Electronic Supplementary Material (ESI) for Nanoscale.

SUPPORTING INFORMATION

Chemical Sporulation and Germination: Cytoprotective Nanocoating of Individual Mammalian cells with Degradable Tannic Acid-Fe^{III} Complex

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Experimental Details

Materials. HeLa cells (HeLa, No. 10002, Korean Cell Line Bank), NIH 3T3 fibroblast cells (NIH 3T3, No. 21658, Korean Cell Line Bank), Jurkat clone E6-1 cells (Jurkat, No. 40152, Korean Cell Line Bank), tannic acid (TA, Sigma), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, Sigma), Dulbecco's modified Eagle's medium (DMEM, Welgene), Roswell Park Memorial Institute 1640 (RPMI, Welgene), fatal bovine serum (FBS, Welgene), penicillin-streptomycin (5,000 U mL^{-1} of penicillin and 5,000 μg mL^{-1} of streptomycin, Welgene), phosphate-buffered saline (PBS, 10 mM, pH 7.4, Welgene), trypsin-ethylenediaminetetraacetic acid solution (1×, 0.05% trypsin, 0.53 mM EDTA·4Na in Hanks' balanced salt solution, Welgene), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Sigma-Aldrich), dimethyl sulfoxide (DMSO, Sigma), Live/Dead® viability/cytotoxicity kit (Life Technologies), Alexa Fluor® 647-conjugated albumin from bovine serum (BSA-Alexa Fluor 647, Life Technologies), 4',6-diamidino-2-phenylindole (DAPI, Vector Laboratories), polyethylenimine (PEI, average Mn: ~10,000 by GPC, average Mw: ~25,000 by LS, Aldrich), ethylenediaminetetraacetic acid (EDTA, Sigma-Aldrich), paraformaldehyde (Sigma-Aldrich), triton X-100 (1%, Life Technologies), bovine serum albumin (BSA, Santa Cruz Biotechnology), and Alexa® 488 palloidin (Life Technologies) were used as received. Ultrapure water (18.3 MΩ·cm) from the Human Ultrapure System (Human Corp.) was used.

Cell culture. HeLa or NIH 3T3 cells were seeded in a cell culture flask with 10 mL of DMEM containing 10% FBS and 1% penicillin-streptomycin, and incubated at 37 ℃ in a humidified atmosphere of 5% CO_2. After the cells were grown to 80% confluence, they were washed twice with PBS. Trypsin (2 mL) was the added to the culture flask, and the cells were incubated at 37 ℃ for 5 min. After the cells were detached from the flask, 3 mL of DMEM was added, and the cells were collected by centrifugation and washed twice with PBS. Jurkat cells were seeded in a cell culture flask with 10 mL of RPMI containing 10% FBS and 1% penicillin-streptomycin, and the cells were incubated at 37 ℃ in a humidified atmosphere of 5% CO_2. After the cells were grown to 80% confluence, they were collected by centrifugation and washed twice with PBS.

TA-Fe^{III} coating. The DMEM (for HeLa and NIH 3T3 cells) or RPMI (for Jurkat cells) solution (0.5 mL) of TA (0.4 mg mL^{-1}) and the DMEM or RPMI solution (0.5 mL) of $FeCl_3 \cdot 6H_2O$ (0.1 mg mL^{-1}) were added sequentially to the collected cells. The resulting suspension was mixed for 10 s and then washed with DMEM or RPMI. The process was repeated up to three times.

Viability test. The viability of the cells was investigated by the MTT colorimetric assay. In a 96-well plate, the cells were seeded in 100 μL of DMEM (for HeLa and NIH 3T3 cells) or RPMI (for Jurkat cells) at a density of 2×10^4 cells mL^{-1}. The MTT solution (10 μL of 5 mg mL^{-1} MTT in PBS) was added to each well, and the cells were incubated for 4 h. The stained cells were collected by centrifugation, and DMSO was added to dissolve the water-insoluble purple formazan product. The absorbance of formazan at 560 nm was measured with a microplate reader (Molecular Devices). The Live/Dead® staining solution was prepared by dissolving 5 μL of calcein AM and 20 μL of ethidium homodimer-1 in 10 mL of PBS (50 mM; pH 7.4). The resulting solution was added to each well, and the cells were incubated for 20 min. After

incubation, the stained cells were observed with a fluorescence microscope (Eclipse Ti, Nikon) or a laser-scanning confocal microscope (LSM 700 META, Carl Zeiss). All the viability tests were performed immediately after coating.

Protein conjugation. The TA-Fe^{III}-coated cells were mixed with an aqueous PBS solution of BSA-Alexa 647 (0.4 mg mL^{-1}), and the resulting solution was incubated for 15 min (at 37.0 ℃ with 5% CO_2). The stained cells were observed with a laser-scanning confocal microscope (LSM 700 META, Carl Zeiss).

Cytoprotection tests. The cytoprotection tests were performed with UV-C and PEI. To test how well the TA-Fe^{III} nanocoat protected the cells from UV-C, a cell-containing cuvette was placed in a home-made dark chamber equipped with a 4 W-filtered UV lamp and irradiated with UV-C light (254 nm) for 50 min. The cell viability was measured every 5 min by the MTT assay. After the cells were collected by centrifugation, the DMEM (for HeLa and NIH 3T3 cells) or RPMI (for Jurkat cells) solutions containing various concentrations of PEI (0.0001, 0.001, 0.01, 0.1, 1, 10, and 30 mg mL^{-1}) were added to the cells, which had been seeded in a 96-well plate at a density of 2×10^4 cells mL^{-1}. The cells were incubated under culture conditions for 24 h, and the viability was determined by the MTT assay.

Cell culture test. The cells were seeded in a 24-well plate at a density of 2×10^4 cells mL^{-1} and then incubated in DMEM (for HeLa and NIH 3T3 cells) or RPMI (for Jurkat cells) containing 10% serum and 1% penicillin-streptomycin. After incubation, the cells were observed with an inverted microscope (AE 2000, Motic).

Immunocytochemistry. The cells were fixed in 4% paraformaldehyde at room temperature for 15 min. The fixed cells were washed with PBS and then treated with triton X-100 (1%) for 15 min to permeabilize the cell membrane. The permeabilized cells were incubated in 6% BSA for 30 min at 37 ^oC , washed with PBS, and then incubated in PBS with 1.5% BSA solution of Alexa® Fluor 488 phalloidin (0.33 μM) for 1 h at 37 ^oC to stain F-actin. The glass slides that were sterilized with 70% ethanol and dried under a stream of argon gas were prepared beforehand. The immunostained samples were washed with PBS and mounted on the glass slides with the mounting solution containing DAPI to stain nuclei. Fluorescent images were taken by laser-scanning confocal microscopy (LSM 700 META, Carl Zeiss).

Characterizations. Scanning electron microscopy (SEM) imaging was performed with a Sirion FEI XL FEG/SFEG microscope (FEI Co.) with an accelerating voltage of 10 kV after sputter-coating with platinum. Transmission electron microscopy (TEM) imaging was performed using a Tecnai-G2 Spirit Twin instrument (FEI Co.). Specimens were fixed with glutaraldehyde and OsO_4 and then dehydrated in ethanol. The fixed samples were embedded in Epon 812/Araldite M resin. Thin sections (ca. 80 nm) were cut by using ULTRACUT UCT ultramicrotome (Leica) and stained with uranyl acetate and lead citrate.

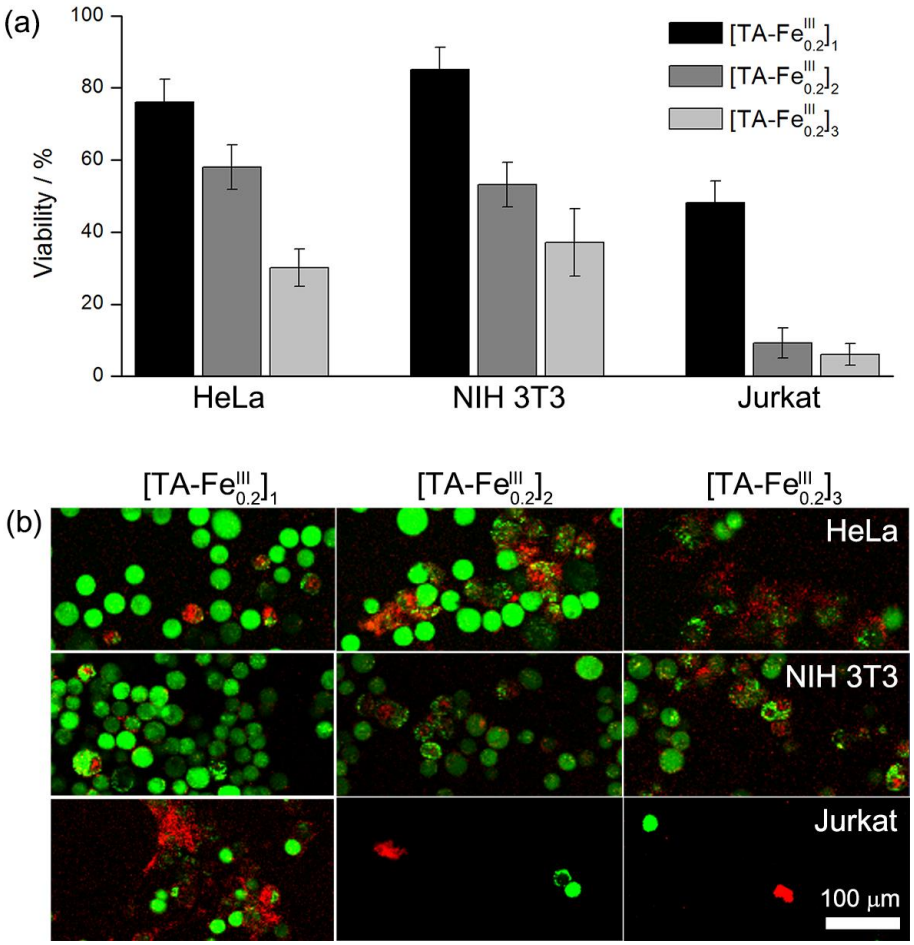


Fig. S1 (a) Viability of HeLa, NIH 3T3, and Jurkat cells after coating with, 0.2 mg mL^{-1} of $FeCl_3 \cdot 6H_2O$, based on the MTT assay. For the viability test, we used an average of 3 biological replicates, and each sample was tested in triplicate. (b) Live/Dead® cell viability assay of HeLa, NIH 3T3, and Jurkat cells before and after coating with 0.2 mg mL^{-1} of $FeCl_3 \cdot 6H_2O$. Green: live; red: dead. For the viability test, we used an average of 3 biological replicates, and each sample was tested in triplicate.

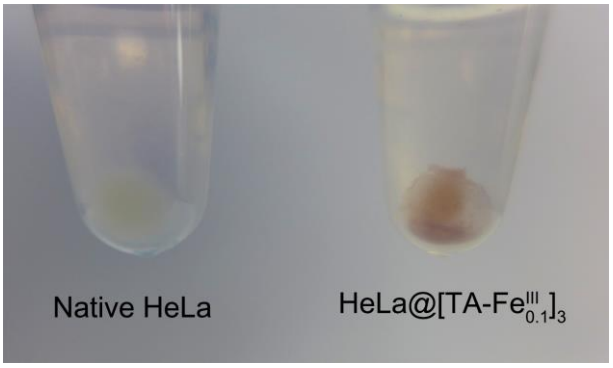


Fig. S2 Optical images of native HeLa and HeLa@[TA-$Fe^{III}_{0.1}$]$_3$ cells.

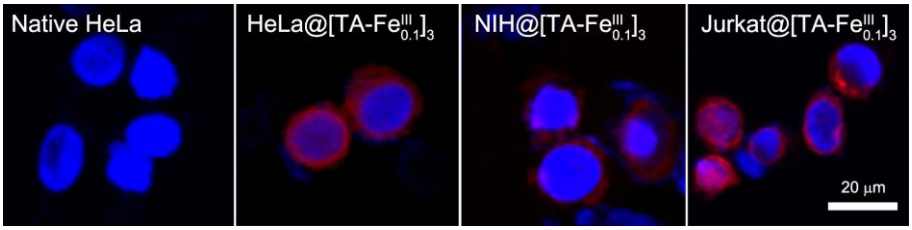


Fig. S3 LSCM images of native HeLa, HeLa@[TA-$Fe^{III}_{0.1}$]$_3$, NIH@[TA-$Fe^{III}_{0.1}$]$_3$, and Jurkat@[TA-$Fe^{III}_{0.1}$]$_3$ cells after treatment with BSA-Alexa Fluor 647 (red). The nuclei were stained with DAPI (blue).

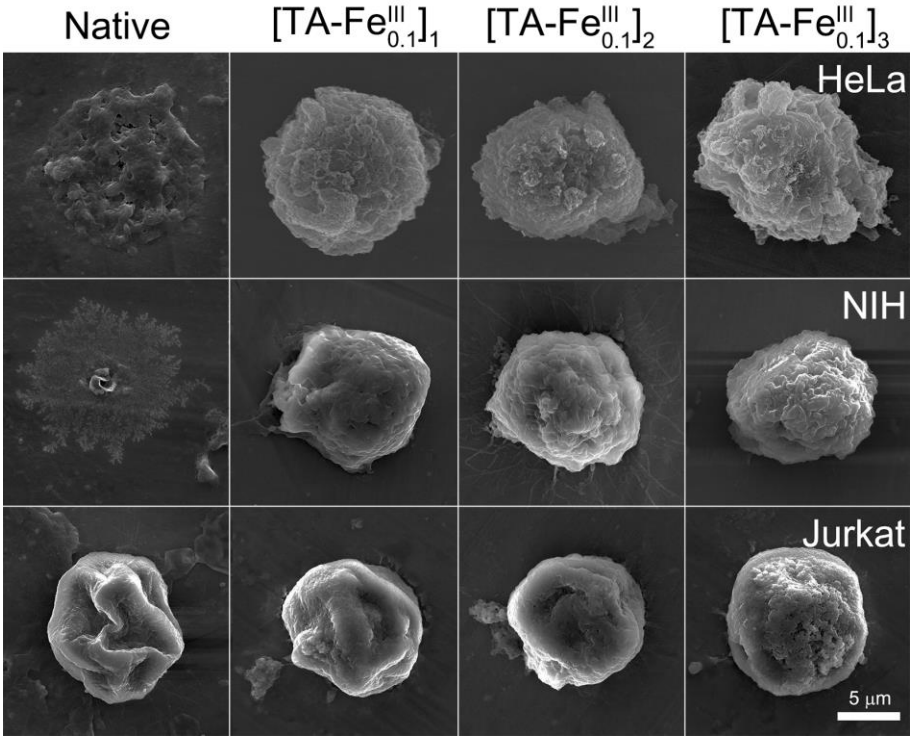


Fig. S4 SEM micrographs of native HeLa, native NIH 3T3, native Jurkat, HeLa@[TA-Fe$^{III}_{0.1}$]$_n$, NIH@[TA-Fe$^{III}_{0.1}$]$_n$, and Jurkat@[TA-Fe$^{III}_{0.1}$]$_n$ cells. n = 1, 2, and 3.

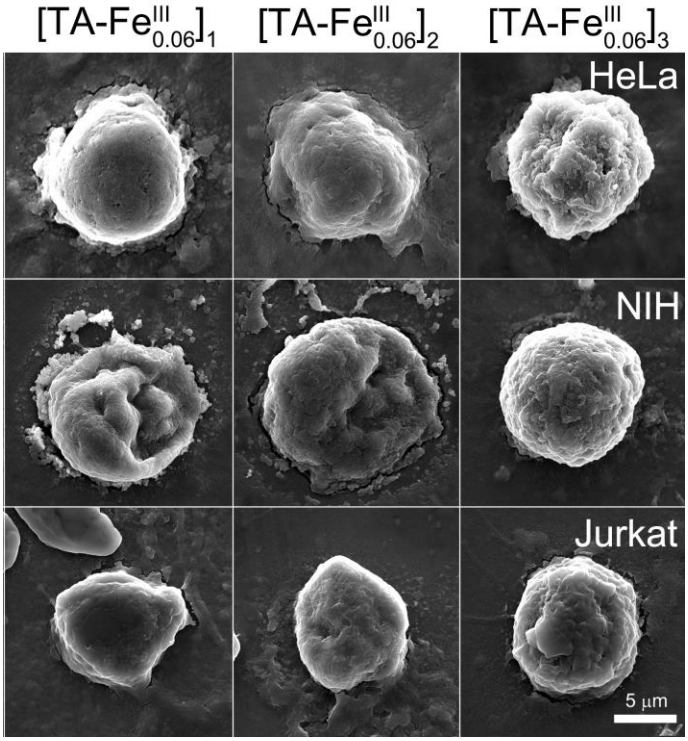
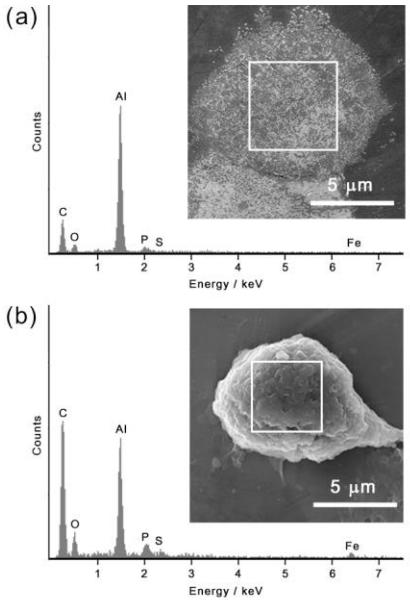


Fig. S5 SEM micrographs of native HeLa, native NIH 3T3, native Jurkat, HeLa@[TA-Fe$^{III}_{0.06}$]$_n$, NIH@[TA-Fe$^{III}_{0.06}$]$_n$, and Jurkat@[TA-Fe$^{III}_{0.06}$]$_n$ cells. n = 1, 2, and 3.



ZAFElement	*Wt%*	*At%*
C K	56.54	72.56
O K	07.53	07.12
Al K	34.06	19.47
P K	01.52	00.76
S K	00.00	00.00
Fe K	00.34	00.09

ZAFElement	*Wt%*	*At%*
C K	72.09	83.03
O K	10.03	08.52
Al K	13.22	06.66
P K	01.82	00.80
S K	00.75	00.32
Fe K	02.08	00.67

Fig. S6 EDX spectra of (a) native HeLa and (b) HeLa@[TA-$Fe^{III}_{0.1}$]$_3$ cells.

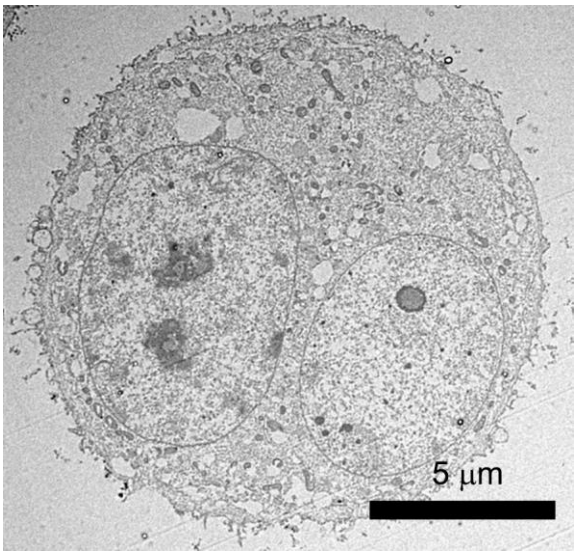


Fig. S7 TEM micrograph of a microtomed native HeLa cell.

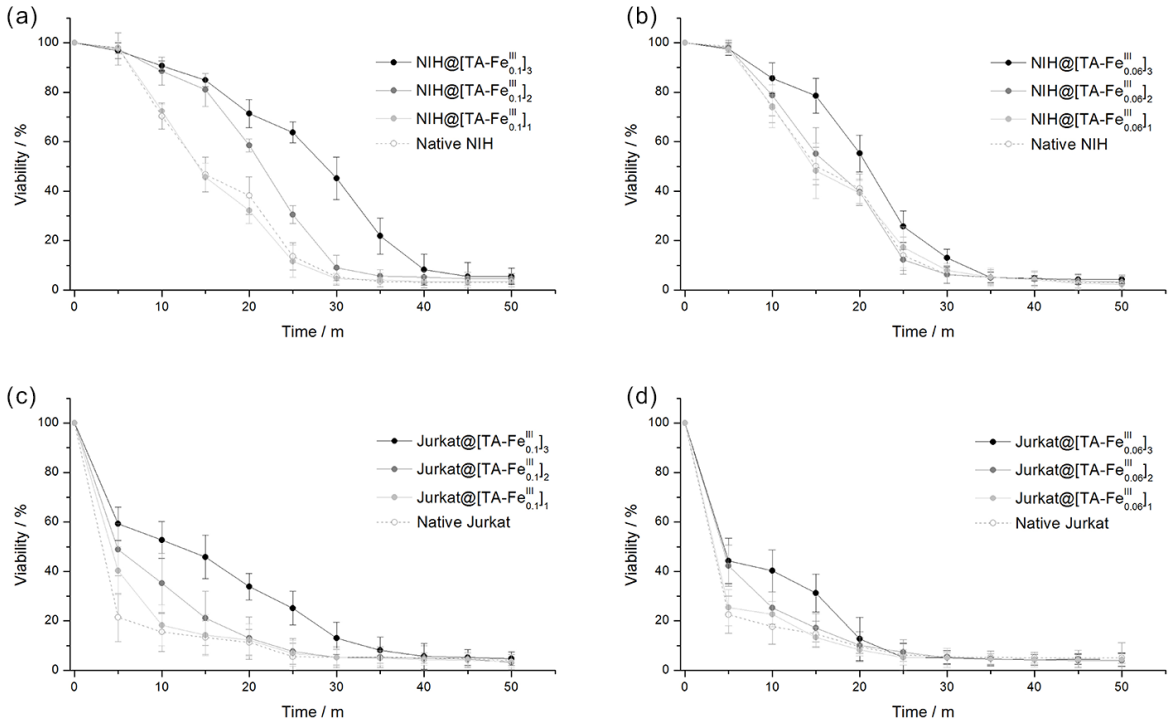


Fig. S8 Viability curves of (a) native NIH 3T3 and NIH@[TA-$Fe^{III}_{0.1}$]$_n$, (b) native NIH 3T3 and NIH@[TA-$Fe^{III}_{0.06}$]$_n$, (c) native Jurkat and Jurkat@[TA-$Fe^{III}_{0.1}$]$_n$, and (d) native Jurkat and Jurkat@[TA-$Fe^{III}_{0.06}$]$_n$ cells after UV-C irradiation. n = 1, 2, and 3. For the viability test, we used an average of 3 biological replicates, and each sample was tested in triplicate.

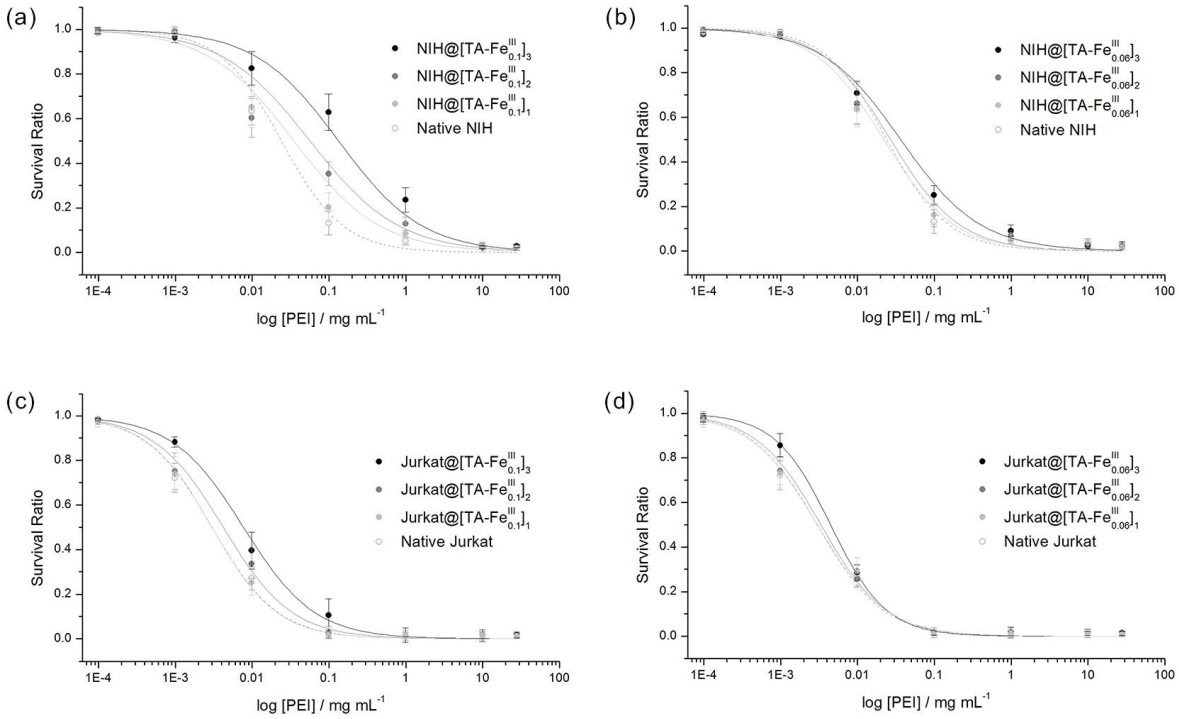


Fig. S9 Survival ratios of (a) native NIH 3T3 and NIH@$[TA\text{-}Fe^{III}_{0.1}]_n$, (b) native NIH 3T3 and NIH@$[TA\text{-}Fe^{III}_{0.06}]_n$, (c) native Jurkat and Jurkat@$[TA\text{-}Fe^{III}_{0.1}]_n$, and (d) native Jurkat and Jurkat@$[TA\text{-}Fe^{III}_{0.06}]_n$ cells after PEI treatment. n = 1, 2, and 3. For the viability test, we used an average of 3 biological replicates, and each sample was tested in triplicate.

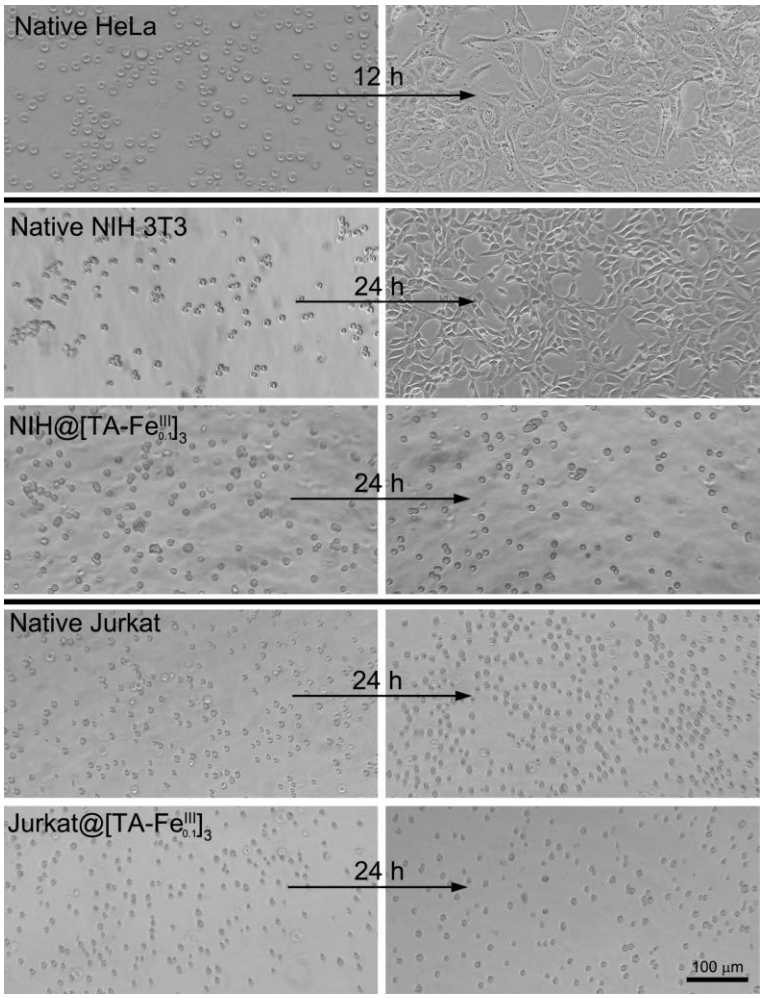


Fig. S10 Optical micrographs of native HeLa, native NIH 3T3, NIH@[TA-$Fe^{III}_{0.1}$]$_3$, native Jurkat, and Jurkat@[TA-$Fe^{III}_{0.1}$]$_3$ cells in culture flasks (left) immediately after cell seeding and (b) after 12-h or 24-h culture.