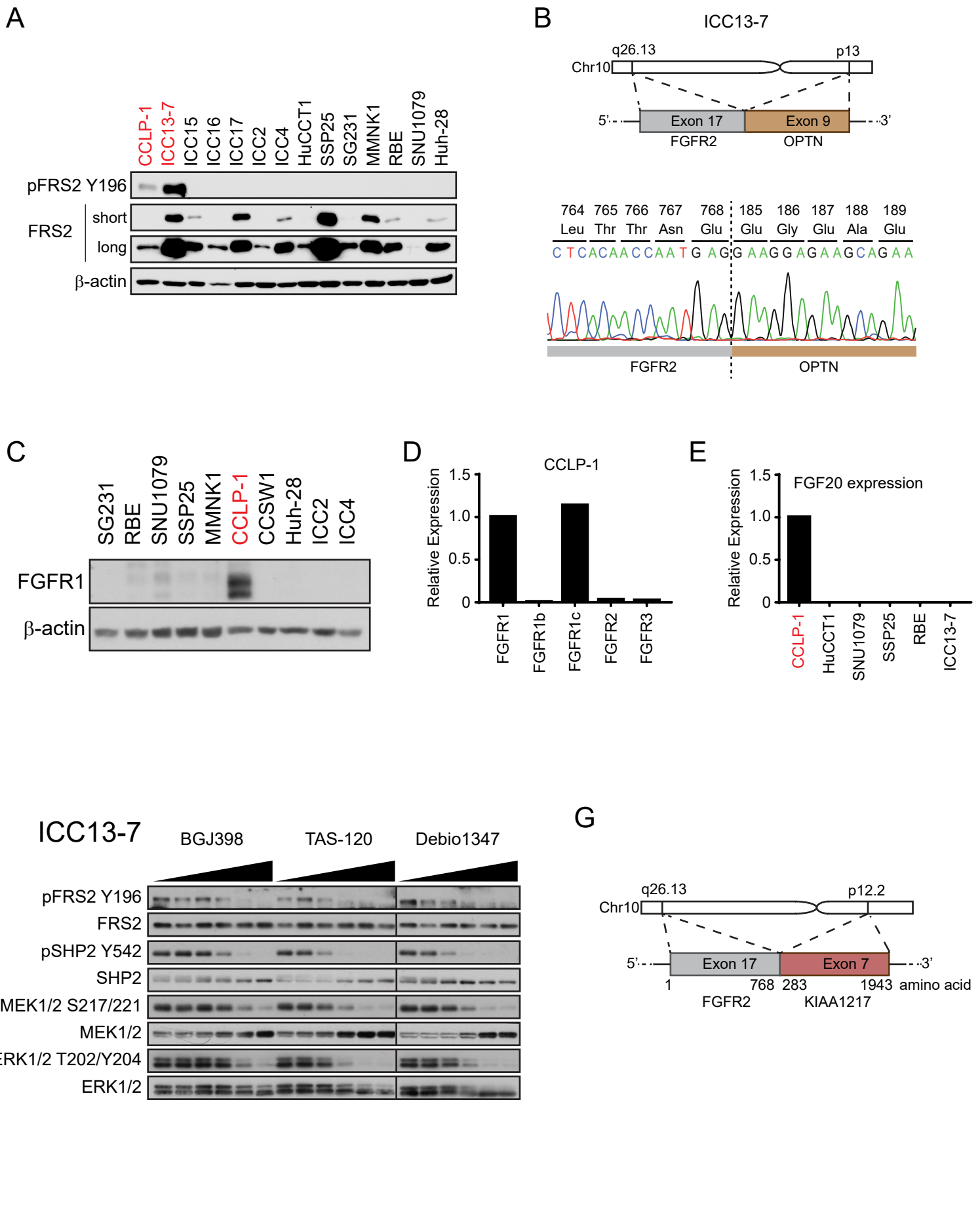
Supplemental Figure 1



Supplementary Figure S1. Characterization of FGFR-driven ICC models

A, Immunoblot of lysates from the indicated biliary tract cancer cell lines and immortalized bile duct cells (MMNK-1). The two FGFR inhibitor sensitive ICC cell lines, CCLP-1 and ICC13-7 (red), show constitutive FRS2-Y196 phosphorylation.

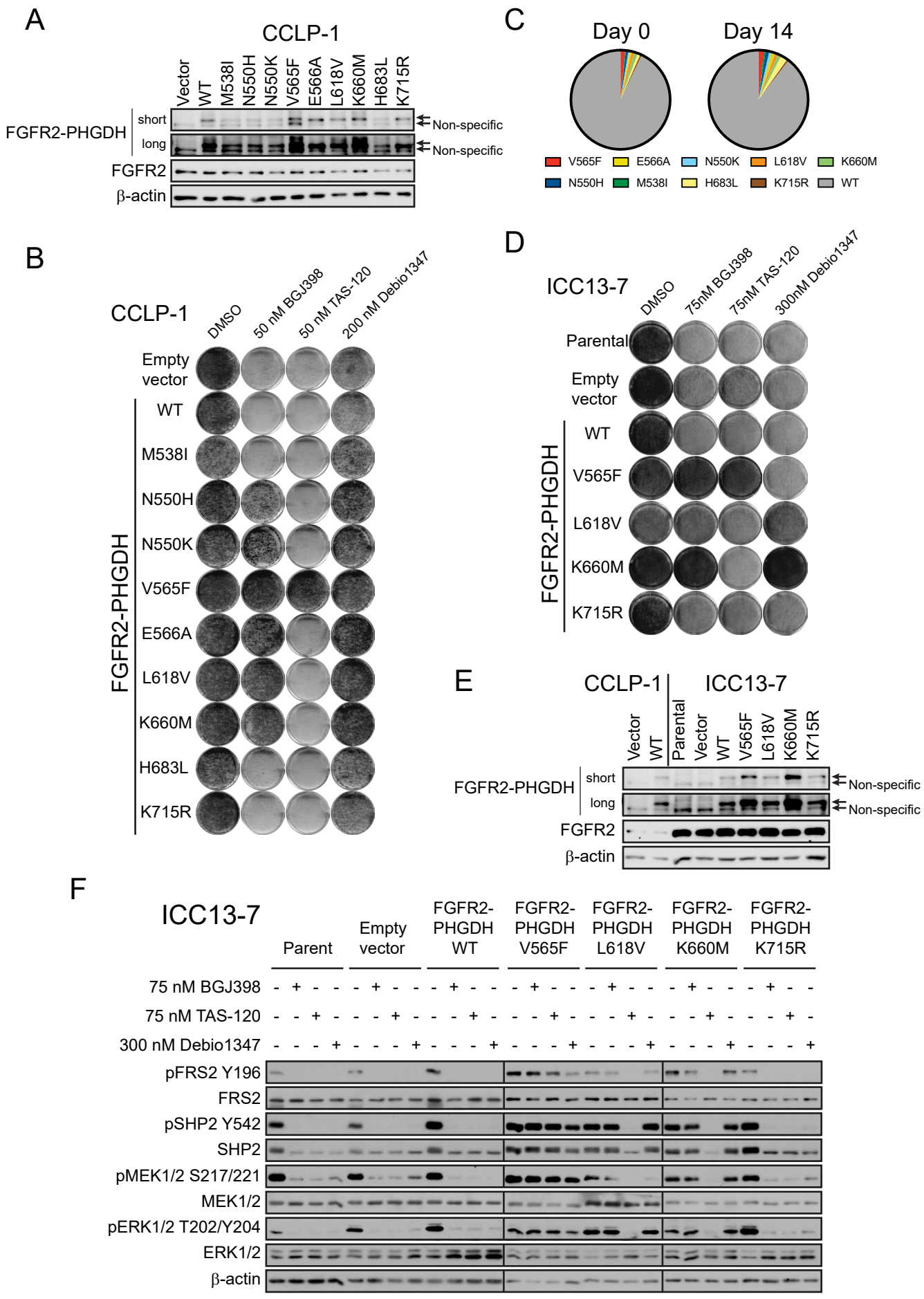
B, Structure of *FGFR2-OPTN* chromosomal fusion in ICC13-7 cells.

C-E, Examination of FGF pathway components in CCLP-1 cells. **C,** Immunoblot for FGFR1 in lysates from a set of human biliary tract cancer cell lines, including CCLP-1. MMNK-1 cells are shown as a reference. **D,** qRT-PCR analysis showing that FGFR1 is expressed as the IIIc isoform in CCLP-1 cells. **E,** Results of qRT-PCR analysis showing FGF20 overexpression in CCLP-1 cells.

F, Immunoblot showing dose-response of the indicated FGFR inhibitors on downstream signaling pathways (dose range: BGJ398: 0.4 nM-250 nM, TAS-120: 0.08-50 nM, Debio1347: 8 nm-5000 nM; for each drug, doses were increased at 5-fold increments).

G, Structure of the *FGFR2-KIAA1217* chromosomal fusion in the MG69 PDX model.

Supplemental Figure 2



Supplementary Figure S2. Activity of FGFR inhibitors against different FGFR2 kinase domain mutations in ICC cells

CCLP-1 cells (**A-C**) and ICC13-7 cells (**D-F**) were established to express the *FGFR2-PHGDH* fusion with a WT kinase domain or with the indicated mutations or expressing empty vector control. In B-D and F, cells were treated with the indicated FGFR inhibitors or DMSO vehicle. **A,** Immunoblot analysis of lysates of CCLP-1 derivative lines grown in the absence of drug treatment. **B,** Crystal violet staining of CCLP-1 cells after 5 days treatment. **C,** Pooled CCLP-1 cell clones of all FGFR2 fusion variants were grown for 14 days in the absence of drug selection. The individual clones were monitored using genomic DNA extracted at day 0 and day 14, using a ddPCR assay specific to each mutation. Data are generated from two independent experiments. **D,** Crystal violet staining of ICC13-7 cells after 14 days treatment. **E,** Immunoblot analysis of lysates from ICC13-7 derivative lines grown in the absence of drug treatment. CCLP-1 cell lysates are shown as a reference. **F,** immunoblot analysis of ICC13-7 cells after 6-8 hour treatment.