Supplemental Materials and Methods

FGFR copy number and gene expression analysis. The copy number of the FGFRs in cell lines was estimated by quantitative PCR (qPCR) using forward and reverse primer pairs specific for *FGFR1* (forward 5'- TTCCTCATCTCCTGCATGGT -3' and reverse 5'-GTGGTGCTGAGTGTGCAAAT 3'), *FGFR2* (5'-ACTTGGGCTGGAGTGATTTG-3' and 5'-AATCCCATCTGCACACTTCC-3'), *FGFR3* (5'- ATCTGGGTCAGAGCCTTCCT-3' and 5'-GAAGACCAACTGCTCCTGCT-3'), *FGFR4* (5'- TCAAACTCCCCACCAAACTC-3' and 5'-ACCAGCTGCTTGAAGGTAGG-3'), and a reference gene, *transketolase,* (5'-CAAAAACATGGCTGAGCAGA-3' and 5'-GAAACAGGCCCCACTTTGTA-3'). FGFR gene expression was analyzed by quantitative RT-PCR (qRT-PCR) with QuantiTect SYBR Green RT-PCR kit (Qiagen) using primers specific for *FGFR1* (5'-AGGCTACAAGGTCCGTTATGC -3' and 5'- TGCCGTACTCATTCTCCACAA -3'), *FGFR2* (5'- TTAAGCAGGAGCATCGCATTG -3' and 5'- GGGACCACACTTTCCATAATGAG -3'), *FGFR2-IIIb* (5'- GCAGAAGTGCTGGCTCTGTT -3' and 5'- TGTTTTGGCAGGACAGTGAG -3'), *FGFR2-IIIc* (5'- CACCACGGACAAAGAGATTG -3' and 5'-CAACCATGCAGAGTGAAAGG -3'), *FGFR3* (5'- CCTCGGGAGATGACGAAGAC -3' and 5'- CGGGCCGTGTCCAGTAAGG -3'), *FGFR4* (5'- TGCAGAATCTCACCTTGATTACA -3' and 5'- GGGGTAACTGTGCCTATTCG -3') and a reference gene *HPRT* (5'-GACACTGGCAAAACAATGCA-3' and 5'- CTTCGTGGGGTCCTTTTCACC-3').

GP369 Binding Specificity. The extracellular domains of human FGFR2-IIIb, human FGFR2-IIIc and mouse FGFR2-IIIb were expressed in CHO cells as human Fc fusion proteins and purified by protein A chromatography. To determine the binding specificity of GP369, FGFR2 fusion proteins (25nM) and a negative control protein, hJag1-hFc (25nM) were captured

on anti-hFc Octet biosensors (ForteBio). Sensograms were recorded when the loaded biosensors were incubated with GP369 (12.5 nM) in Octet sample diluent (ForteBio).

Soft Agar Assay. For HCC95 soft agar assay, the 12-well plates were first coated with a bottom layer of agarose consisting of a 1:1 solution of 1.6% agarose and 2XRPMI supplemented with Penicillin/Streptomycin and Fungizone (0.25 ug/ml) and 5% FBS. The agarose layer was allowed to solidify at room temperature for 30 min or overnight at 37°C. The plates were then plated with a top agarose layer consisting of a 1:1 mix of 1% agarose and HCC95 cells in RPMI, 5% FBS and 30μg/ml of either mIgG or GP369. Plates were incubated overnight at 37°C and fed with RPMI containing 5% FBS the following day. Each well was seeded with 20,000 cells. The plates were replenished with fresh media containing 30μg/ml of either mIgG or GP369 every 3-4 days. Images were taken after 15 days and the number of colonies was counted. Similarly, KATO III soft agar assay was carried out except using the DMEM medium instead of RPMI.

***Western Blotting*.** Cells were lysed in NP-40 lysis buffer containing 1% NP-40, 20 mM Tris-HCl (pH8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, protease inhibitors (Roche Applied Science), and Halt phosphatase inhibitors (Thermo Scientific). The protein lysates were analyzed by Western blot with anti-FGFR2 (sc-122) (Santa Cruz Biotechnology), anti-phospho-FGFR (Y653/Y654) (R&D Systems), anti-FRS2 (Santa Cruz Biotechnology), anti-phospho-FRS2 (Y196) (Cell Signaling Technology), anti-ERK1/2 and anti-phospho-ERK1/2 (Cell Signaling Technology), and anti-β-tubulin (Millipore Corporation) antibodies.

Figure Legends for supplementary Tables

Supplementary Table 1. *FGFR* copy number of human cancer cell lines. qPCR was carried out using primers specific for *FGFRs* or a reference gene, *transketolase 1 (TKT1)*, and copy number was calculated.

Figure Legends for supplementary Figures

Supplementary Figure 1. Alignment of the entire antigens used for immunization. The antigens consist of the extracellular portion of the IIIb or IIIc isoforms of FGFR2 beta (without the first Ig domain or the acid box) followed by a Factor Xa cleavage site, human IgG1 Fc, a 6X His tag, and an HA tag. The following alignment displays the entire amino acid sequence of the two antigens used. The FGFR2 extracellular domains are from positions 1 through 244 of the alignment. The amino acids highlighted in yellow denote identical residues and the ones highlighted in green denote similar residues.

Supplementary Figure 2. Specificity of ligand responsiveness of FDCP-1 cells expressing the IIIb or IIIc isoform of FGFR2. FDCP-1 cells expressing FGFR2-IIIb or FGFR2-IIIc were seeded in a 96-well plate without IL3. Various amounts of FGFs plus heparin (5ug/ml) were added, and MTT assays were carried out after 2 days.

Supplementary Figure 3. Inhibition of soft agar colony formation by GP369. KATO III (panel A and B) and HCC95 cells (panel C and D) were plated in 12-well plates in soft agar and were

treated with 30μg/ml of either GP369 (panel B and D) or control mouse IgG (mIgG) (panel A and C). The experiment was done in triplicate and representative images were shown.

Supplementary Figure 4. Stimulation of tyrosine phosphorylation of FGFR2 by FGF7 in SNU-16 cells *in vitro* detected by protein array analysis. SNU-16 cells were either untreated or treated with FGF7 (30 ng/mL) and heparin (20ug/ml) for 10 min and protein lysates were analyzed with a phospho-RTK array (R&D Systems) according to manufacturer's instructions.