SUPPLEMENTARY INFORMATION.

Supplementary Materials

Humanized Archaeal ferritin as a tool for cell targeted delivery.

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Figures and Table

Figure S1: Structure-based sequence alignment of AfFt, HumAfFt and HuHF.

Complete sequence alignment of AfFt, HumAfFt and HuHF. Elements of secondary structure for the AfFt are shown on the top. White characters in a red background indicate strict conservation while residues with poor conservation are drawn in black on a white background.

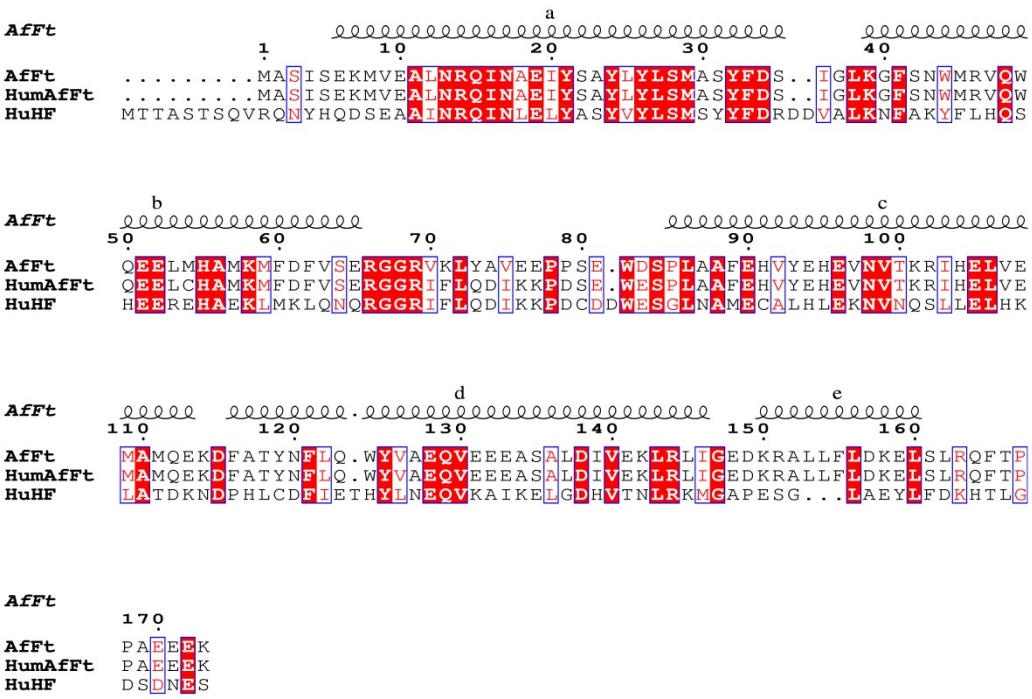

TABLE S1

Table S1: Data collection and refinement statistics[a]

[a]Numbers in parentheses refer to data in the highest resolution shell. R_{free} was computed omitting 5% of the reflections as a test set.

Data Collection	
Space Group	$C222_1$
Cell Dimensions a, b, c (Å)	185.80 190.65 176.54
Resolution (Å)	48.86 - 2.94 (3.00 - 2.94)
R_{merge}	0.134 (0.751)
Unique Reflections	59926 (4378)
$I/\sigma I$	7.8 (1.8)
Completeness (%)	95.8 (95.6)
Redundancy	4.2 (4.3)
Refinement	
Resolution (Å)	49.69 - 2.94 (3.00 - 2.94)
No. reflections	56823 (346)
R_{factor} R_{free}	0.27 (0.61) 0.30 (0.59)
Ion (Mg^{2+})	2
water	13
R.m.s deviations	
Bond lengths (Å)	0,007
Bond angles (°)	0,99

FIGURE S2

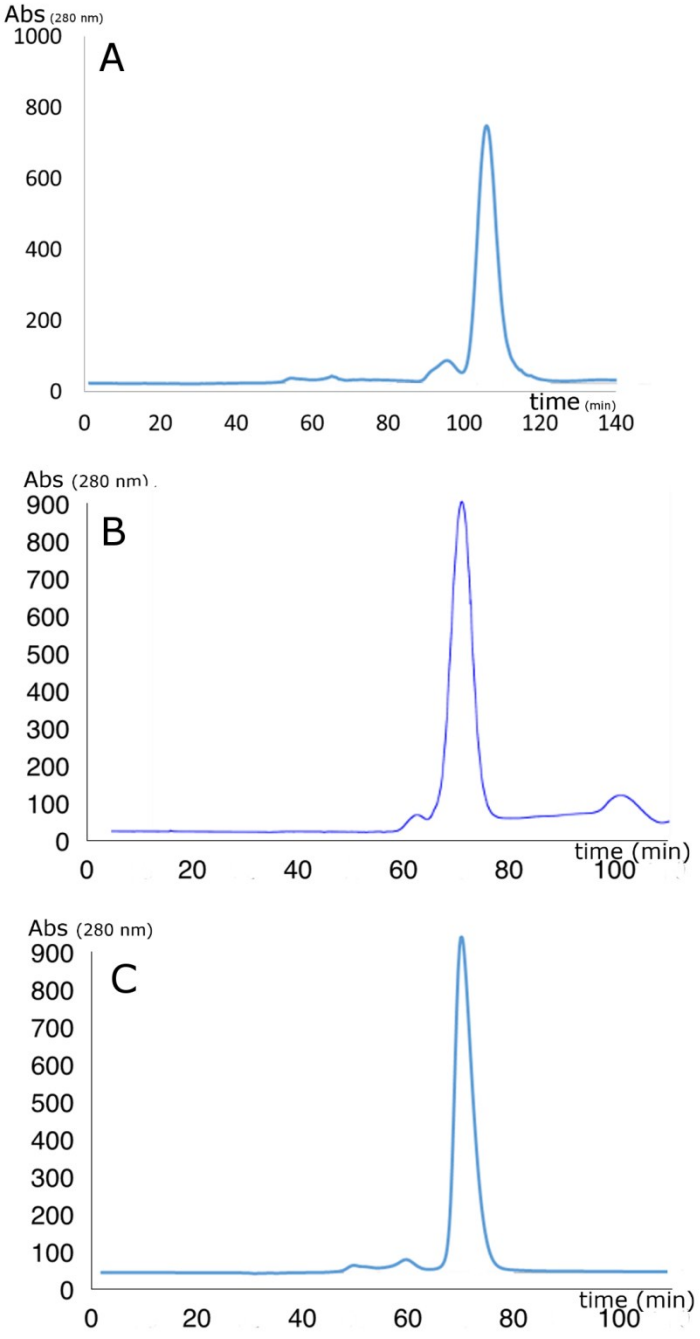

FIGURE S3

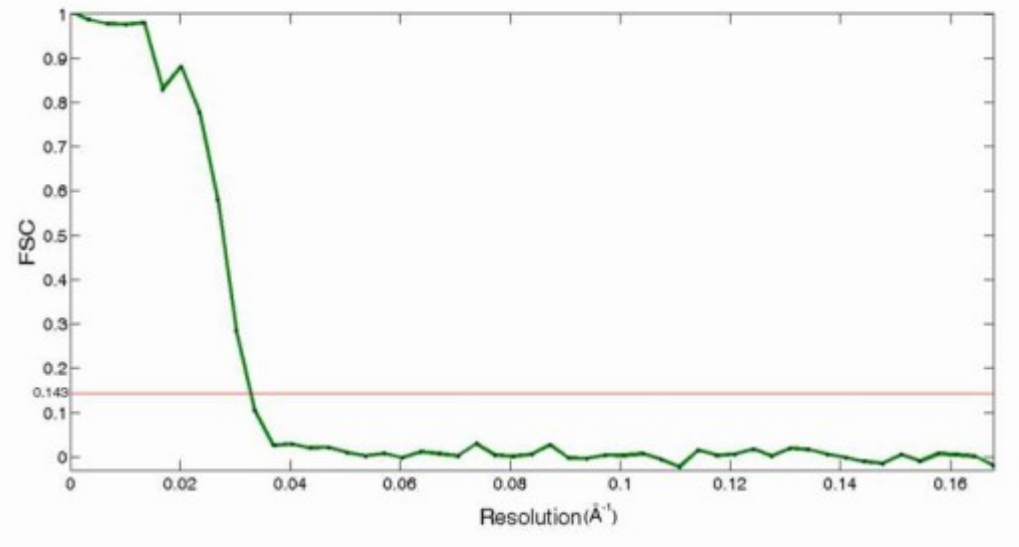

Figure S3: Cryo-EM FSCs curve.

Gold standard Fourier Shell Correlation (FSC) curve of the HumAfFt three-dimensional reconstruction. Red line represents the resolution (equivalent to 33.1 Å) at which FSC=0.143.

FIGURE S4

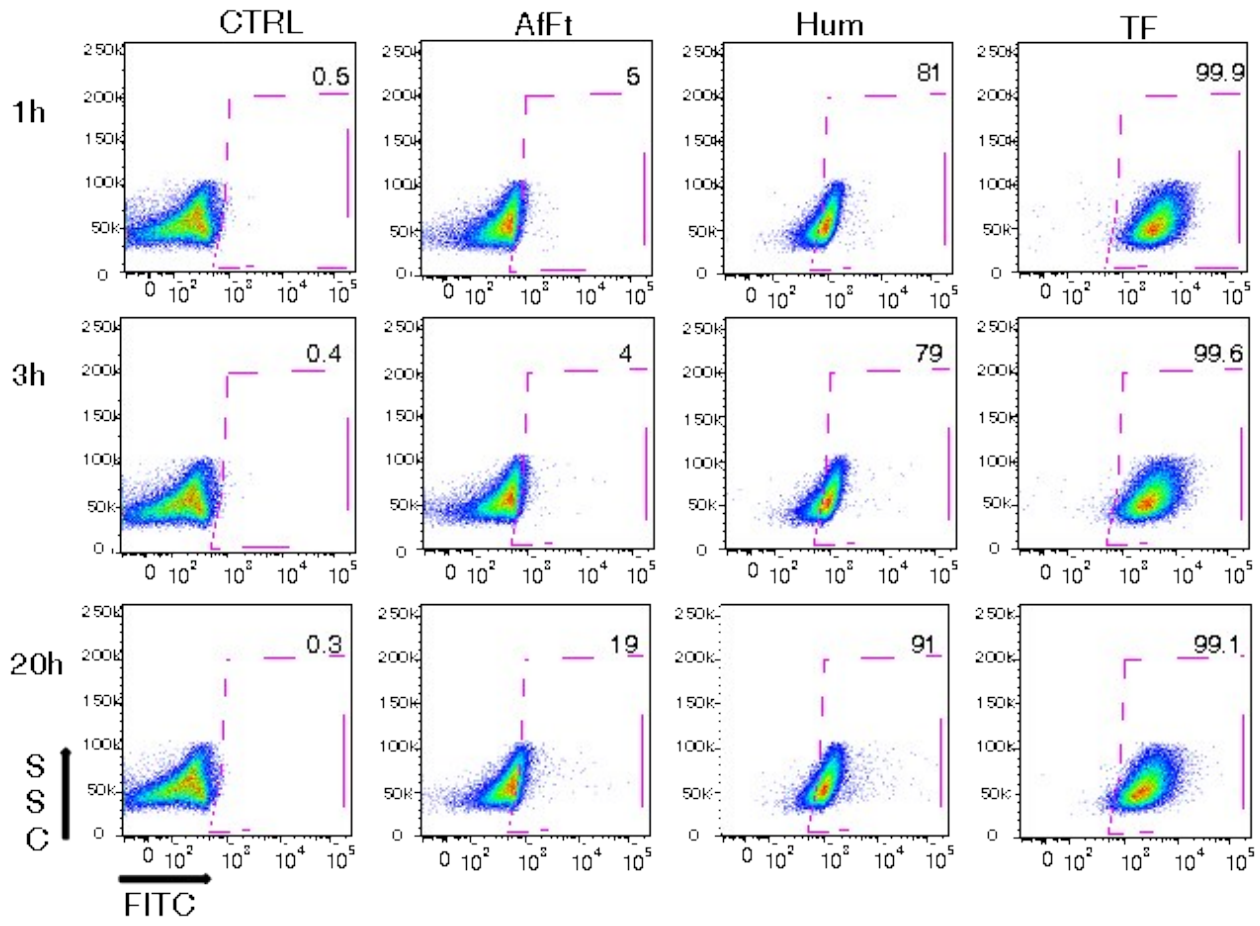

Figure S4: Ferritin internalization analysis by flow cytometry.

Detailed FACS plot from the data presented in Fig.4. FACS analysis of HeLa cells show the increased uptake of HumAfFt compared to the original AfFt in the population at different time. Cells were treated and data were acquired as described in the Material and Methods section. As shown in the plots, the gate for the final detection was set in the control sample (CTRL) and the percentage of FITC positive cells for AfFt, HumAfFt and Transferrin (Tf) is indicated in each plot. For each sample 30,000 events gated on live cells were acquired.

FIGURE S5

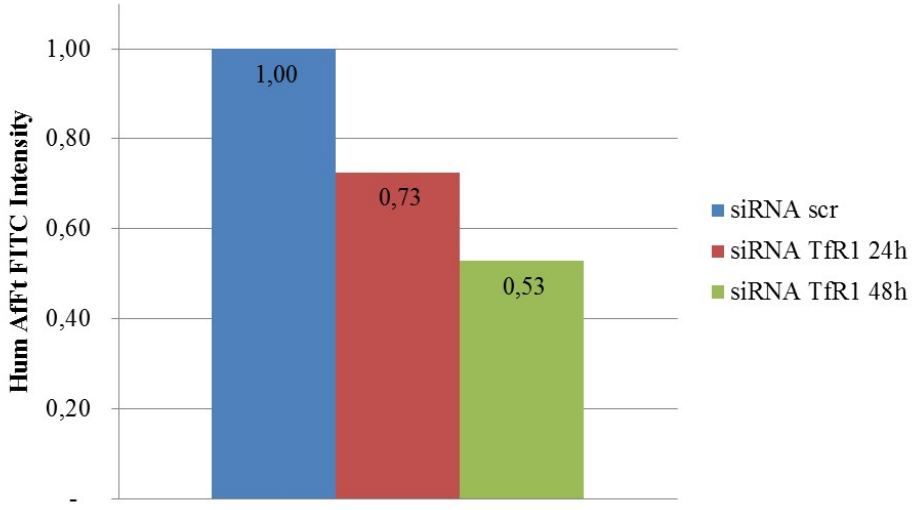

Figure S5: TfR1 silencing causes a decreased Hum AfFt uptake.

HeLa cells transfected with control (scr) and specific anti-TfR1 (TfR1) siRNA for 24 and 48 hours, were incubated with Hum AfFt-FITC. After 3 hours cells were collected, washed and analyzed at the cytometer for the FITC fluorescent intensity. Compared to the FITC intensity in control cells (scr) we obtained an uptake reduction of almost 50% after 48 hours of RNAi.

FIGURE S6

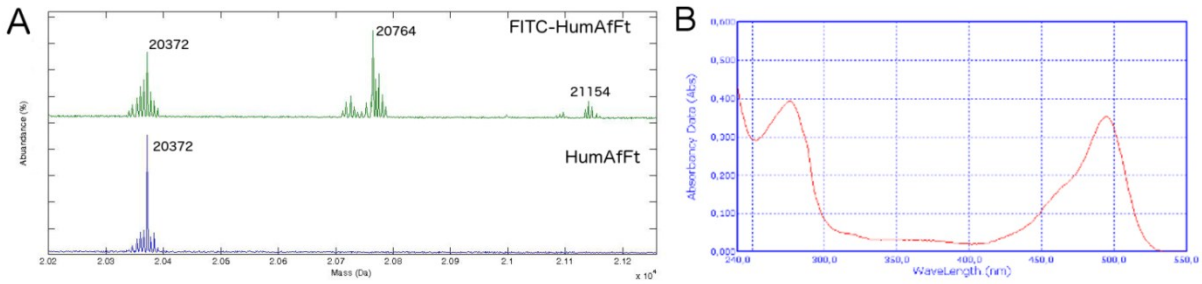

Figure S6: Analyses of FITC content.

A) LC–MS measurements carried out on HumAfFt (blue) and FITC-HumAfFt (green). Each protein is eluted as monomer (20372 Da) and FITC reacted protein shows a shifted peak of +389 ± 4 Da, and a second peak of +778± 4 Da in agreement with the expected molecular weight of one and two molecules of FITC per monomer corresponding to 52% and 9% respectively. B) Uv-Vis spectrum of the FITC-HumAfFt after purification on a G25 gel filtration column (GeHealthcare).

Supplemental Materials and Methods

TfR1 silencing in HeLa cells.

Hela cells were transfected with Lipofectamine® RNAiMAX (Life Technologies) following standard procedure. The siRNA against TfR1 was purchased from Sigma (product# NM_003234, siRNA ID: SASI_Hs01_00059217). Cells were then incubated with 30µg/ml of Hum AfFt-FITC, treated and analized as described in the methods session. The FITC intensity was normalized for the FITC intensity of the scr samples.

LC–MS measurements.

LC–MS was performed on protein samples reacted with 10 molar excess of FITC per ferritin monomer in comparison with unreacted protein. Protein samples were diluted with distilled water in the presence of 1 mM EDTA and loaded on a Waters Acquity UPLC connected to Waters Acquity Single Quad Detector. A BEH300 C_4 column was used: 1.7 µm, 2.1 × 150 mm at 220 nm observation wavelength; mobile phase: 0.1% TFA in water: MeCN (0.1% TFA); gradient 20:65% over 60 min; flow rate: 0.25 ml min^{-1}; MS mode was set at a scan range: m/z=200 – 2,000 (ES+); scan time: 0.5 s, electrospray source with a capillary voltage of 3.0 kV and a cone voltage of 45 V. N_2 gas was used as nebulizer and desolvation gas at a total flow of 200 l/hours. Ion series were generated by integration of the ultraviolet-absorbance (at 220 nm) chromatogram over 2.4–2.8 min range. Mass spectra were reconstructed from the ion series by using MassLynx software program.