Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary information.

Targeting ferritin receptors for the selective delivery of imaging and therapeutic agents to breast cancer cells.

S. Geninatti Crich, M. Cadenazzi, S. Lanzardo, L. Conti, R. Ruiu, D. Alberti, F. Cavallo, J.C. Cutrin, S. Aime.*

Figure S1

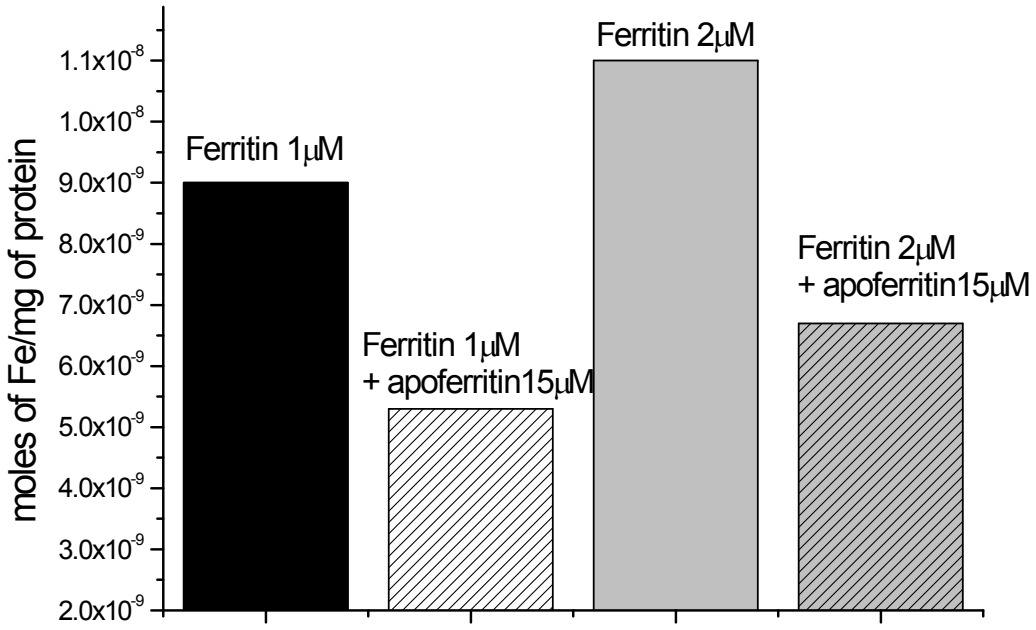

Figure S1. Competition studies with free apoferritin. Free apoferritin (15μM) was added to the culture medium of MCF-7 cells 1.5 h before the addition of ferritin (1 and 2 μM, respectively). After 6 hours incubation cells were washed with cold PBS, detached with trypsin/EDTA and the amount of Fe taken-up by cells was measured by ICP-MS.

Figure S2

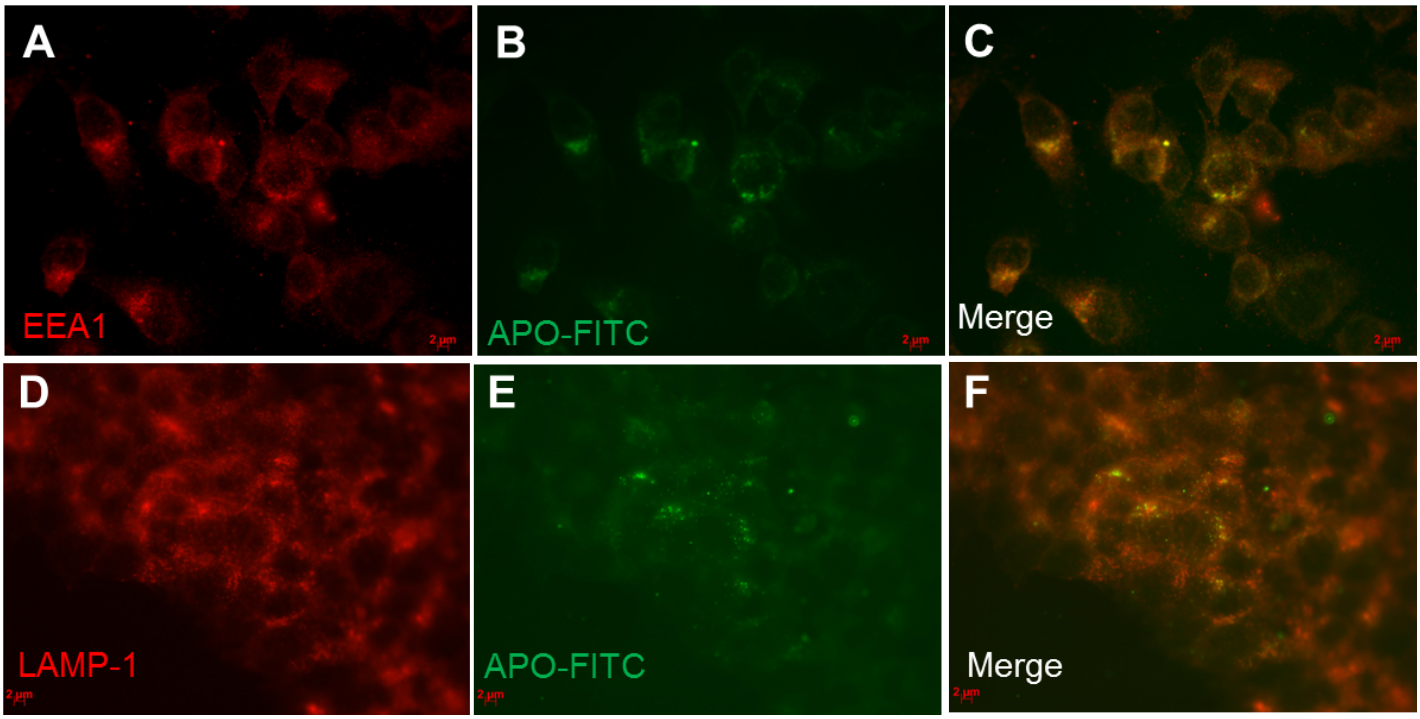

Figure S2. MCF-7 cells were stained for the early endosome marker EEA1 (upper panels) and the lysosome marker LAMP-1 (lower panels) after 30 minutes or 24 h of incubation with APO-FITC, respectively. Images were acquired with an Apotome fluorescent microscope (Leica), 63X.

APO-FITC preparation.

A 2mg/ml apoferritin solution in 0.1 M sodium carbonate buffer at pH 9 was prepared. The fluorescein isothiocyanate (FITC, Sigma-Aldrich) was dissolved in anhydrous DMSO at 1 mg/ml; for each mg of protein, 50 µl of FITC solution were added slowly in 5 µl aliquots while stirring the protein solution.When all the required amount of FITC solution was added, the reaction mixture was incubated in the dark for 16 hours at 4°C. Then NH_4Cl was added to the solution to a final concentration of 50 mM, andit was incubated for other 2 hours at 4°C.

The obtained APO-FITC solution was purified from non-entrapped FITC in two steps: gel filtration using a G25 sephadex column, followed by dialysis. After purification, the solution was characterized in terms of protein concentration using the Bradford assay. The FITC concentration was determined by measuring fluorescence at 492/517 nm ex/em in triton 0.1%. .

Immunofluorescence protocol. For the intracellular detection of APO-FITC, $3x10^5$ MCF-7 cells were plated on glass coverslips and left to adhere ON at 37°C in a 5% CO_2. The day after, cells were incubated with 150 μl/ml of APO-FITC (FITC 6.02 M, APO 2.35 μM) for different intervals and then washed twice with PBS. Coverslips were fixed with acetone (Sigma-Aldrich) for 10 min at -20°C and washed twice with PBS. Non-specific binding was blocked with 10% bovine serum albumin (BSA, from Sigma-Aldrich) in PBS for 20 minutes at room temperature. The anti-EEA1 antibody (*BD Biosciences*) or the anti-LAMP-2 antibody were respectively diluted 1/200 or 1/50 in PBS containing 1% BSA and added to the coverslips for 1 hour at room temperature. Cells were rinsed twice with PBS and then incubated with Texas Red conjugated goat anti-mouse Ig (Invitrogen; 1/1000) in PBS containing 1% BSA for 1 hour at room temperature. Cells were rinsed three times with PBS and air dried.
Finally, coverslips were mounted with Fluoromount mounting medium (Sigma-Aldrich) and visualized with an Apotome fluorescent microscope (Leica). Photographs were taken using a digital CCD camera and images were processed using the AxioVision (Zeiss, V. 4.4), Adobe Photoshop and Microsoft PowerPoint softwares. Software program ImageJ was used to quantify fluorescence intensity.