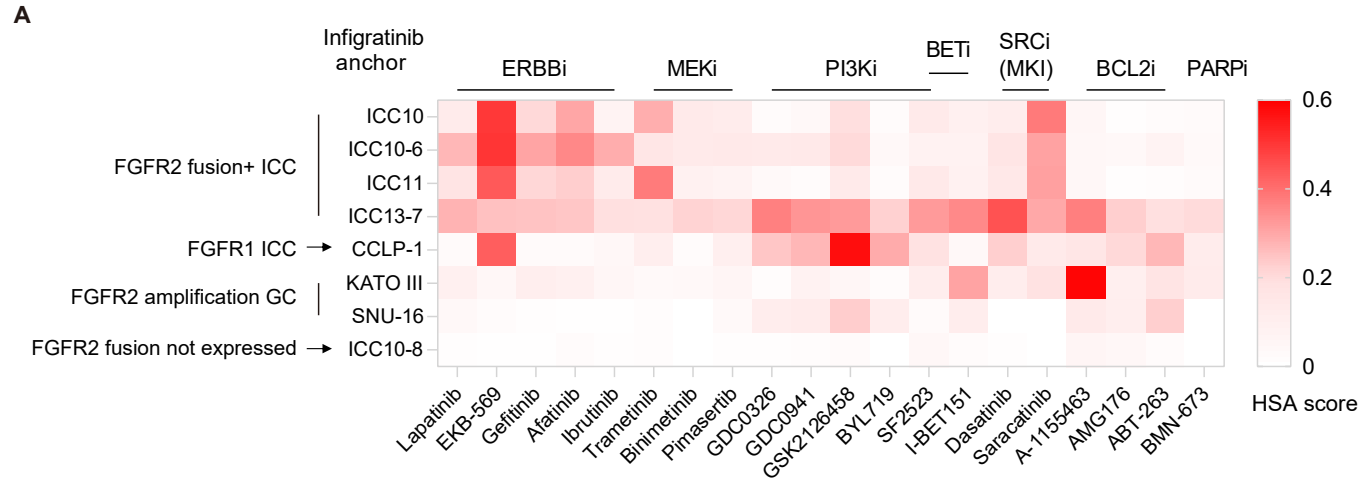


Compound	Target
Gefitinib	**EGFR**
Afatinib	**EGFR/HER2/HER4**
Lapatinib	**EGFR/HER2**
Ibrutinib	**EGFR/BTK/HER4**
SHP099	SHP2
GDC-0152	IAP
LTT462	ERK
KD025	ROCK2
Nutlin	MDM2

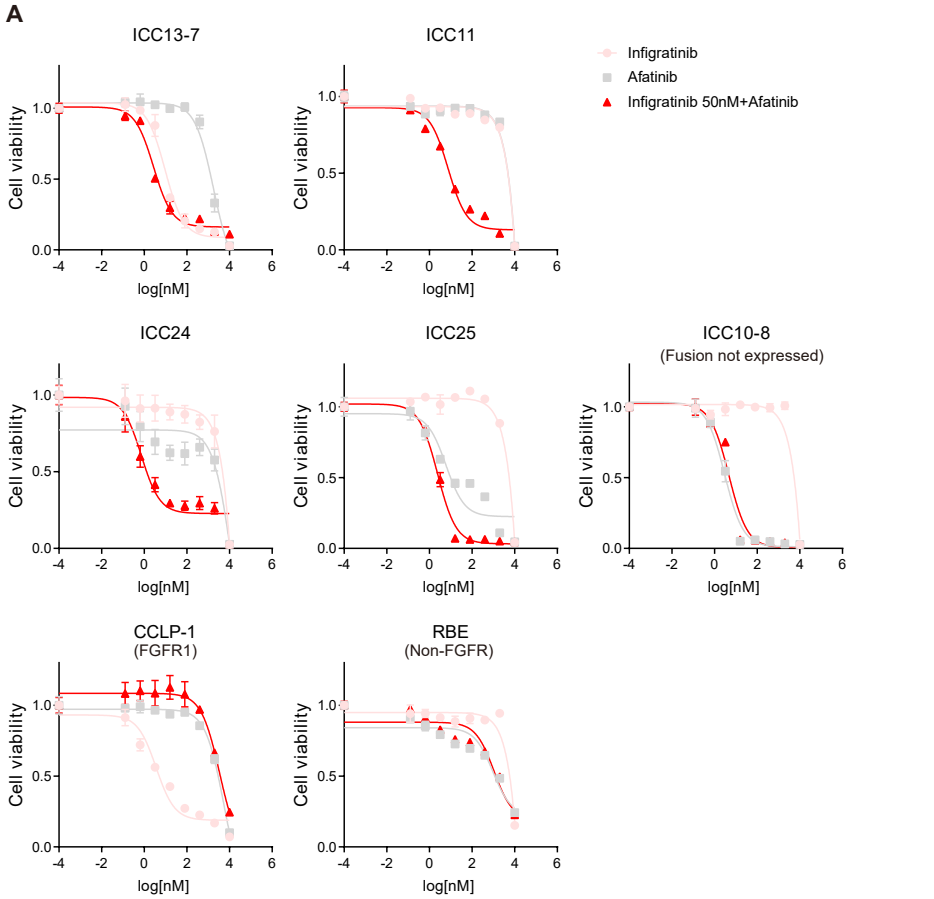
Supplementary Figure 2. High-throughput drug combination screen reveals compounds that potentiate the ability of FGFR inhibitors to induce cell death in FGFR2+ ICC models. A. Results of combination screen of the four FGFR2-fusion+ ICC cell lines tested using rogaratinib (1000 nM). The chart shows the cooperative induction of cell death across the 111 drugs screened based on second highest single agent (HSA) score. Inhibitors of WT EGFR/ERBB signaling are highlighted red. Mutant selective EGFR inhibitor is highlighted in black. Other inhibitors are color coded grey. **B**. Heat map and hierarchical cluster analysis generated in Morpheus (https://software.broadinstitute.org/morpheus/) based on second HSA score with rogaratinib for cell death induction across all cell lines screened (color scheme is based on the minimum and maximum HSA values in each row). The top ranked combinations are presented as blown-up images on the right.

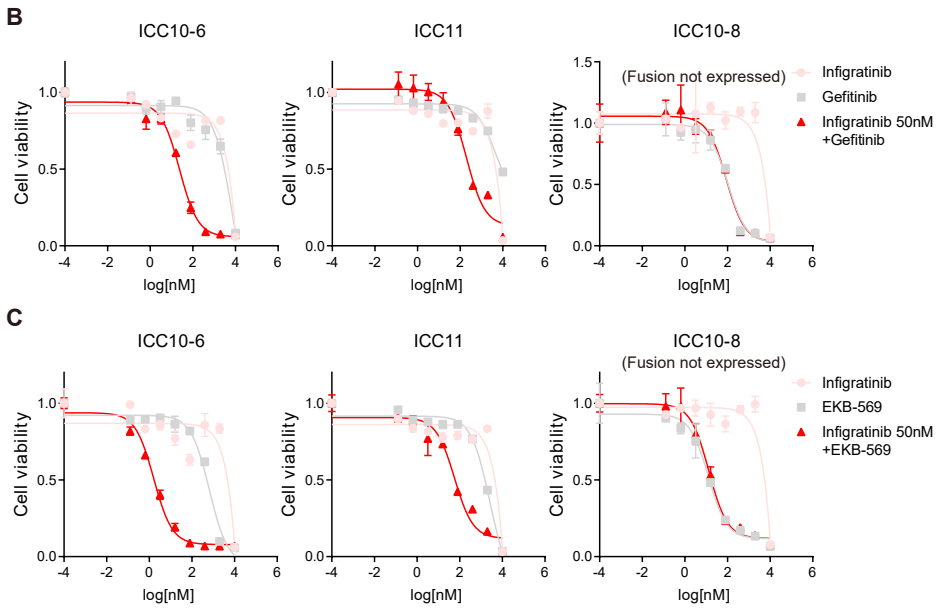
Supplementary Figure 3.

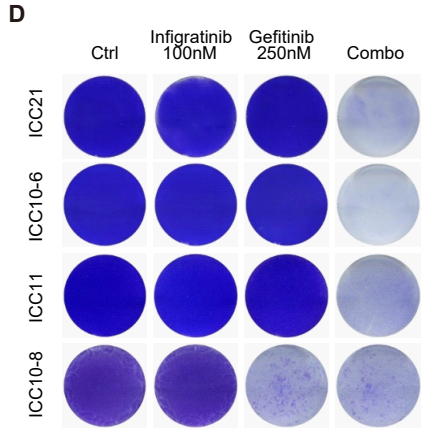


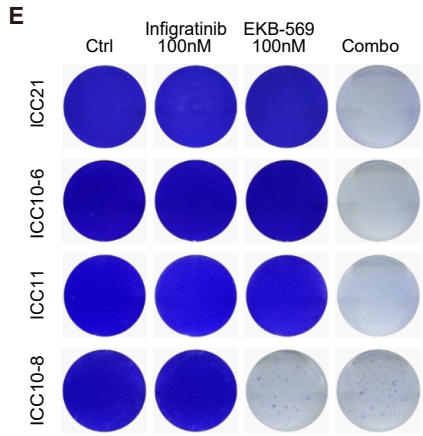
Supplementary Figure 3. Compounds potentiating the effects of the FGFR inhibitor, infigratinib, in FGFR2-fusion+ ICC models. A. Screen data depicting the top 20 compounds potentiating the ability of infigratinib to induce cell death in ICC13-7 cells, an FGFR2-fusion+ ICC model that is sensitive to infigratinib monotherapy. The heatmap shows the cooperativity of these compounds with infigratinib, based on second highest single agent (HSA) score, across all cell lines tested in the screen. MKI: multi-kinase inhibitor.

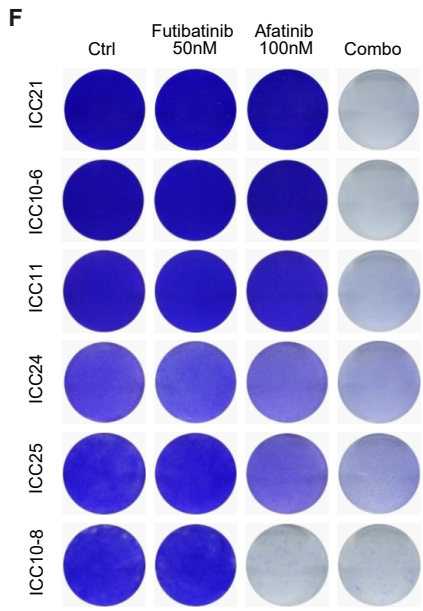
Supplementary Figure 4.

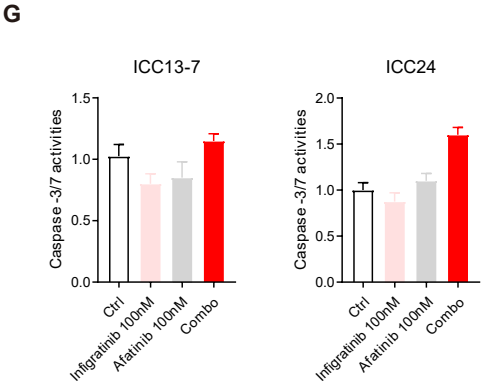


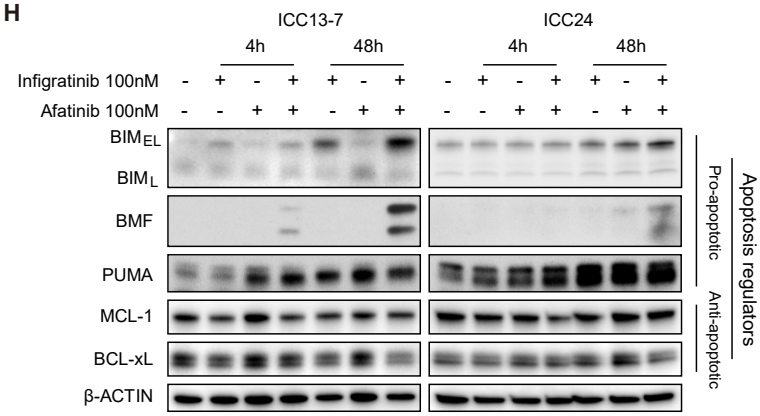






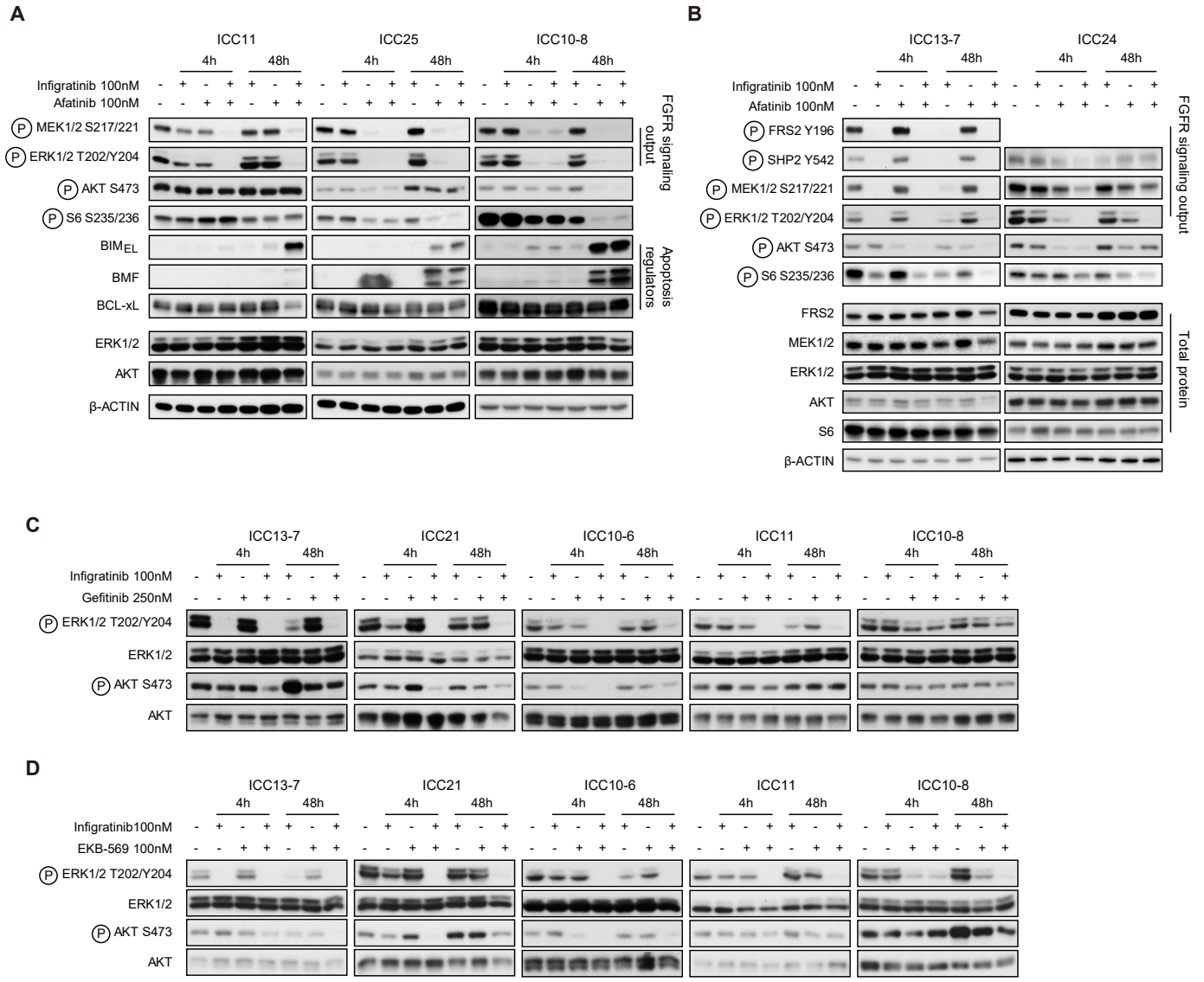






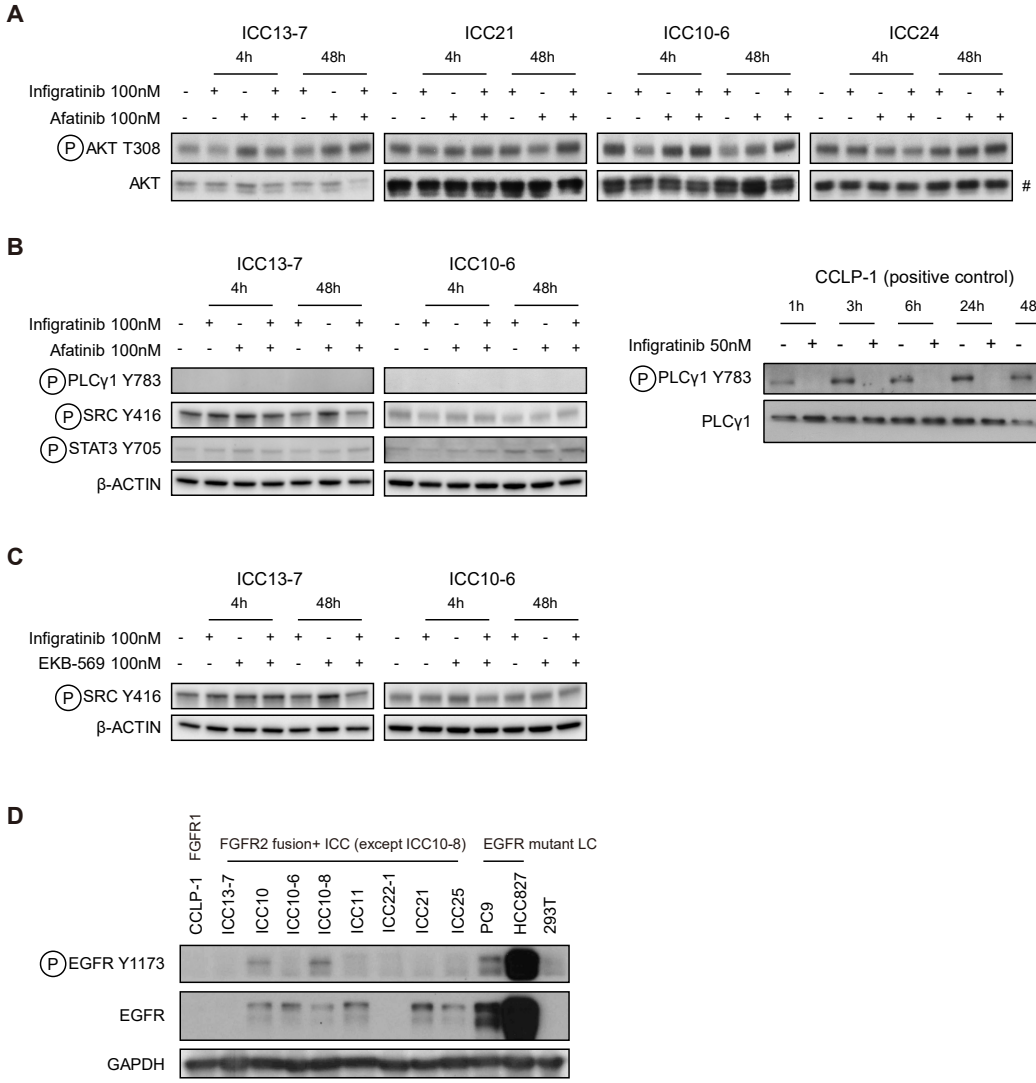
Supplementary Figure 4. Potentiating effects of combined inhibition of FGFR and EGFR/ERBB in FGFR2-fusion+ ICC models. A-C. IC50 assay for evaluating sensitivity of the indicated ICC cell lines to treatment with infigratinib or the indicated EGFR/ERBB inhibitors, or to 50 nM infigratinib combined with a range of doses of each EGFR/ERBB inhibitor. Each point on the dose-response curves represents 2 technical replicates. Data are shown as Mean ± SD. **D-F**. The indicated cell lines were tested for cell viability via crystal violet staining after 10 days treatment with indicated FGFR inhibitor, EGFR/ERBB inhibitors, or the combination, or vehicle control. **G**. Induction of apoptosis assessed by Caspase-3/7 activity after 3 days treatment with the indicated single agents or combination. Error bars on the graph represent 4 technical replicates. Data are shown as Mean± SD. **H.** Immunoblot analysis of pro-apoptotic (BIM, BMF, PUMA) and anti-apoptotic proteins (MCL-1, BCL-xL) in FGFR2-fusion+ ICC cell lines at the indicated timepoints.

Supplementary Figure 5.



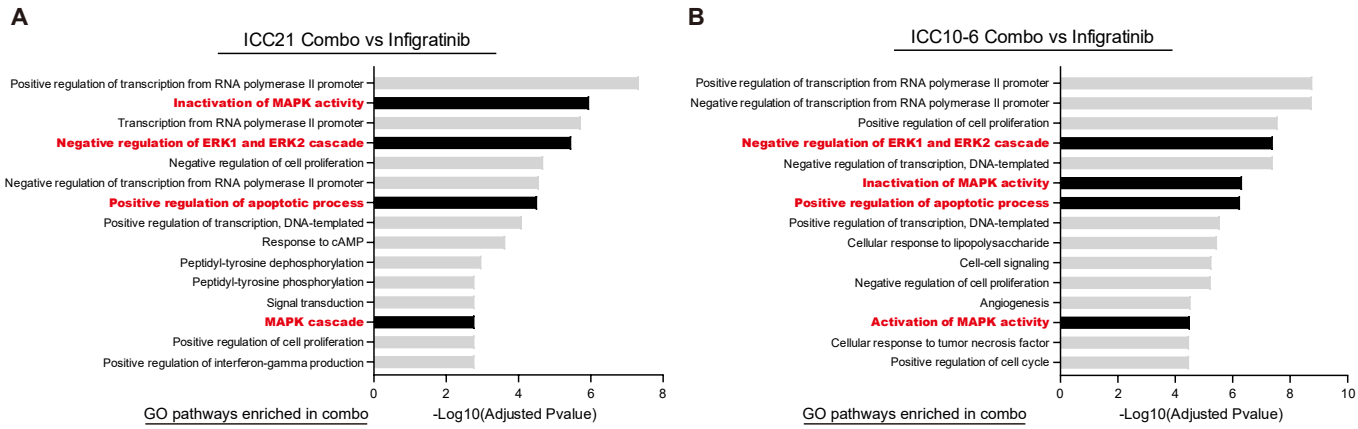
Supplementary Figure 5. Combined inhibition of FGFR and EGFR/ERBB leads to durable suppressed MAPK signaling. A-D. Immunoblot analysis of cell lysates from the indicated cell lines, using specific antibodies against indicated proteins upon treatment with vehicle, infigratinib, an EGFR/ERBB inhibitor or the combination for 4 hours or 48 hours. The EGFR/ERBB inhibitors were (A-B) afatinib, (C) gefitinib, and (D) EKB-569. ICC24 has a low level of phospho-Y196 FRS2.

Supplementary Figure 6.



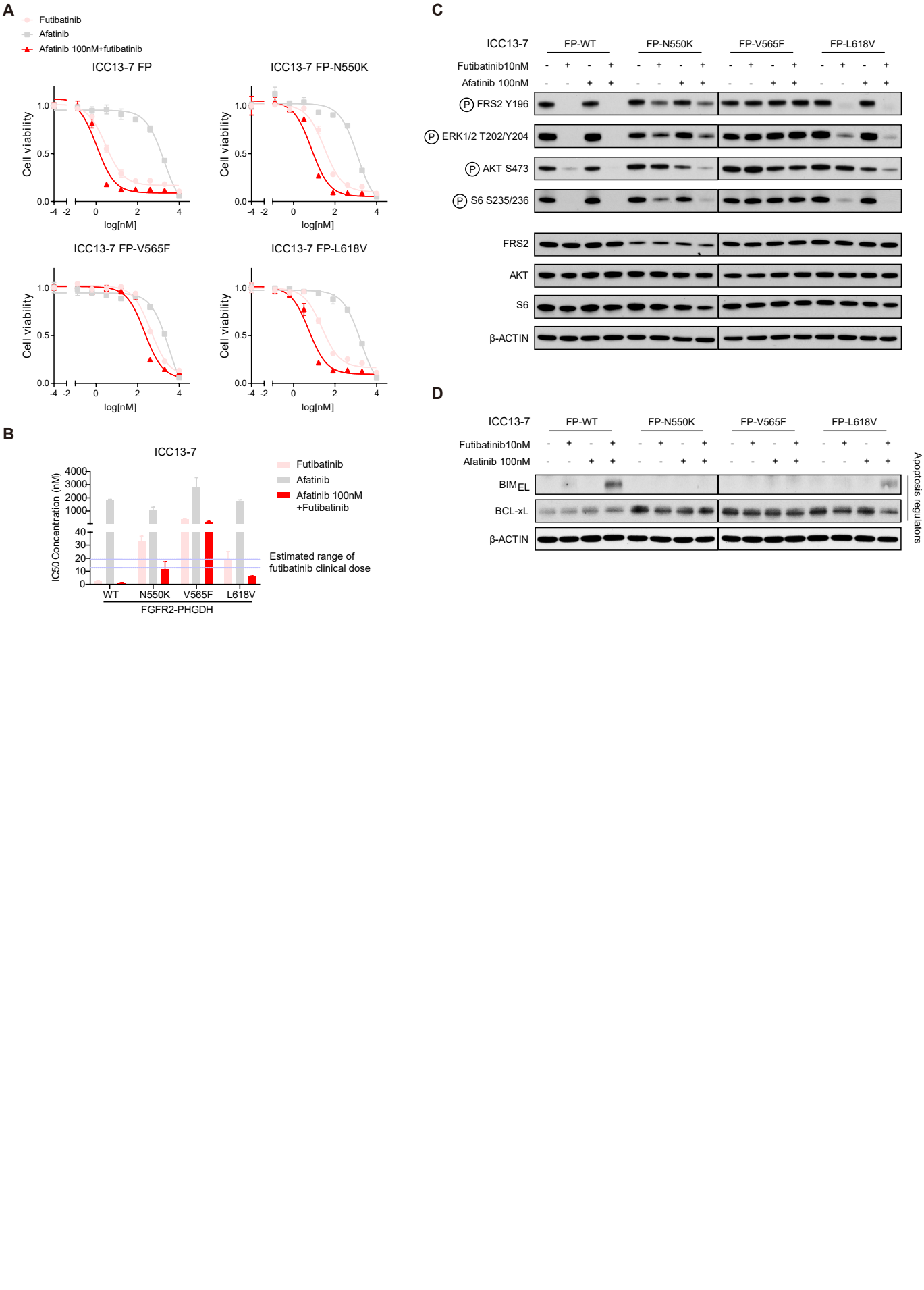
Supplementary Figure 6. Examination of FGFR signaling outputs. A-C. Immunoblot analysis of the indicated signaling proteins in lysates from FGFR2-fusion+ ICC cell lines (ICC13-7/ICC21/ICC10-6/ICC24) treated for 4 hours or 48 hours with vehicle or with the indicated FGFRi (100 nM infigratinib) and/or EGFRi/ERBBi (100 nM afatinib in [A and B] and 100 nM EKB-569 in [C]). Note: in (A) the total AKT blots (#) are the same as those shown in Figure 3F and Supplementary Figure 5B. In (B), immunoblot analysis of lysates from the FGFR1-driven ICC cell line, CCLP-1, treated with vehicle or 50 nM infigratinib is shown as a positive control for assessment of PLCγ signaling. **D.** Immunoblot analysis of EGFR signaling from the indicated cell lines. Human EGFR mutant lung cancer (LC) cell lines and 293T cells were used as references. Among ICC cell lines, the EGFR-dependent ICC10-8 cell line has the highest level of phospho-EGFR.

Supplementary Figure 7.



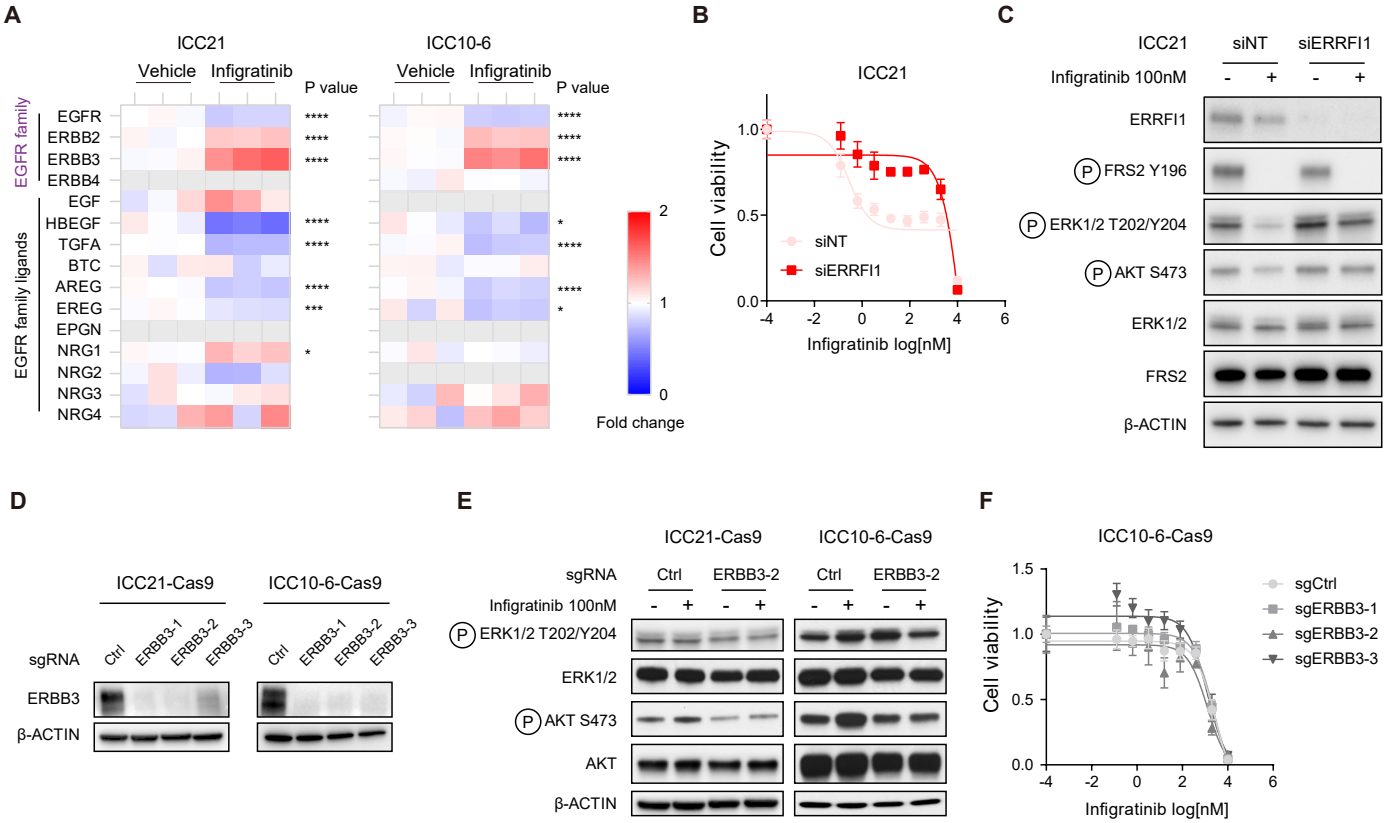
Supplementary Figure 7. Gene ontology analysis of combined inhibition of FGFR and EGFR in FGFR2-fusion+ ICC. A-B. ICC21 (A) and ICC10-6 (B) cells were treated with vehicle, 100 nM infigratinib, 100 nM afatinib, or the combination for 4 hours and then profiled by RNA sequencing. Pathway enrichment in infigratinib/afatinib combination versus infigratinib was determined by Gene Ontology analysis. Signatures related to MEK/ERK signaling and induction of apoptosis are highlighted.

Supplementary Figure 8.



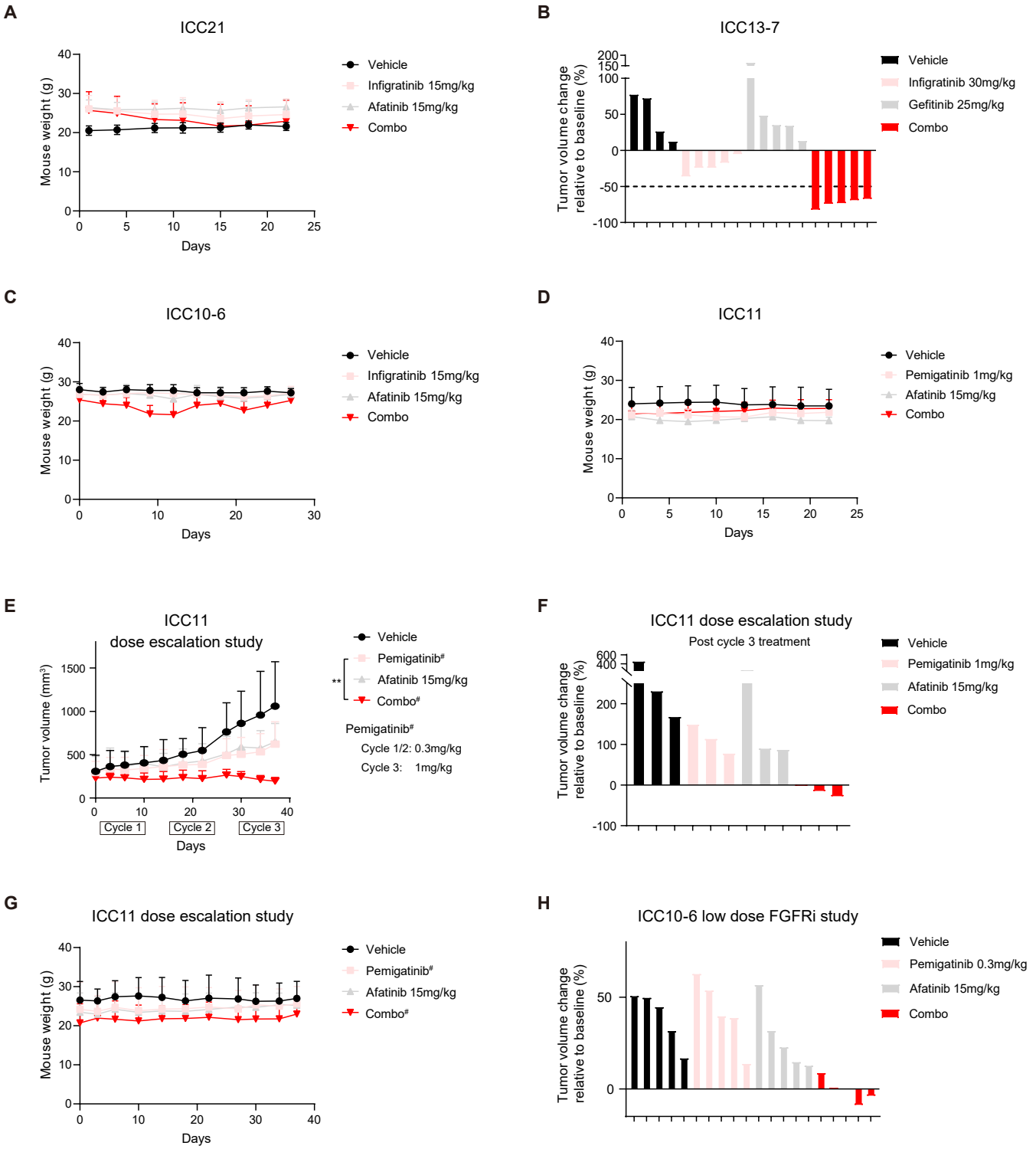
Supplementary Figure 8. EGFR inhibition potentiates the activity of futibatinib against secondary FGFR2 kinase mutations. A-D. ICC13-7 cells were engineered to express the FGFR2-PHGDH fusion with a WT FGFR2 kinase domain or with the indicated kinase domain mutations. **A, B.** IC50 assays for sensitivity to futibatinib, afatinib, or futibatinib in combination with 100 nM afatinib. (A) Representative IC50 response curves. (B) Graph of IC50 values. IC50 values were generated from two independent experiments and determined by GraphPad Prism 9. **C** and **D.** Immunoblot of lysates isolated after treatment of cells for 4 hours (C) or 48 hours (D) with 10 nM futibatinib, 100 nM afatinib, or the combination. Lysates were analyzed for (C) the indicated signaling proteins and (D) the indicated apoptotic regulators.

Supplementary Figure 9.



Supplementary Figure 9. Feedback modulation of EGFR signaling upon FGFR inhibition in FGFR2+ ICC. A. Heatmap showing relative expression of EGFR family members and ligands in the indicated FGFR2-fusion+ ICC models (ICC21 and ICC10-6 cells) treated with vehicle or an FGFR inhibitor (100 nM infigratinib for 4 hours). *P<0.05, ***P<0.001, ****P<0.0001. Gray color indicates not expressed. Each column shows a biological replicate (3/condition). **B** and **C.** ICC21 cells were transfected with ERRFI1 siRNA or non-targeting control siRNA and treated with infigratinib or vehicle. (B) Representative IC50 response curve showing that complete extinction of ERRFI1 leads to FGFRi resistance. Each point on the dose-response curves represents 4 technical replicates. Experiments were repeated twice. Data are shown as Mean ± SD. (C) Immunoblot analysis showing that complete ERRFI1 loss boosts feedback reactivation of ERK signifying upon infigratinib treatment (analyzed after 4 hours treatment). **D**-**F**. Derivatives of ICC21 and ICC10-6 cells were subject to CRISPR-CAS9 gene editing using three distinct sgRNAs targeting ERBB3 or with non-targeting control sgRNA. (D) Immunoblot analysis showing effective ERBB3 knockout. (E) Immunoblot analysis after 24 hours (ICC10-6) or 48 hours (ICC21) treatment with 100 nM infigratinib showing the ERBB3 status does not strongly affect downstream signaling. (F) IC50 response curve evaluating the sensitivity of ICC10-6 cells with ERBB3 sgRNAs or non-targeting control sgRNA to FGFR inhibitor infigratinib. Each point on the dose-response curves represents 3 technical replicates. Data are shown as Mean ± SD.

Supplementary Figure 10.



Supplementary Figure 10. In vivo efficacy of combined inhibition of FGFR and EGFR/ERBB in FGFR2-fusion+ ICC models. A. Body weight change of ICC21 tumor-bearing mice (5/group except vehicle 4/group) treated daily for 21 days at the indicated dose of infigratinib and/or afatinib. In the combination group, one mouse was euthanized at 20 days due to weight loss and another was given a 4-day drug holiday beginning at 15 days. **B.** Waterfall plot showing tumor volume changes of ICC13-7 subcutaneous xenografts upon treatment with vehicle (n=4), infigratinib 30 mg/kg (n=5), gefitinib 25 mg/kg (n=5), or the combination of both drugs (n=5) for 10 days. **C.** Body weight change of ICC10-6 tumor-bearing mice treated daily at the indicated dose of infigratinib and/or afatinib, for cycles of 10 days on treatment followed by 4 days off treatment. One mouse was euthanized after the first treatment cycle due to weight loss secondary to injury unrelated to the medication. **D**. Body weight change of ICC11 tumor-bearing mice (6/group except combination 8/group) treated with pemigatinib (1 mg/kg), afatinib (15 mg/kg), or the combination of both drugs, or with vehicle for 2 cycles of 10 days each, separated by an interval of 4 days. **E-G.** Mice bearing ICC11 subcutaneous xenograft tumors were treated with vehicle, pemigatinib, afatinib 15 mg/kg, or the combination of both drugs. Treatment was given daily for 10-day cycles with intervening 4-day drug holidays. Pemigatinib was administered at 0.3 mg/kg for cycles 1 and 2 and at 1 mg/kg for cycle 3 in both the single agent and combination groups. (E) serial monitoring of tumor size. (F) waterfall plot of tumor volume changes at the end of the third cycle of treatment. (G) Body weight change. n = 3 mice per group. Two-way ANOVA multiple comparisons with Tukey correction were used to analyze the data. **P<0.01. Data are shown as Mean± SD. **H.** Waterfall plot showing tumor volume changes of ICC10-6 subcutaneous xenografts upon treatment with vehicle, pemigatinib 0.3 mg/kg, afatinib 15 mg/kg, or the combination of both drugs for 10 days. n = 5 mice per group.