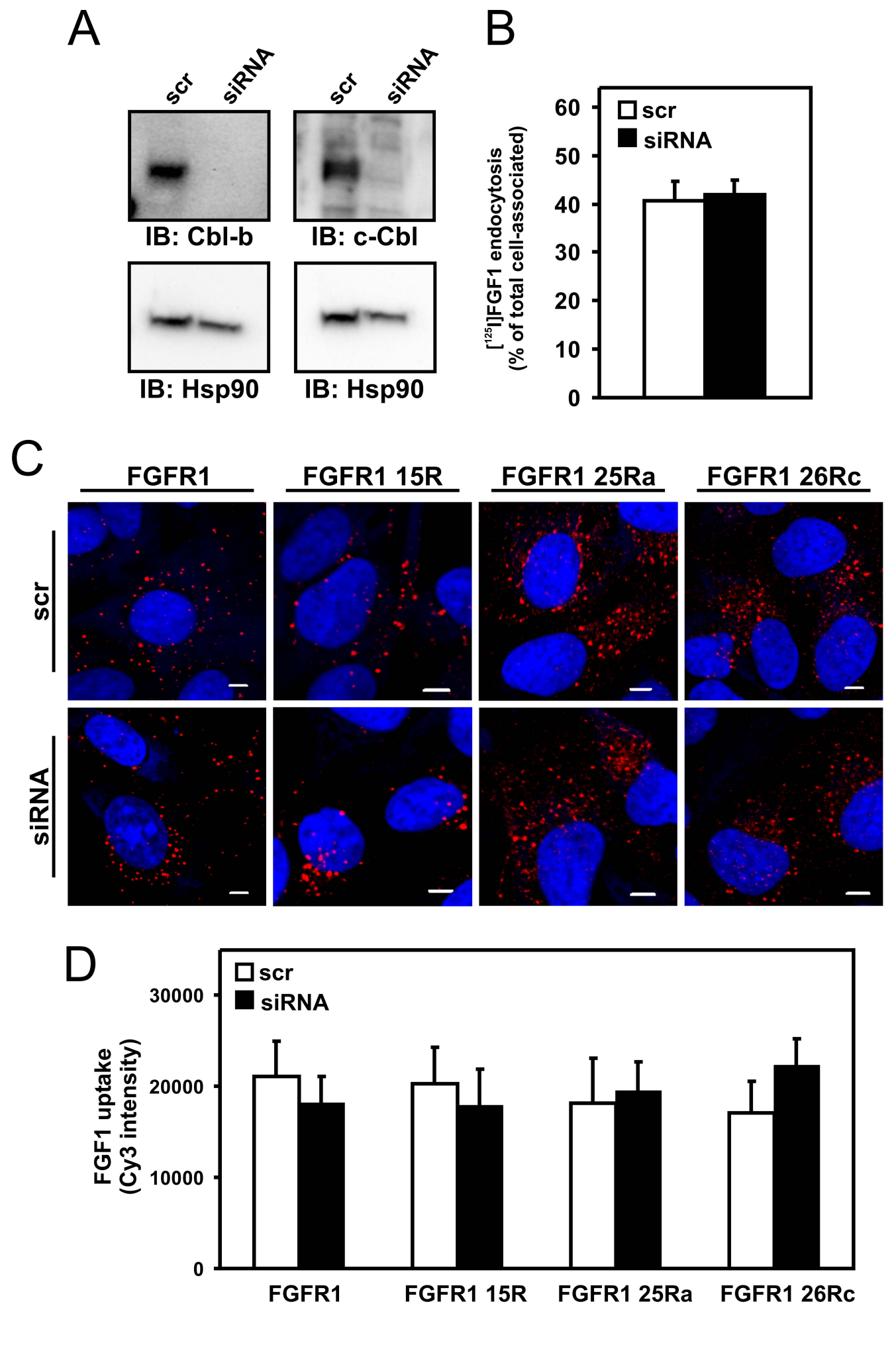
Supplementary figure legends

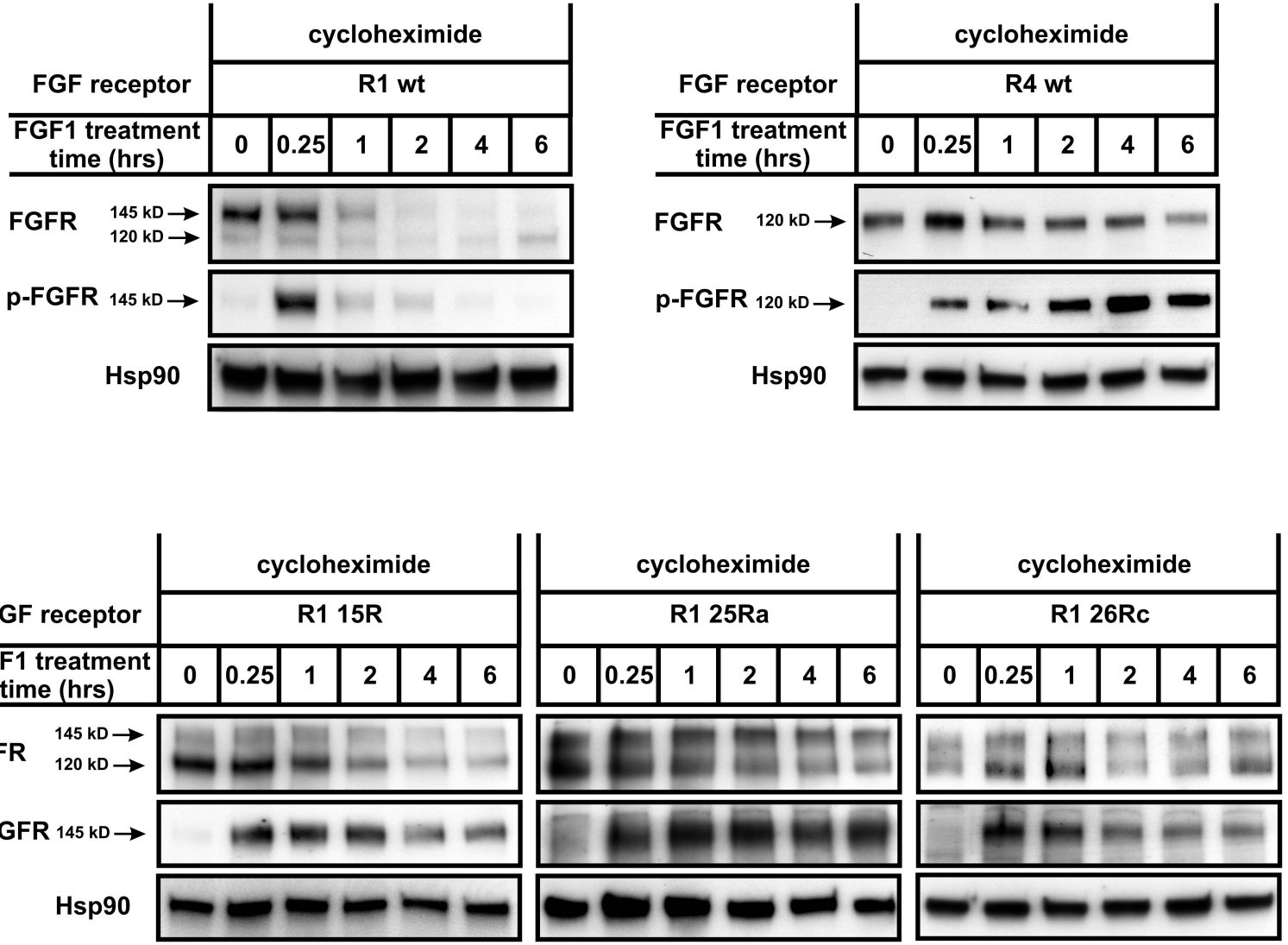
Supplementary Figure 1. The effect of c-Cbl and Cbl-b depletion on FGF1 internalization. **(A)** To knock down c-Cbl and Cbl-b, U2OS cells stably expressing FGFR1 were transfected with oligo-based siRNA against c-Cbl and Cbl-b or scramble (scr) siRNA and incubated for 2 days. Then the cells were re-transfected and incubated for 2 additional days. Cell lysate from transfected cells was analyzed by SDS-PAGE and immunoblotting with the indicated antibodies. **(B)** U2OS cells stably expressing FGFR1 and transfected as in (A) were grown on gelatinized plates and then incubated in HEPES medium with 5-10 ng/ml [^{125}I]FGF1, 20 U/ml heparin and 0.2% gelatine at 37°C for 10-15 minutes. Internalized and surface-bound [^{125}I]FGF1 were separated as described in Materials and methods. The degree of endocytosis was calculated as percentage of total cell associated [^{125}I]FGF1. The mean of three independent experiments are shown. Error bars denote the standard error. **(C)** U2OS cells, stably expressing FGFRs as indicated and transfected as in (A) were incubated with Cy3-FGF1 and 50 U/ml heparin at 37°C for 20 minutes before fixation. The cells were stained with DRAQ5 and examined with confocal microscopy. Confocal scanning and picture processing was performed with identical settings for each receptor construct. Bar, 5 μM. **(D)** The uptake of Cy3-FGF1 was measured as Cy3 intensity in scrambled (□) or c-Cbl siRNA and Cbl-b siRNA transfected (■) U2OS cells stably expressing indicated FGFR constructs treated as in (C). The cells were also stained with anti-FGFR1 antibody. Cells expressing similar amounts of FGFRs were selected for quantification based on their intensity of anti-FGFR1 antibody staining and 5-13 cells were quantified in each case. Confocal scanning was performed with identical settings. The mean intensity in each case is presented in the histogram and error bars denote the standard error.

Supplementary Figure 2. Half-life of phosphorylated FGFR in the presence of cycloheximide. U2OS cells, stably expressing various types of FGF receptors, were serum-starved for 24 hours and then left untreated or treated with 100 ng/ml FGF1 and 10 U/ml heparin, in the presence or absence of 20 μg/ml cycloheximide. Cells were lysed and the cellular material was analyzed by SDS-PAGE and immunoblotting with the indicated antibodies. A p in front of the name of the antibody indicates that it recognizes the phosphorylated form of the protein.

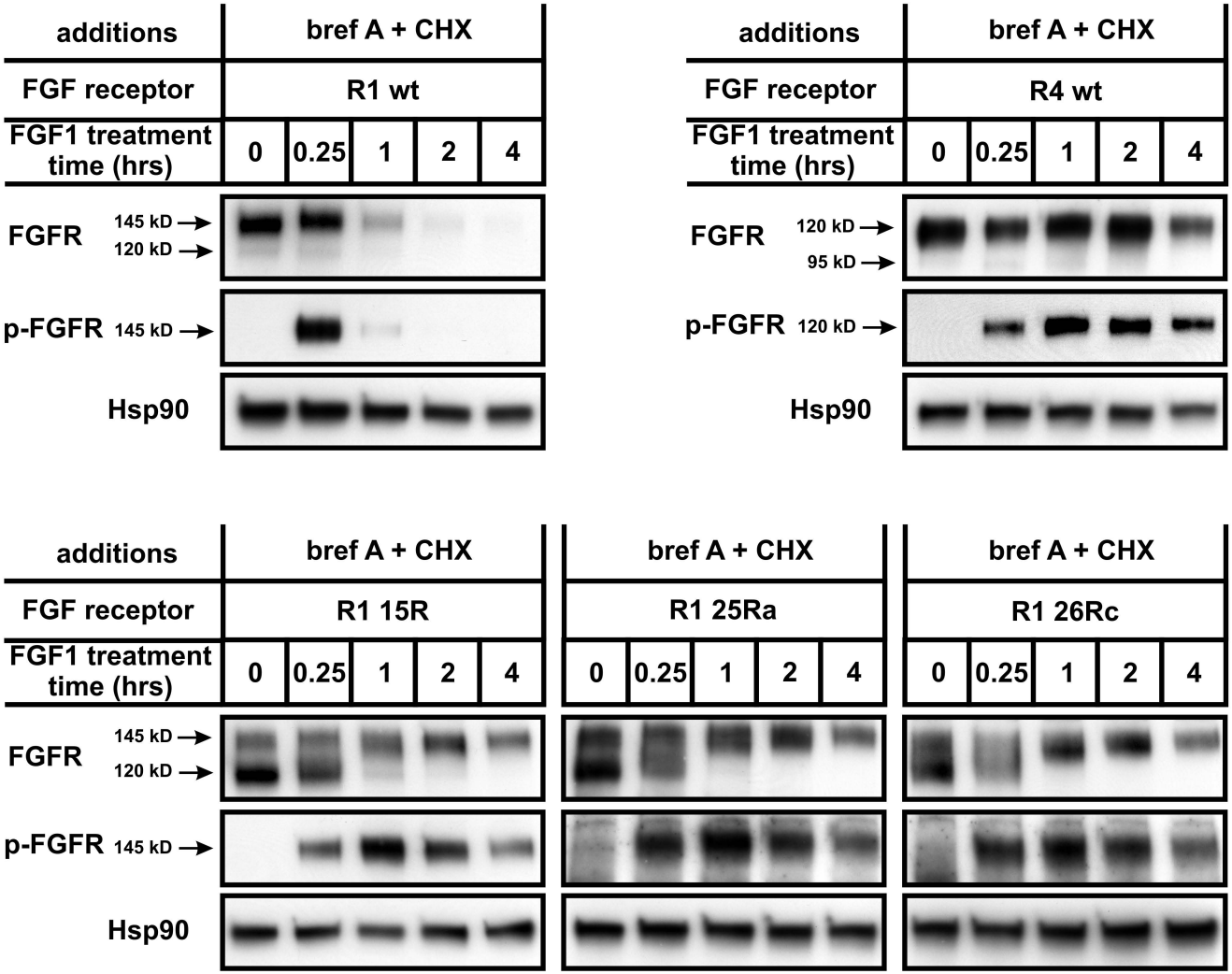
Supplementary Figure 3. Half-life of phosphorylated FGFR in the presence of cycloheximide and brefeldin A. U2OS cells, stably expressing various types of FGF receptors, were serum-starved for 24 hours and then left untreated or treated with 100 ng/ml FGF1 and 10 U/ml heparin, in the presence or absence of 20 μg/ml cycloheximide (CHX) and 2 μg/ml brefeldin A (bref A). Cells were lysed and the cellular material was analyzed by SDS-PAGE and immunoblotting with the indicated antibodies. A p in front of the name of the antibody indicates that it recognizes the phosphorylated form of the protein.



Supplementary Fig 1



Supplementary Fig 2



Supplementary Fig 3