Supplemental Information for:

Antibody-induced dimerization of FGFR1 promotes receptor endocytosis independently of its kinase activity

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Fig. S1. Antibody fragments interact with FGFR1 produced by U2OSR1 cells

A. Interaction of antibody fragments in the scFv format with the FGFR1 produced by U2OSR1 cells. Antibody fragments were bound to anti-c-Myc agarose and incubated with the lysates prepared from U2OSR1 cells that overproduce FGFR1. Proteins that interact with scFvs were eluted and the level of FGFR1 was assessed by Western blotting. B. Interaction of antibody fragments in the scFv-Fc format with the FGFR1 produced by U2OSR1 cells. Antibody fragments were bound to Protein A Sepharose and incubated with the lysates prepared from U2OSR1 cells that overproduce FGFR1. Proteins that interact with scFv-Fc proteins were eluted and the level of FGFR1 was assessed by Western blotting.

Fig. S2. The level of FGFR1 in studied cell lines

Equal amounts of U2OS, U2OSR1 and NIH3T3 cells were lysed and the level of FGFR1 was assessed by Western blotting.

Fig. S3. Antibody fragments bind to D1 domain of FGFR1

scFvD1 and scFvE2 (myc-tagged) were bound to the anti-c-Myc agarose and incubated with either purified full length extracellular part of FGFR1 fused with Fc fragment (FGFR1 D1-D2-D3-Fc) or with the Fc-fusion of the extracellular part of FGFR1 lacking domain D1 (FGFR1 D2-D3-Fc). Proteins bound to scFvE2 and scFvC1 were analyzed with anti-Fc antibodies.

Fig. S4. SPR analysis of the competitive binding of antibody fragments and FGF1 to the extracellular portion of FGFR1

SPR sensograms showing epitope binning analysis of scFvs and FGF1. Monomeric fractions of scFvC1, scFvD2 and FGF1 at 1 μM were tested in pairwise combinations over a CM5 sensor chip coated with about 650 RU of FGFR1-Fc protein.

Fig. S5. Antibody fragments and FGF1 do not compete for the binding to FGFR1 produced by model cells

U2OSR1 cells were incubated with FGF1 to allow for the formation of the growth factor-FGFR1 complexes. Cells were lysed and incubated with Protein A Sepharose-bound scFv-Fc antibody fragments. Proteins bound to scFv-Fcs were eluted and analyzed by Western blotting. Since antibody fragments do not interact with FGF1 (data not shown), detection of FGF1 in the eluates of scFv-Fc pull down suggest the presence of ternary complexes: FGF1-FGFR1-scFv-Fcs and thus distinct binding sited for FGF1 and scFv-Fc proteins on FGFR1.

Fig. S6. Antibody fragments block interaction of FGFR1 with β-Klotho

BLI technique was used to assess the interaction of FGFR1 with β-Klotho co-receptor. Extracellular part of FGFR1 was chemically immobilized on AR2G biosensors, reaction was subsequently quenched and sensors were used for binding analysis either with β-Klotho alone (A), or first saturated with the excess of scFv proteins and then incubated with β-Klotho in the presence of appropriate scFv (B-D).

Fig. S7. scFcD2-Fc is internalized by NIH3T3 cells

Serum starved NIH3T3 cells were incubated with scFvD2.myc or scFvD2-Fc to allow for formation of FGFR1-antibody fragment complexes. Cells were shifted to 37ºC to induce endocytosis. Reaction was stopped by cooling down the cells on ice. Surface-bound, non-internalized antibody fragments were removed by washing the cells with

HSLP buffer. Internalized antibody fragments were recovered with anti-c-Myc agarose and Protein-A-Sepharose.

Fig. S8. Full length blots used in the main figures

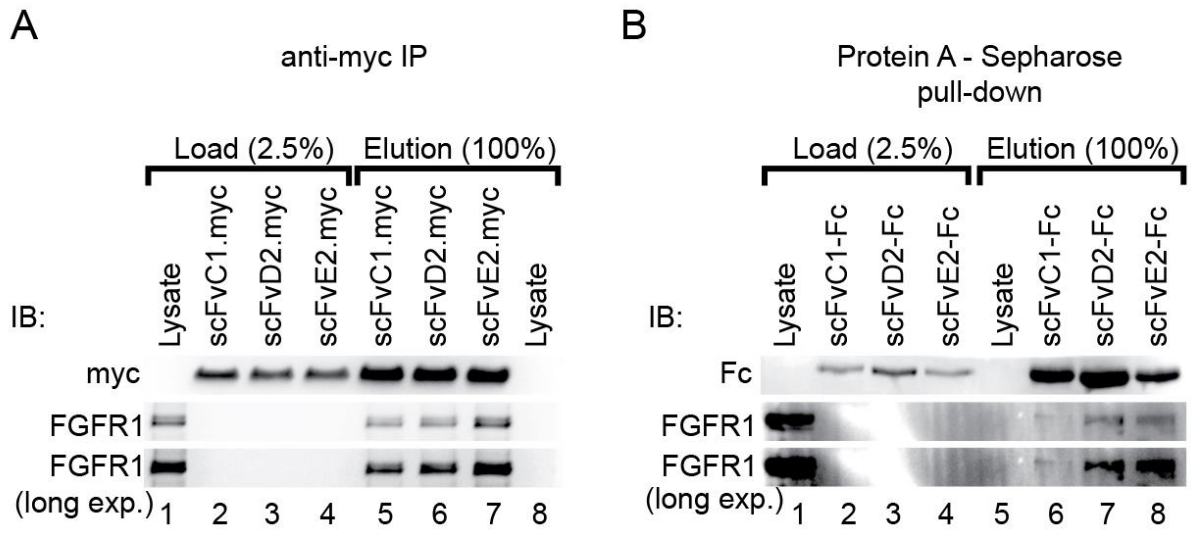

A
anti-myc IP
Load (2.5%)
Elution (100%)
Lysate
scFvC1.myc
scFvD2.myc
scFvE2.myc
scFvC1.myc
scFvD2.myc
scFvE2.myc
Lysate
IB:
myc
FGFR1
FGFR1
(long exp.)
1 2 3 4 5 6 7 8
B
Protein A - Sepharose
pull-down
Load (2.5%)
Elution (100%)
Lysate
scFvC1-Fc
scFvD2-Fc
scFvE2-Fc
Lysate
scFvC1-Fc
scFvD2-Fc
scFvE2-Fc
IB:
Fc
FGFR1
FGFR1
(long exp.)
1 2 3 4 5 6 7 8

Fig. S1

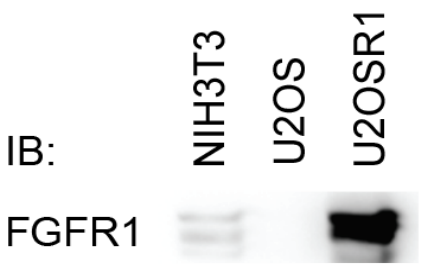


Fig. S2

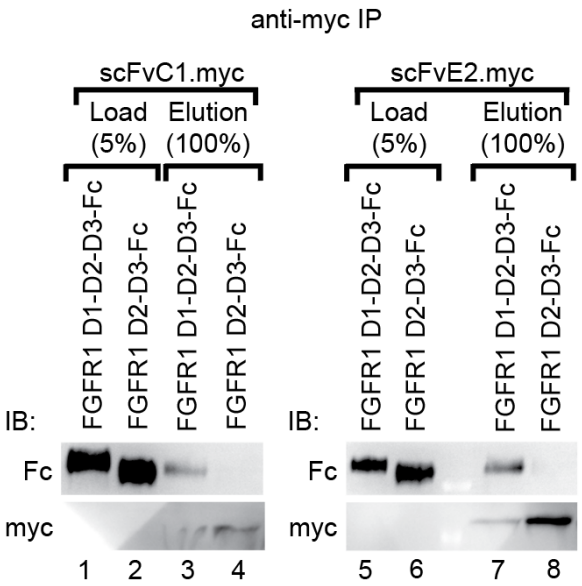
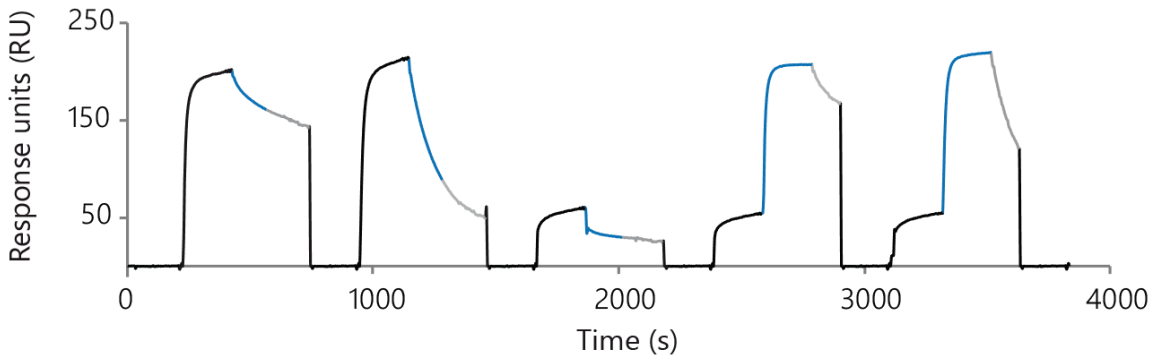

anti-myc IP
scFvC1.myc
Load (5%)
Elution (100%)
scFvE2.myc
Load (5%)
Elution (100%)
FGFR1 D1-D2-D3-Fc
FGFR1 D2-D3-Fc
FGFR1 D1-D2-D3-Fc
FGFR1 D2-D3-Fc
FGFR1 D1-D2-D3-Fc
FGFR1 D2-D3-Fc
FGFR1 D1-D2-D3-Fc
FGFR1 D2-D3-Fc
IB:
Fc
myc
IB:
Fc
myc
1 2 3 4
5 6 7 8

Fig. S3



Analyte 1:	scFvD2	scFvC1	FGF1	FGF1	FGF1
Analyte 2:	Buffer	Buffer	Buffer	scFvD2	scFvC1
Dissociation:	Buffer	Buffer	Buffer	Buffer	Buffer

Fig. S4

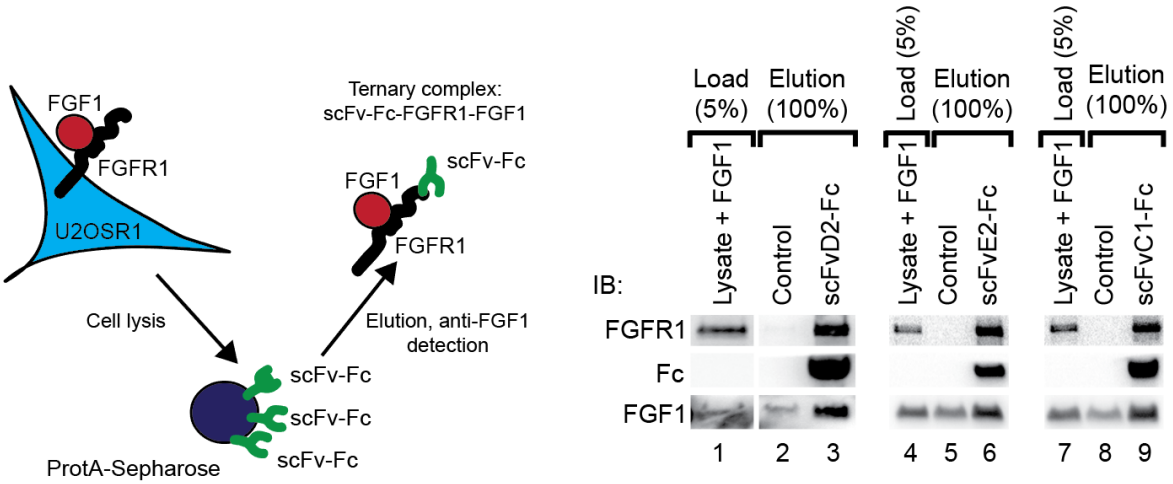

FGF1
FGFR1
U2OSR1
Ternary complex:
scFv-Fc-FGFR1-FGF1
FGF1
scFv-Fc
FGFR1
Cell lysis
Elution, anti-FGF1
detection
scFv-Fc
scFv-Fc
scFv-Fc
ProtA-Sepharose
IB:
Load (5%)
Elution (100%)
Load (5%)
Elution (100%)
Load (5%)
Elution (100%)
Lysate + FGF1
Control
scFvD2-Fc
Lysate + FGF1
Control
scFvE2-Fc
Lysate + FGF1
Control
scFvC1-Fc
FGFR1
Fc
FGF1
1 2 3 4 5 6 7 8 9

Fig. S5

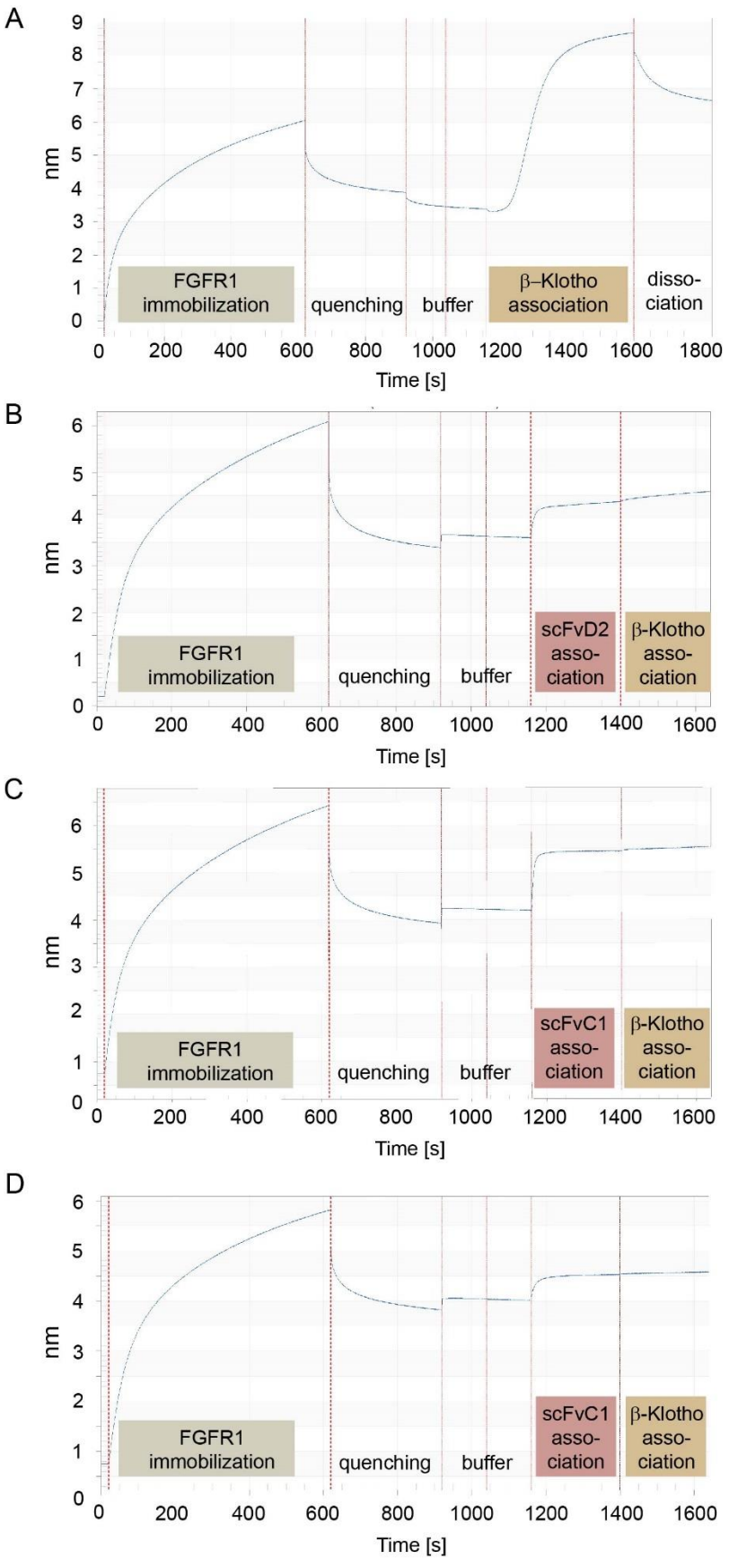

A
nm
FGFR1 immobilization
quenching
buffer
β–Klotho association
disso-ciation
Time [s]
B
nm
FGFR1 immobilization
quenching
buffer
scFvD2 asso-ciation
β-Klotho asso-ciation
Time [s]
C
nm
FGFR1 immobilization
quenching
buffer
scFvC1 asso-ciation
β-Klotho asso-ciation
Time [s]
D
nm
FGFR1 immobilization
quenching
buffer
scFvC1 asso-ciation
β-Klotho asso-ciation
Time [s]

Fig. S6

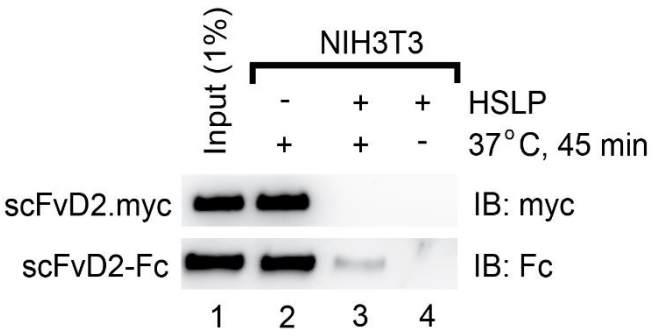

Input (1%)
NIH3T3
-
+
+
HSLP
+
+
-
37°C, 45 min
scFvD2.myc
IB: myc
scFvD2-Fc
IB: Fc
1
2
3
4

Fig. S7

Figure 1B

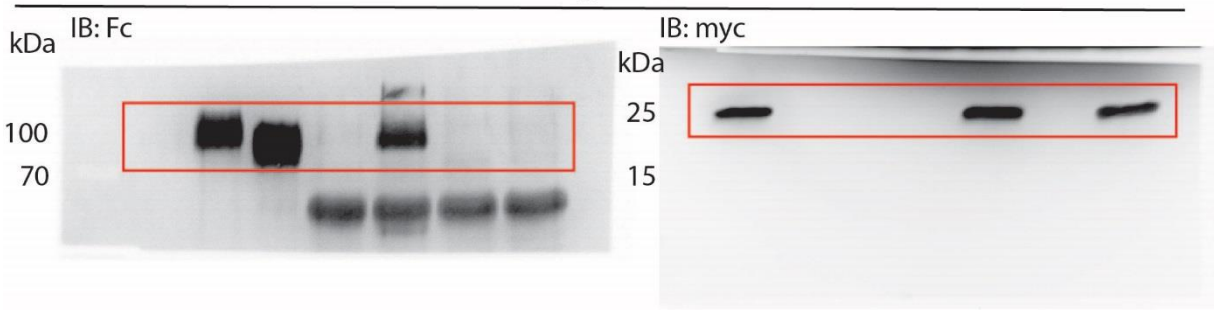


Figure 1C

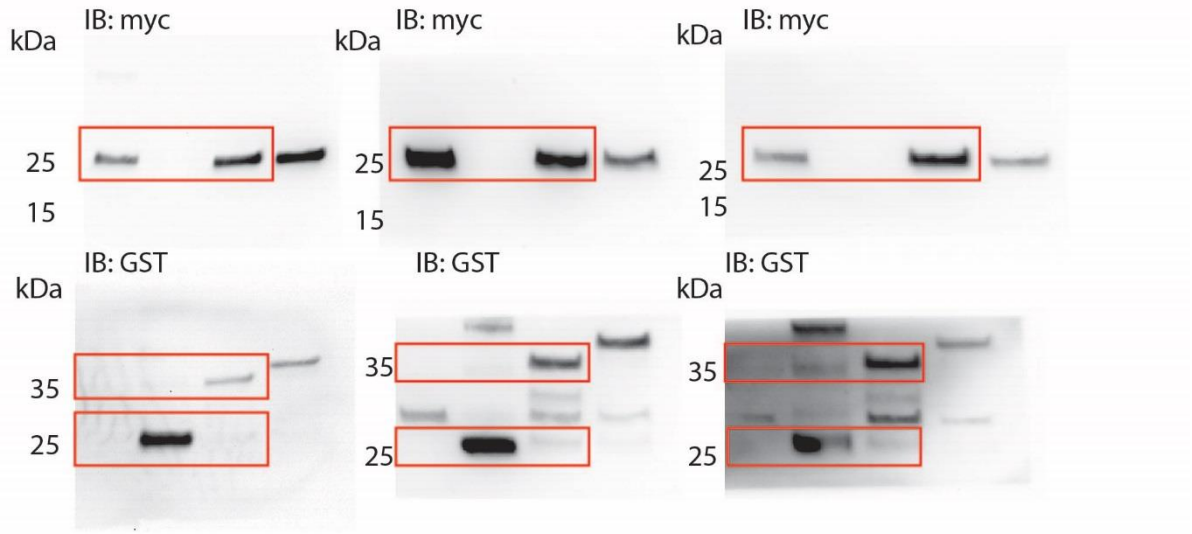


Figure 2A - top panel

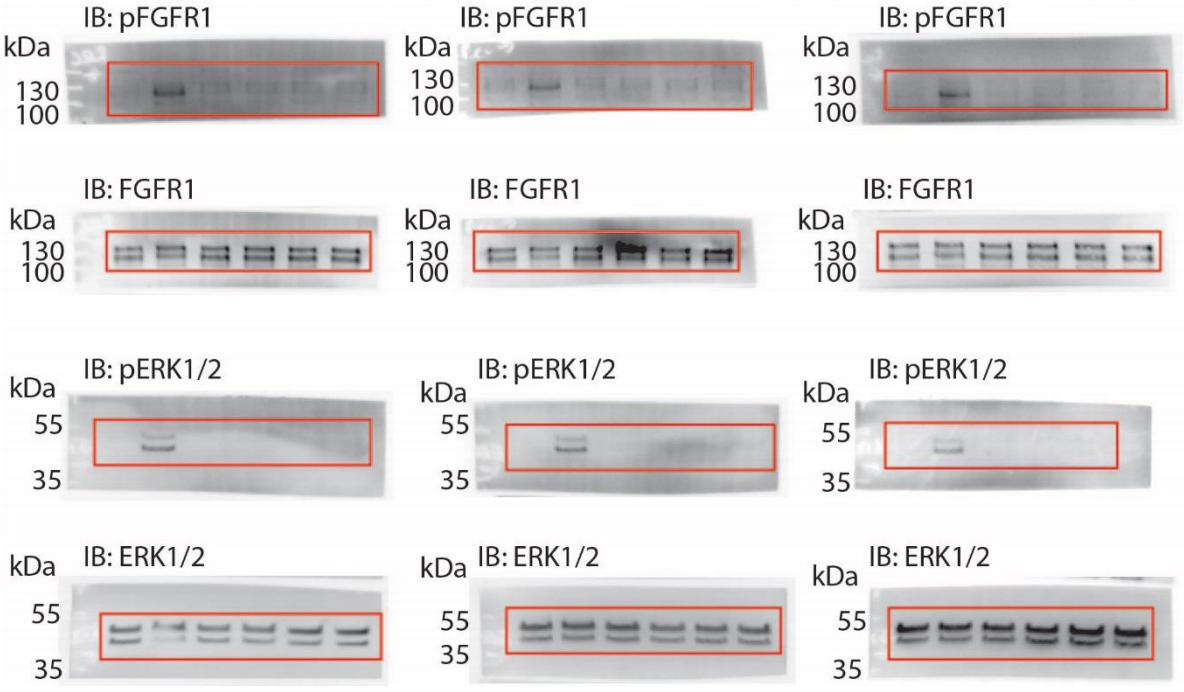


Figure 2A - bottom panel

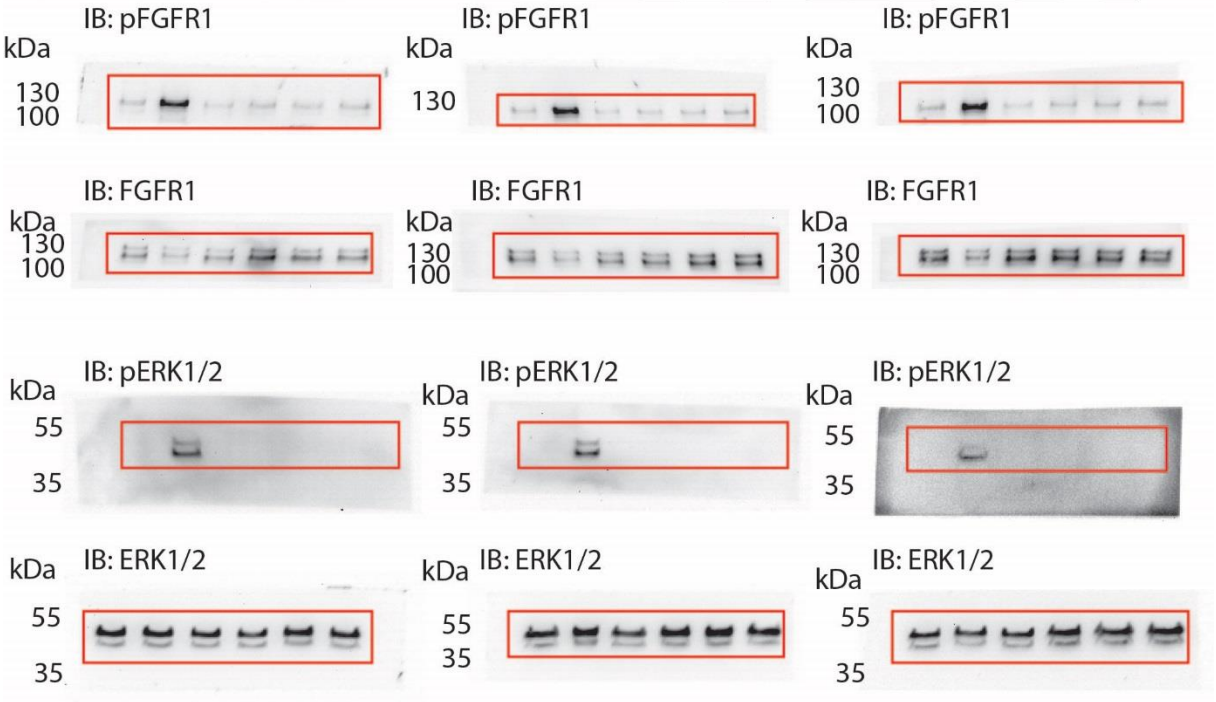


Figure 2B - left panel

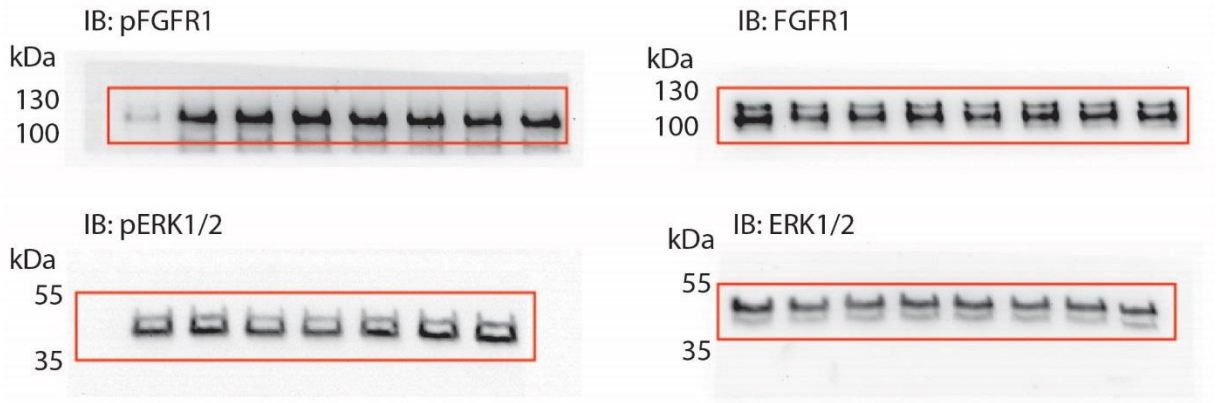


Figure 2C

Figure 2C, left panel

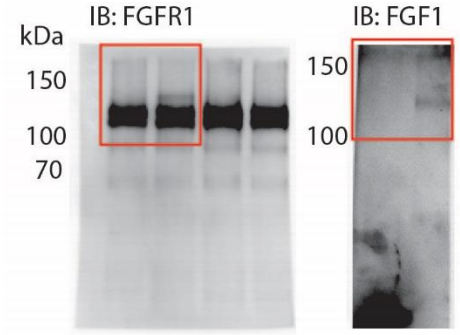


Figure 2C, right panel

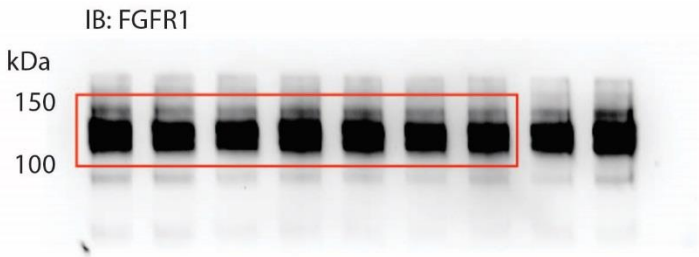


Figure 3A

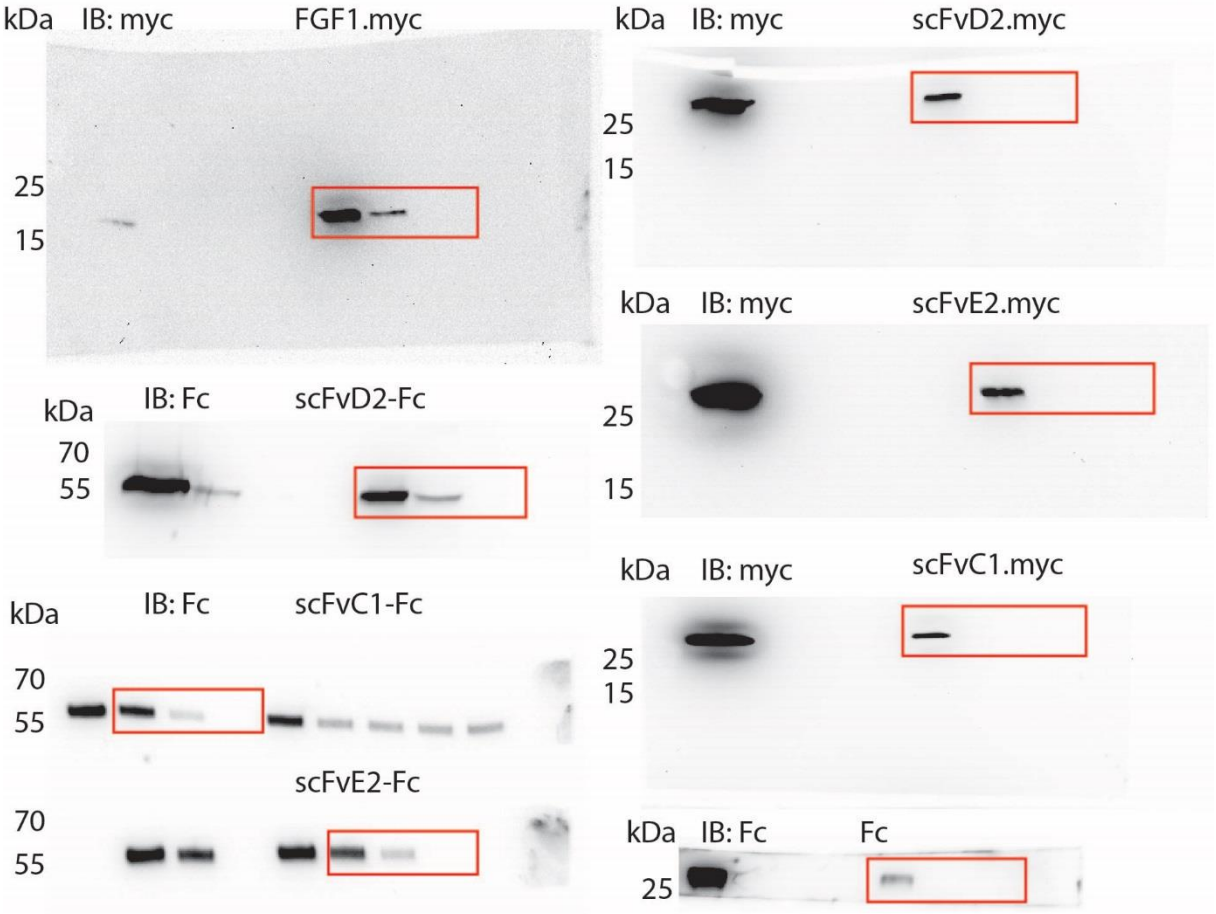


Figure 3B

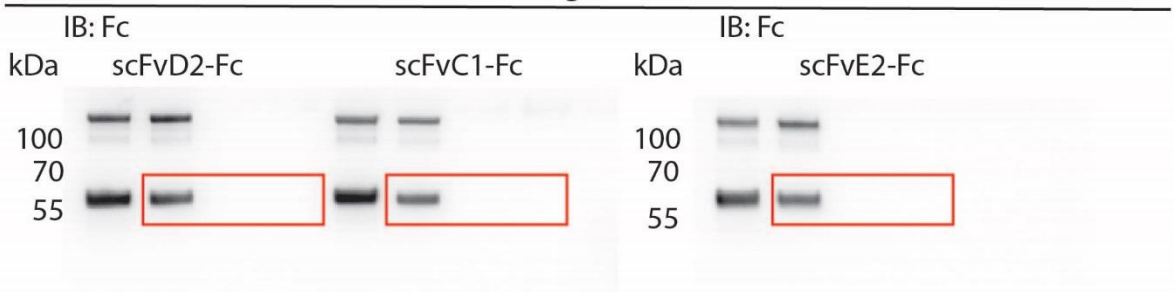


Figure 3C

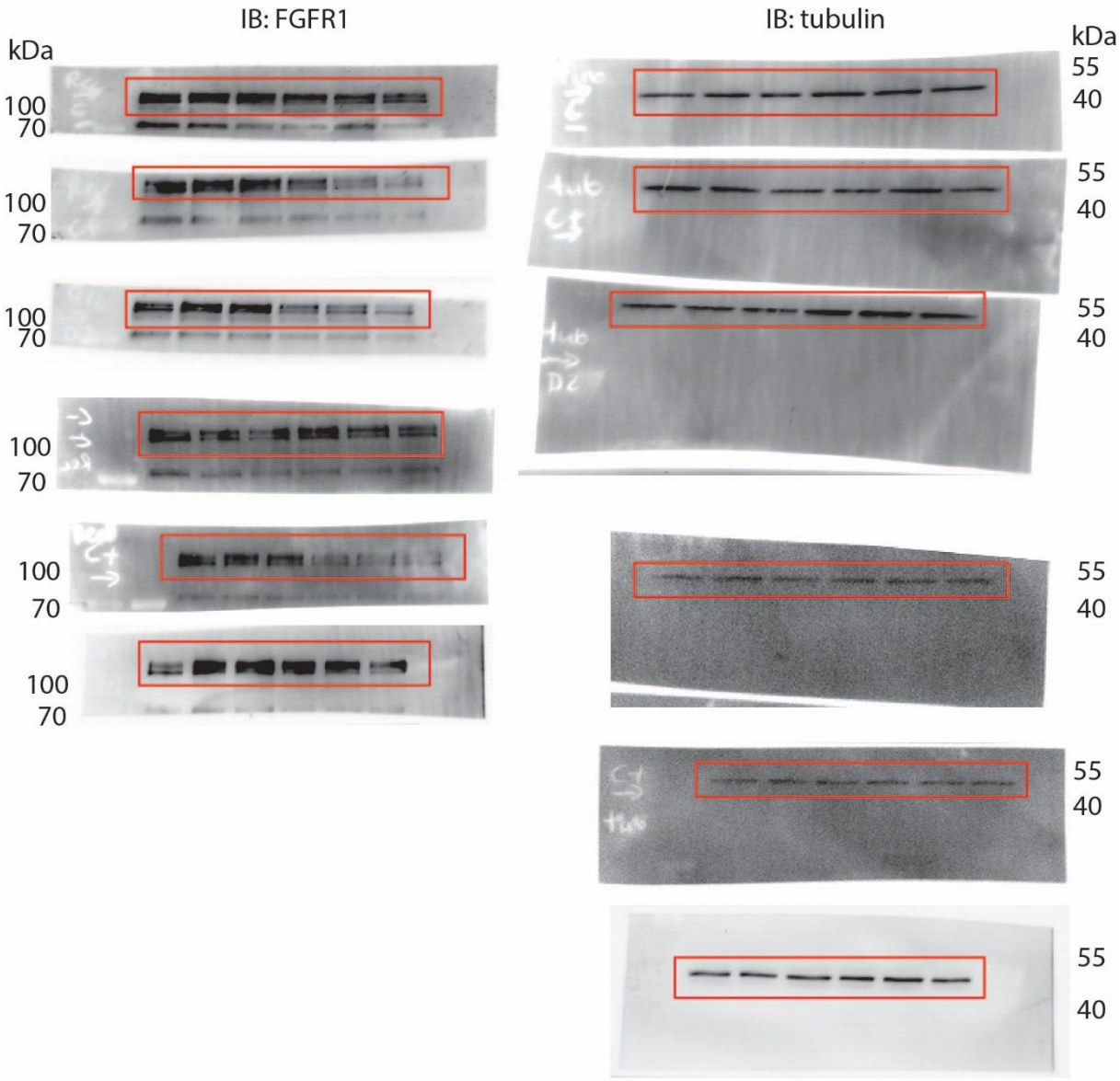


Figure 4A

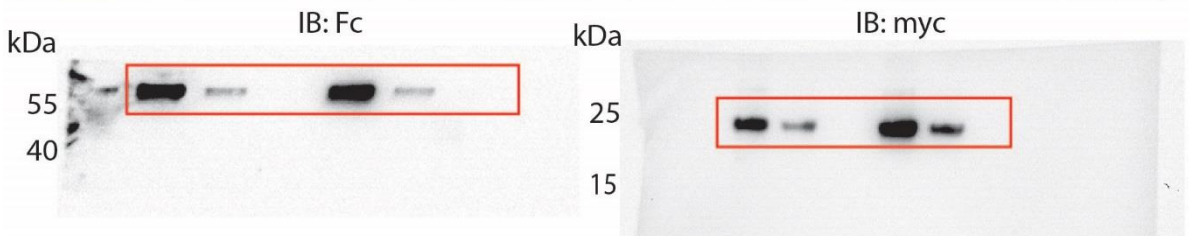


Figure 4B

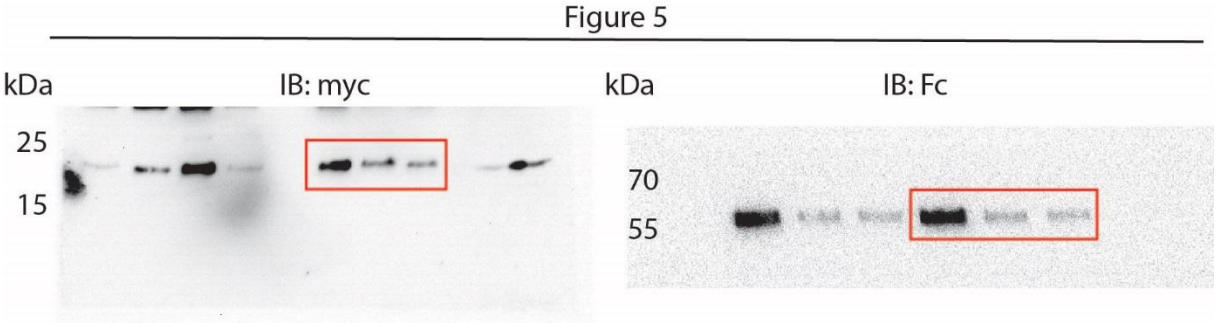


Fig. S8