Electronic Supplementary Information

Diagnostic nanoparticle targeting of the EGF-receptor in complex biological conditions using single-domain antibodies

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Figure S1. (A) Quantification of EGFR level by RT-qPCR. FaDu cells were treated with two siRNA (siEGF1 and siEGF2) and neg siRNA. The RNA was extracted after 48 h post-transfection. Transcription of EGFR mRNA was analysed using RT-qPCR assay. The relative mRNA amount was calculated in relation to negative silencing cells (n =4, mean ± S.D.). (B) Uptake of Alexa Fluor® 488-EGF in FaDu cells by flow cytometry. FaDu cells were treated with siEGFR and neg-siRNA for 48 h. The cells were then exposed to fluorescently labelled EGF (200 ng/mL at 37°C for 4 h. Shown is the median cell fluorescence intensity (MFI) obtained by flow cytometry of FaDu cells. (C & D) Confocal microscopy images of FaDu cells exposed to fluorescently labelled EGF protein (Alexa Fluor® 488-conjugated). Cells were exposed to 200 ng/mL of protein, for 2 h, then fixed and the nuclei was stained with DAPI. Control cells (non-EGFR receptor silenced) show a high EGF protein uptake (C), while silenced cells show significantly low uptake of protein (D).

Figure S2. Confocal microscopy of single-domain antibody silica NPs (SiO_2-sdAbs) in A431 cells. A431 cells were silenced 48 h with negative silencer control (A-D) and for EGFR (E-H) prior to exposure to SiO_2-sdAbs (100 µg/mL) in serum free DMEM for 4 h at 37°C. Blue: DAPI to visualize nucleus, green: LAMP1 to visualize lysosome, red: SiO_2-sdAbs. The overlay is shown in D and H for negative silenced cells and EGFR silenced cells respectively.

Figure S3. Determination of EGF concentration in human serum by dilution (A) and serum spiking (B). The results shown in (A) indicate that the ELISA assay is not able to correctly determine the concentration of EGF in samples with high serum concentrations. At sufficiently high dilutions of serum the results agree with one another (red; ~1280 pg/mL). Observed reading in for samples of serum spiked with EGF (B). Based on the given equation, the concentration of EGF can be calculated mathematically (~1145 pg/mL). Additions of EGF were made on a 1:5 dilution of serum.

Figure S1.

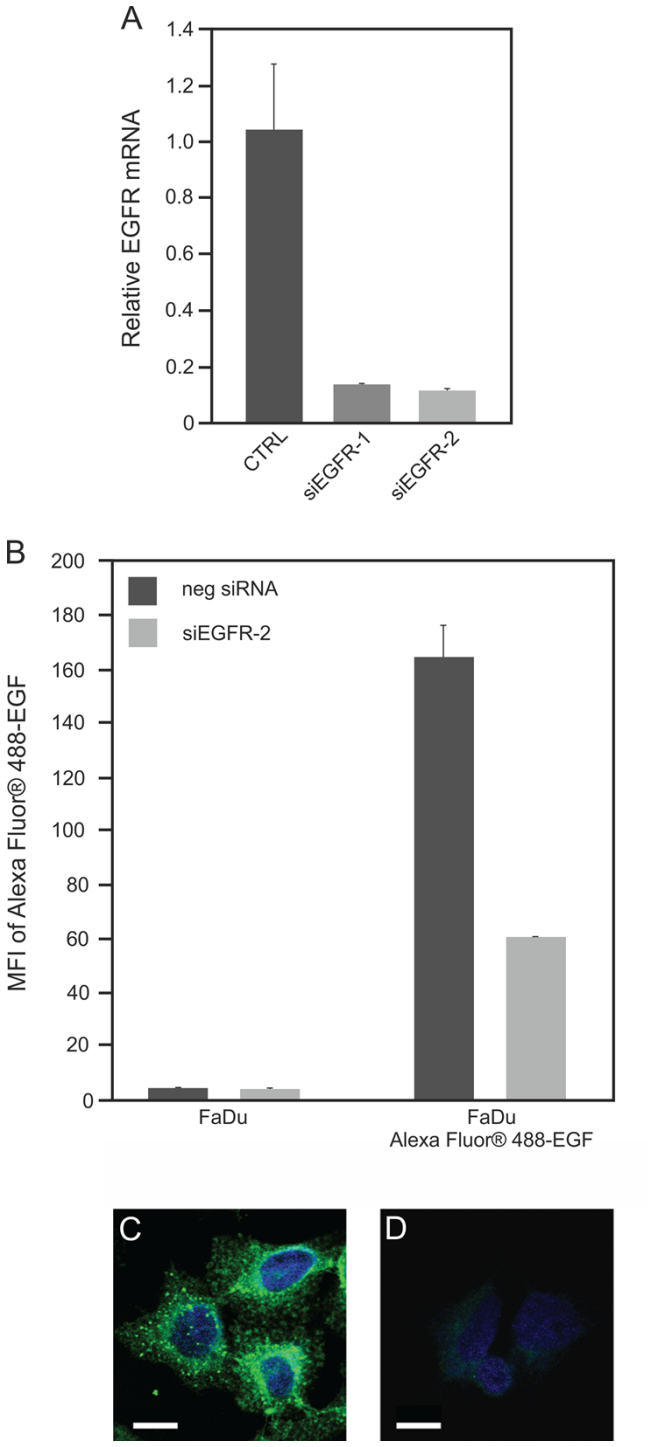

A
Relative EGFR mRNA
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
CTRL
siEGFR-1
siEGFR-2
B
MFI of Alexa Fluor® 488-EGF
200
180
160
140
120
100
80
60
40
20
0
neg siRNA
siEGFR-2
FaDu
FaDu
Alexa Fluor® 488-EGF
C
D

Figure S2.

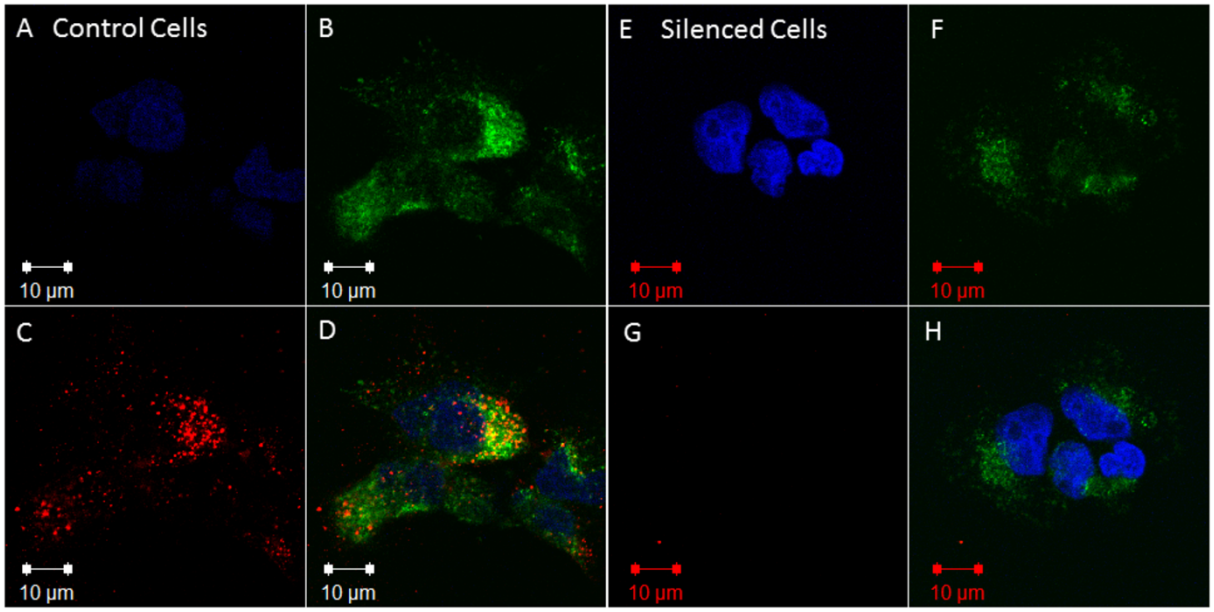
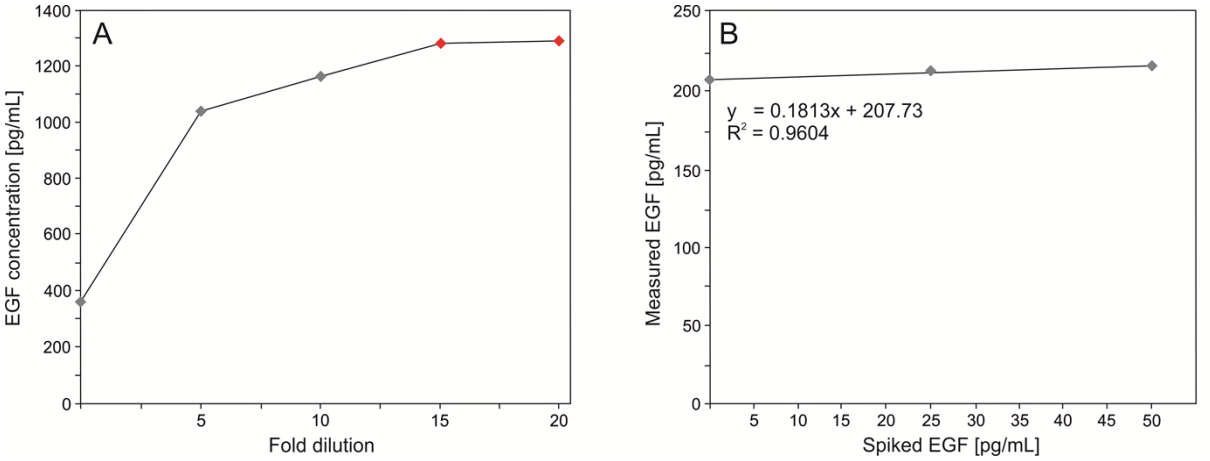

A Control Cells
B
E Silenced Cells
F
10 µm
10 µm
10 µm
10 µm
C
D
G
H
10 µm
10 µm
10 µm
10 µm

Figure S3


A
EGF concentration [pg/mL]
Fold dilution
0
200
400
600
800
1000
1200
1400
5
10
15
20
B
Measured EGF [pg/mL]
Spiked EGF [pg/mL]
y = 0.1813x + 207.73
R² = 0.9604
0
50
100
150
200
250
5
10
15
20
25
30
35
40
45
50