Supplementary Figure legends

Table S1. Proteins identified by quantitative LC MS/MS.

Table S2. List 1. Proteins significantly ($p<0.05$) enriched more than 10X in S1 (U2OS-R1-BirA* cells) compared to C1 (U2OS-R1 control cells) and C2 (U2OS-R1 cells co-expressing BirA*)

Table S3. List 2. Proteins significantly ($p<0.05$) enriched more than 10X in S2 (U2OS-R1-BirA* cells stimulated with FGF1) compared to C1 (U2OS-R1 control cells), C2 (U2OS-R1 cells co-expressing BirA*), and C3 (U2OS-R1 cells co-expressing BirA* stimulated with FGF1)

Table S4. List 3. Proteins significantly ($p<0.05$) enriched more than 10X in C3 (U2OS-R1 + BirA* cells, FGF1 stimulated) compared to C1 (U2OS-R1 control cells). Sorted on enrichment compared to C2 (U2OS-R1 cells co-expressing BirA*)

Table S5. Previously reported human FGFR1 protein-protein interactions were downloaded from BioGRID (v3.4, www.thebiogrid.org/). Overlap with our Mass Spectrometry data are indicated in colours.

Table S6. Phosphopeptides identified in the MS dataset. Note that enrichment of phosphopeptides was not performed.

Figure S1. Efficiency of biotinylation in osteosarcoma cells stably expressing FGFR1-BirA* and BirA*. U2OS-R1 cells, U2OS-R1-BirA* cells or U2OS-R1 cells coexpressing BirA* (U2OS-R1 + BirA*) were left untreated or treated with 50 mM biotin and/or 100 ng/ml FGF1 in the presence of 10 U/ml heparin as indicated for 24 hours. The cells were then lysed and the cellular material was analyzed by SDS-PAGE and western blotting using the indicated antibodies.

Figure S2. Correlation between independent biological replicates of the BirA* mass spectrometry experiments. The scatter plots show the correlation between independent BirA*-MS experiments. The mean intensity (log values) of the three technical replicates in each independent biological replicate is plotted. BR, biological replicate.

Figure S3. (A) U2OS-R1 cells were treated with PTPN1/PTPN13 siRNAs (#1-#3) or a control siRNA (scr) for 72 hours. The cells were then serum-starved for 2 hours and subsequently stimulated with 10 ng/ml FGF1 in the presence of 10 U/ml heparin for 15 minutes or left untreated. Next, the cells were lysed and subjected to SDS-PAGE and western blotting using indicated antibodies. One of three indepependent experiments is shown, whereas the graphs below represent mean values ± SD.. Knockdown efficiency of PTPN13 was assessed using real time PCR. Cells transfected with indicated siRNAs for 72 hours were lysed and RNA isolation, cDNA synthesis and qRT-PCR were performed as described in materials and methods. The amount of mRNA was calculated relative to the housekeeping gene SDHA and is expressed as fraction of scr. The graphs represent the mean + SD of three independent experiments. ***$p \leq 0.001$. **(B)** G292 cells transfected with indicated siRNAs for 72 hours were lysed and RNA isolation, cDNA synthesis and qRT-PCR were performed as described in materials and methods. The amount of mRNA was calculated relative to the housekeeping gene SDHA and is expressed as fraction of scr. The graphs represent the mean + SD of three independent experiments. **$p \leq 0.01$. **(C)** RH30 cells transfected with indicated siRNAs for 72 hours were lysed and RNA isolation, cDNA synthesis and qRT-PCR were performed as described in materials and methods. The amount of mRNA was calculated relative to the housekeeping gene SDHA and is expressed as fraction of scr. The graph represent one experiment. **(D)** RH30 cells were treated with PTPRG siRNAs (#1-#3) or

control siRNA (scr) for 72 hours. The cells were then serum-starved for 2 hours before stimulation with FGF1 in the presence heparin for 15 minutes. Next, the cells were lysed and the lysates were subjected to SDS-PAGE followed by western blotting using denoted antibodies. **(E,F)** U2OS-R2 and U2OS-R3 cells were treated with siRNAs againts PTPRG (#1-#3) or control siRNA (scr) for 72 h before the cells were starved for 2 h and stimulated with 10 ng/ml FGF1 in the presence of 10 U/ml heparin. Then the cells were lysed in SDS-PAGE sample buffer, subjected to electrophoresis and analyzed by western blotting indicated antibodies. A representative result of three independent experiments is shown.

Figure S4. PTPRG is a major tyrosine dephosphorylating enzyme of activated FGFR1. U2OS-R1 cells were transfected with siRNA againts PTPRG (#1) or control siRNA (scr) for 72 h. Then the cells were serum starved for 2 h, pretreated 5 min with 2 mM sodium orthovanadate and treated 15 min with 10 ng/ml FGF1 in the presence of 10 U/ml heparin. After the indicated time points the cells were lysed in SDS-PAGE sample buffer. The lysates were subjected to SDS-PAGE followed by western blotting using denoted antibodies. The bands were quantified and normalized to γ-tubulin or total FGFR1. The graphs represent mean values ± SD of four independent experiments, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

Figure S5. (A) U2OS-R1 cells were treated with siRNAs against PTPRG (#1) or control siRNA (scr) for 72 hours. Then the cells were serum-starved for 2 hours and stimulated with various concentrations of FGF1 in the presence of 10 U/ml heparin for 15 minutes, lysed and the lysates were subjected to SDS-PAGE followed by western blotting using denoted antibodies. One representative experiment is shown. For quantification see Fig. 7A. **(B)** U2OS-R1 cells were treated with siRNAs against PTPRG (#1) or control siRNA (scr) for 72 hours, serum-starved for 2 hours and stimulated with 10 ng/ml FGF1 in the presence of 10

U/ml heparin and various concentrations of AZD4547 for 15 minutes. The cells were then lysed and the lysates were subjected to SDS-PAGE followed by western blotting using denoted antibodies. One representative experiment is shown. For quantification see Fig. 7D.

Figure S1

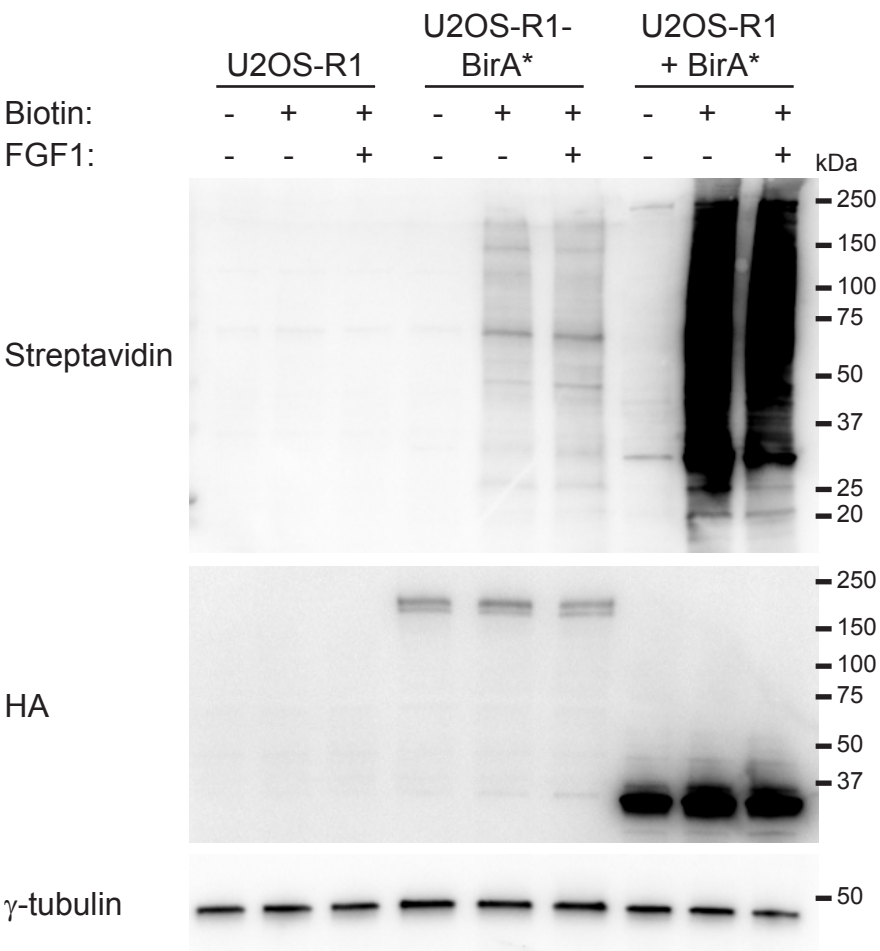
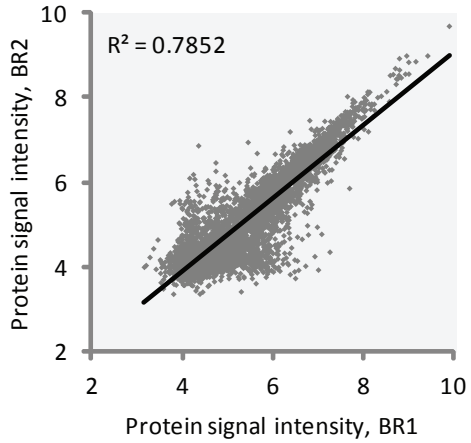
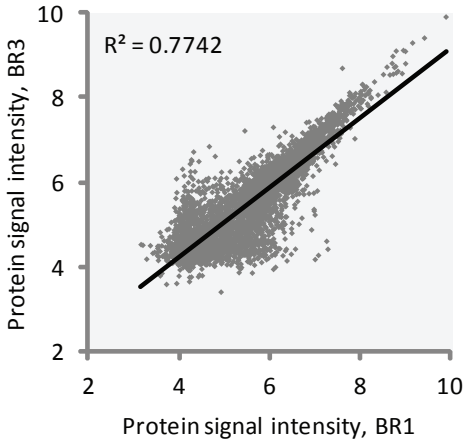
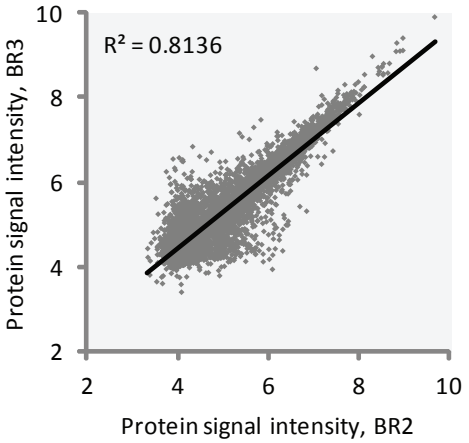

U2OS-R1
U2OS-R1-BirA*
U2OS-R1 + BirA*
Biotin: - + + - + + - + +
FGF1: - - + - - + - - +
kDa
Streptavidin
250
150
100
75
50
37
25
20
HA
250
150
100
75
50
37
γ-tubulin
50

Figure S2

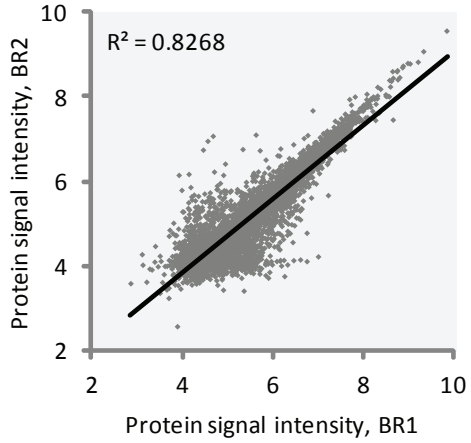
S1: U2OS-R1-BirA*

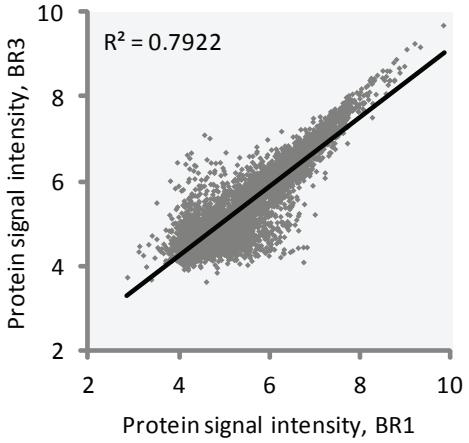


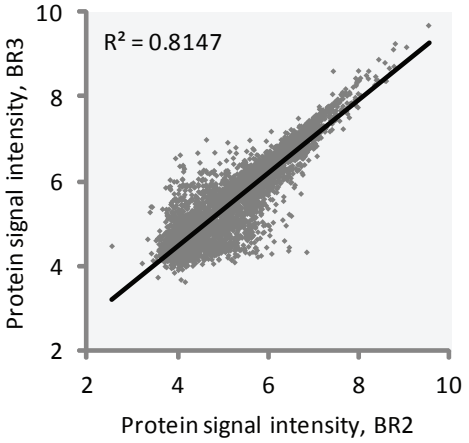




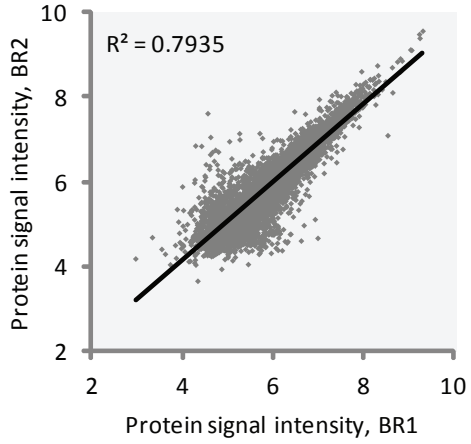
S2: U2OS-R1-BirA*, FGF1 stimulated

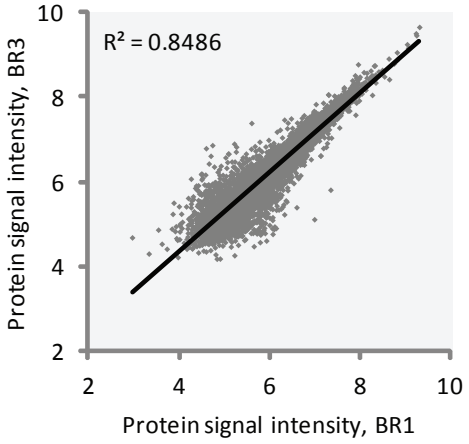






C3: U2OS-R1 + BirA*, FGF1 stimulated





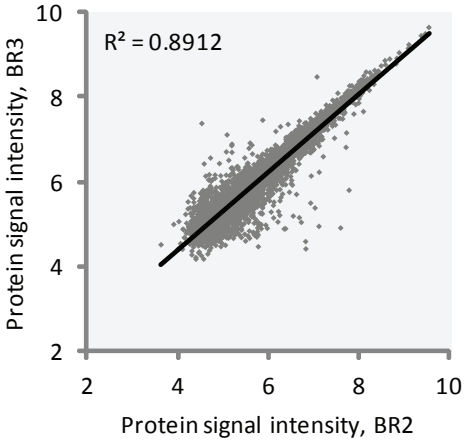


Figure S3

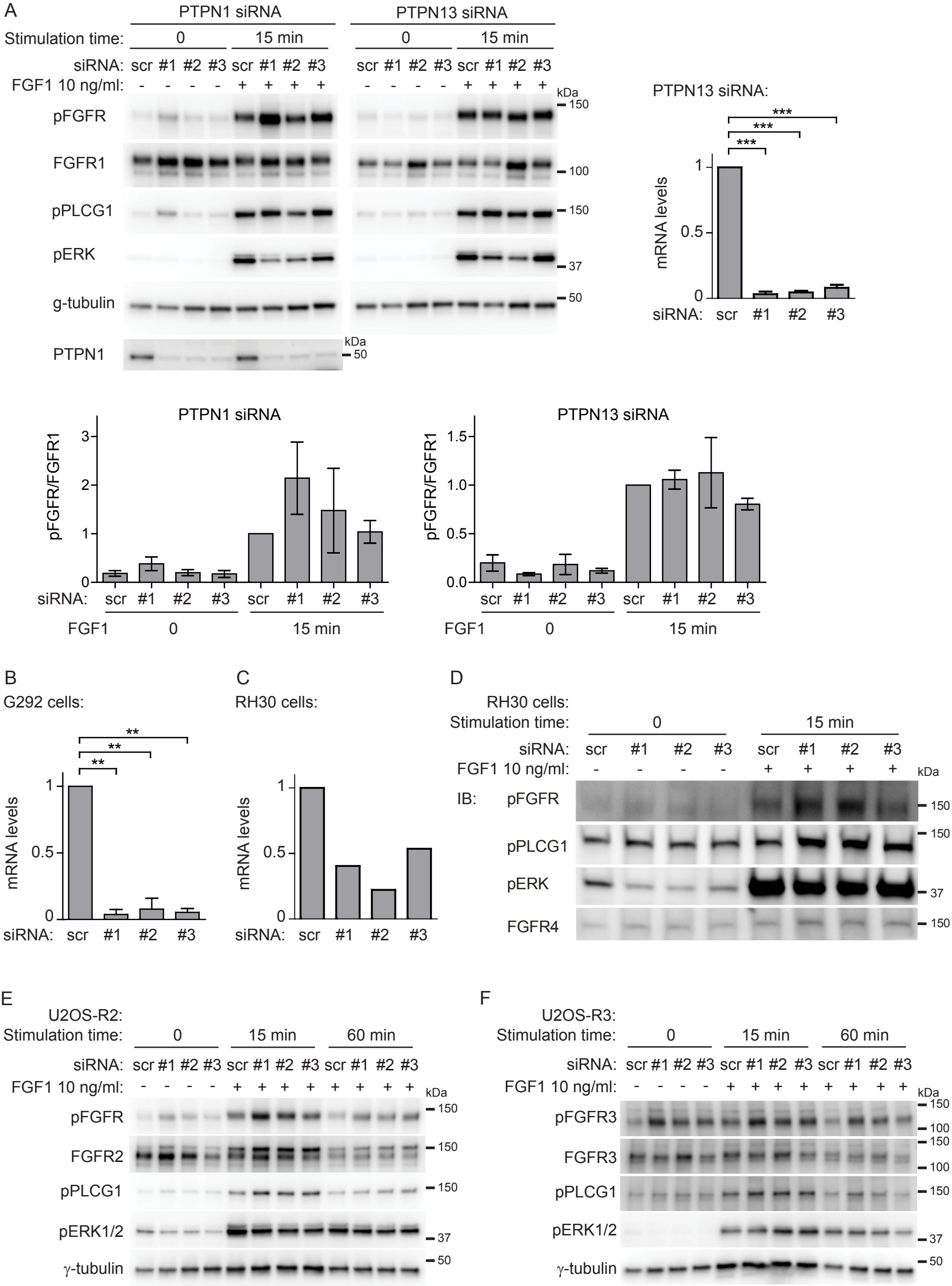

A
PTPN1 siRNA
PTPN13 siRNA
Stimulation time:
0
15 min
siRNA: scr #1 #2 #3 scr #1 #2 #3
FGF1 10 ng/ml: - - - - + + + +
kDa
pFGFR
FGFR1
pPLCG1
pERK
g-tubulin
PTPN1
150
100
37
50
PTPN13 siRNA:
mRNA levels
***
1
0.5
0
siRNA: scr #1 #2 #3
PTPN1 siRNA
PTPN13 siRNA
pFGFR/FGFR1
3
2
1
0
1.5
1.0
0.5
0.0
FGF1
0
15 min
B
G292 cells:
**
mRNA levels
C
RH30 cells:
D
RH30 cells:
IB:
pFGFR
pPLCG1
pERK
FGFR4
E
U2OS-R2:
60 min
FGFR2
pERK1/2
γ-tubulin
F
U2OS-R3:
pFGFR3
FGFR3

Figure S4

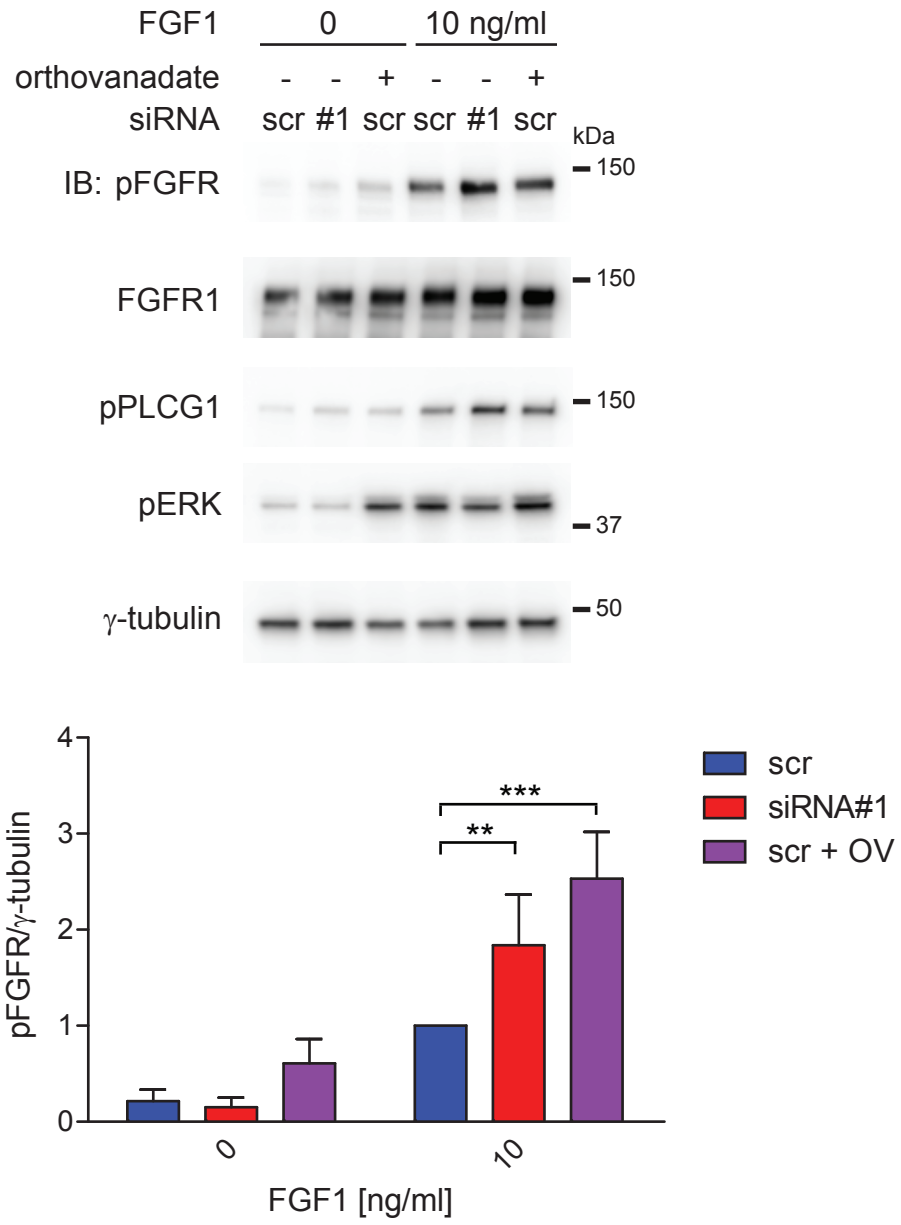
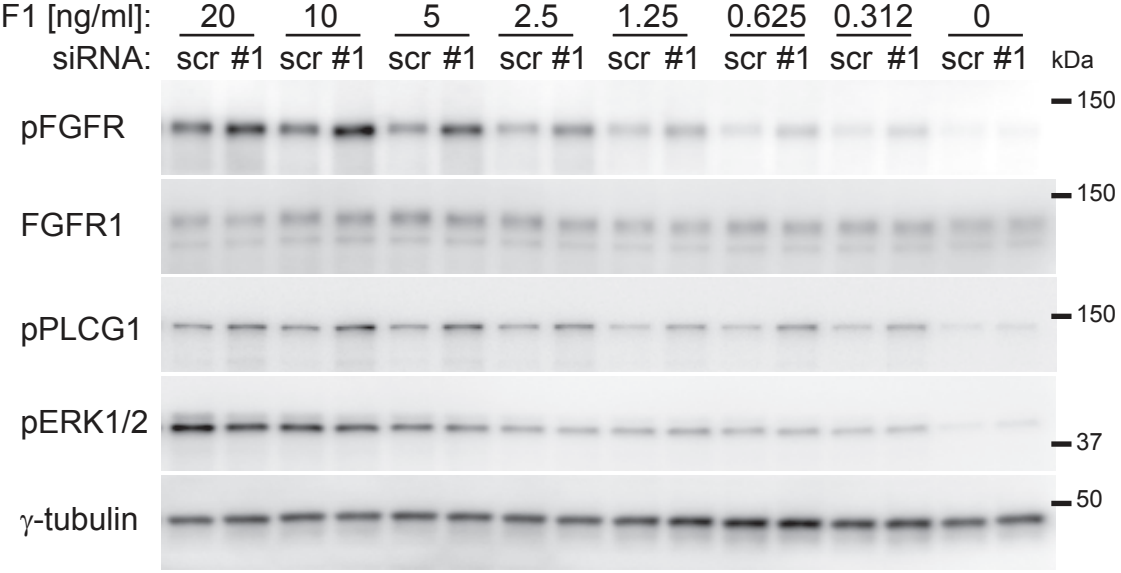
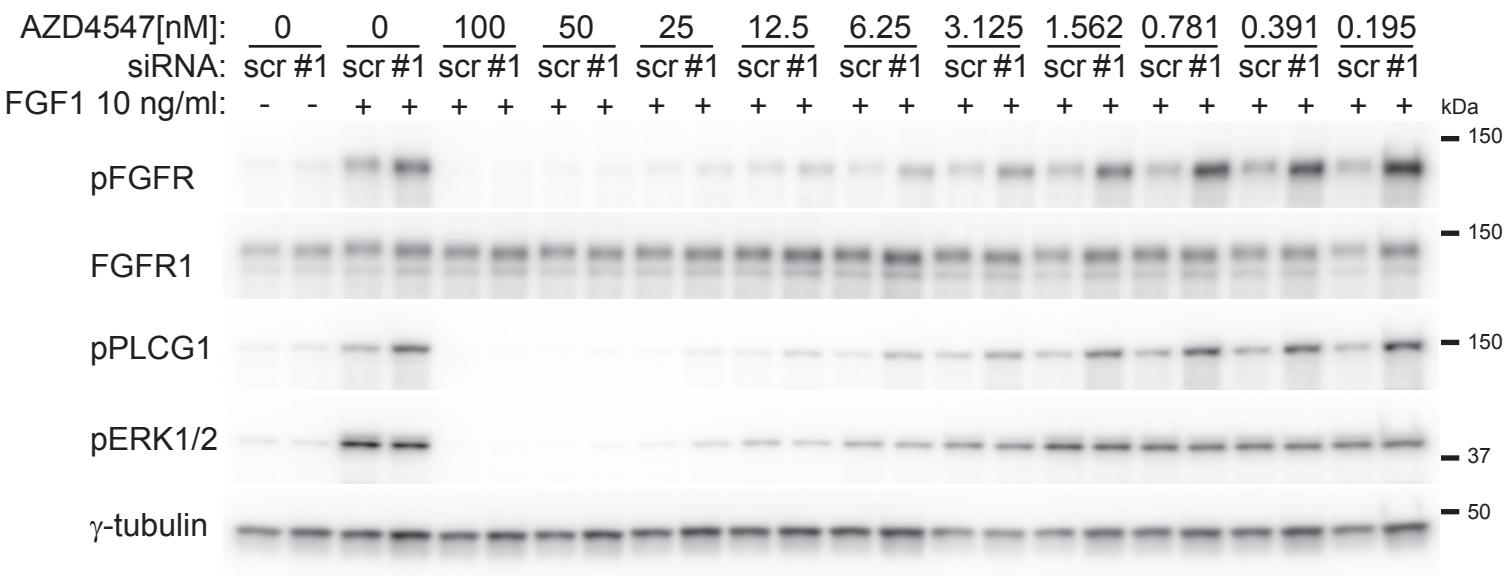

FGF1
0
10 ng/ml
orthovanadate
- - + - - +
siRNA
scr #1 scr scr #1 scr
kDa
IB: pFGFR
150
FGFR1
150
pPLCG1
150
pERK
37
γ-tubulin
50
pFGFR/γ-tubulin
4
3
2
1
0
***
**
0
10
FGF1 [ng/ml]
scr
siRNA#1
scr + OV

Figure S5

A


FGF1 [ng/ml]: 20 10 5 2.5 1.25 0.625 0.312 0
siRNA: scr #1 scr #1 scr #1 scr #1 scr #1 scr #1 scr #1 scr #1
kDa
pFGFR
FGFR1
pPLCG1
pERK1/2
γ-tubulin
150
150
150
37
50

B


AZD4547[nM]: 0 0 100 50 25 12.5 6.25 3.125 1.562 0.781 0.391 0.195
siRNA: scr #1 scr #1 scr #1 scr #1 scr #1 scr #1 scr #1 scr #1 scr #1 scr #1 scr #1 scr #1
FGF1 10 ng/ml: - - +
kDa
pFGFR
FGFR1
pPLCG1
pERK1/2
γ-tubulin
150
150
150
37
50