Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.


*Supplementary Information*

# Application of biocompatible and ultrastable superparamagnetic iron(III) oxide nanoparticles doped with magnesium for efficient magnetic fluid hyperthermia in lung cancer cells

Anna M. Nowicka,[1] Monika Ruzycka-Ayoush,[2] Artur Kasprzak,[3] Agata Kowalczyk,[1] Magdalena Bamburowicz-Klimkowska[2], Malgorzata Sikorska[2], Kamil Sobczak,[4] Mikolaj Donten,[1] Anna Ruszczynska,[4] Julita Nowakowska[5] and Ireneusz P. Grudzinski[2*]

[1] Faculty of Chemistry, University of Warsaw, Pasteura 1 Str., PL 02-093, Warsaw, Poland

[2] Faculty of Pharmacy, Medical University of Warsaw, Banacha 1 Str., PL 02-097 Warsaw, Poland

[3] Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3 Str., PL 00-664 Warsaw, Poland

[4]Faculty of Chemistry, Biological and Chemical Research Centre, University of Warsaw, Zwirki i Wigury 101 Str., PL 02-089 Warsaw, Poland

[5] Faculty of Biology, University of Warsaw, Miecznikowa 1 Str., PL 02-096 Warsaw, Poland

[*] Correspondence to: ireneusz.grudzinski@wum.edu.pl

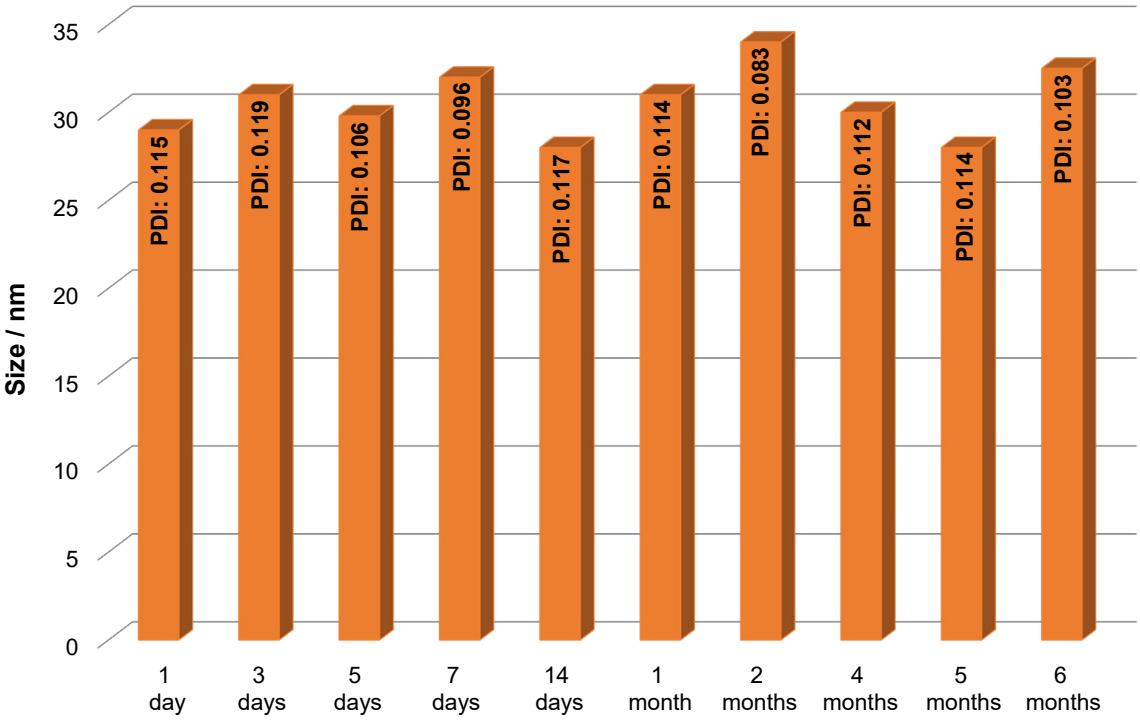


**Figure S1.** Mean size and polydispersity index (PDI) of iron(III) oxide nanoparticles doped with magnesium ($Mg_{0.1}$-γ-$Fe_2O_3$(mPEG-silane)$_{0.5}$) dispersed in water and measured for 6 months.

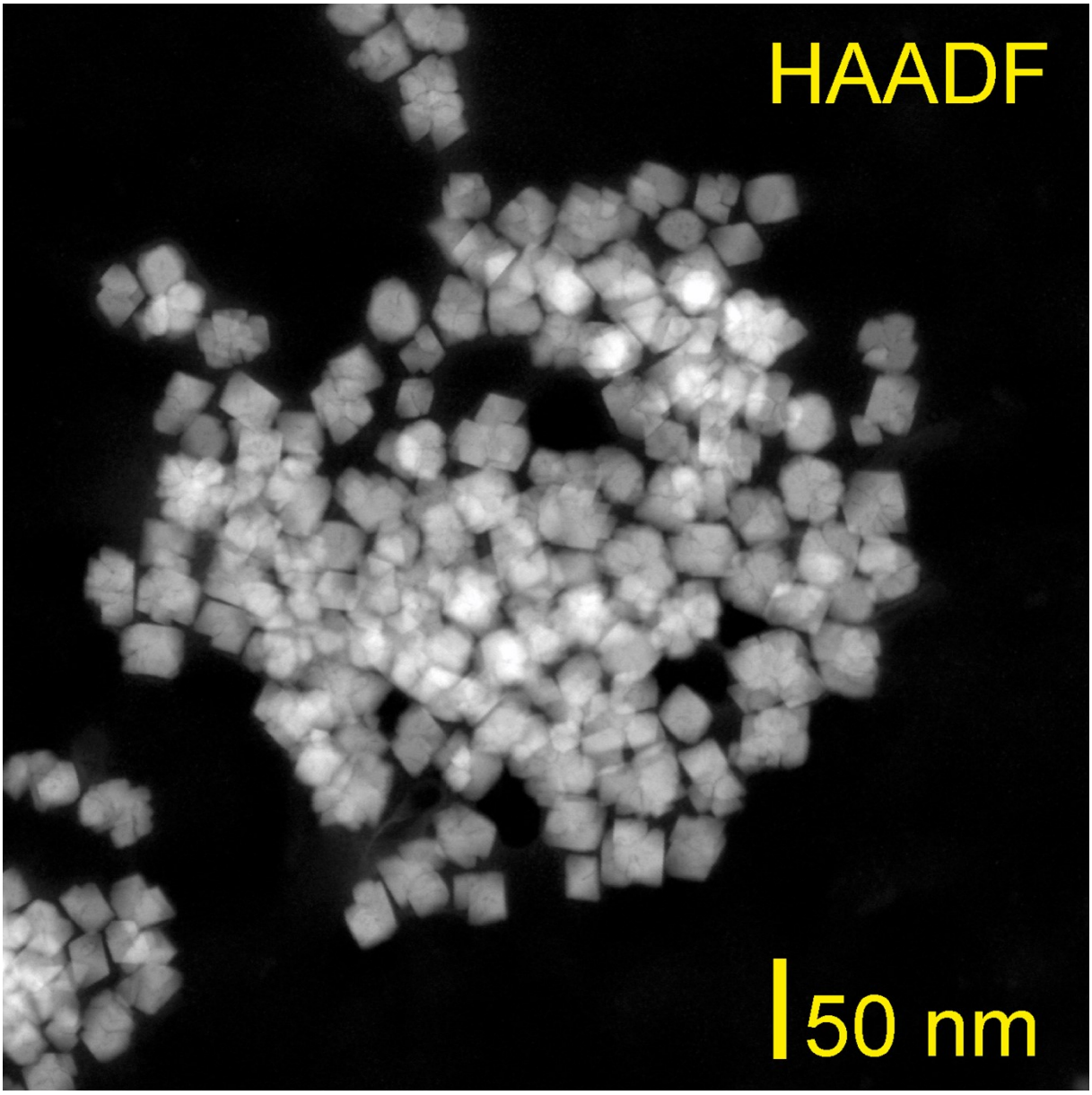


**Figure S2.** Representative high-angle annular dark-field (HAADF)-STEM image of iron(III) oxide nanoparticles doped with magnesium ($Mg_{0.1}$-γ-$Fe_2O_3$(mPEG-silane)$_{0.5}$).

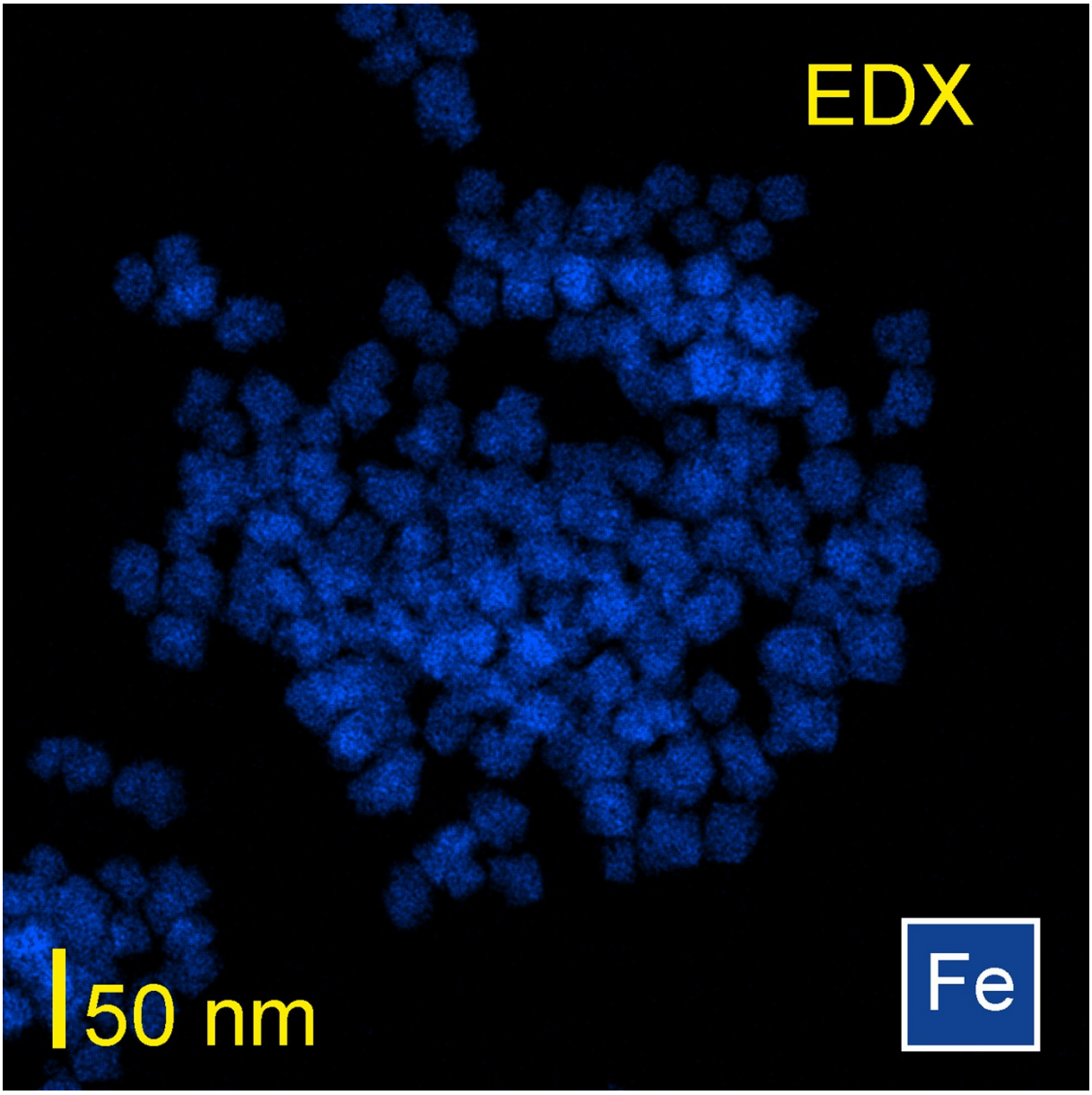


**Figure S3.** Representative high-angle annular dark-field (HAADF)-STEM image and energy dispersive X-ray (EDX) distribution map of iron (Fe) in iron(III) oxide nanoparticles doped with magnesium ($Mg_{0.1}$-γ-$Fe_2O_3$(mPEG-silane)$_{0.5}$).

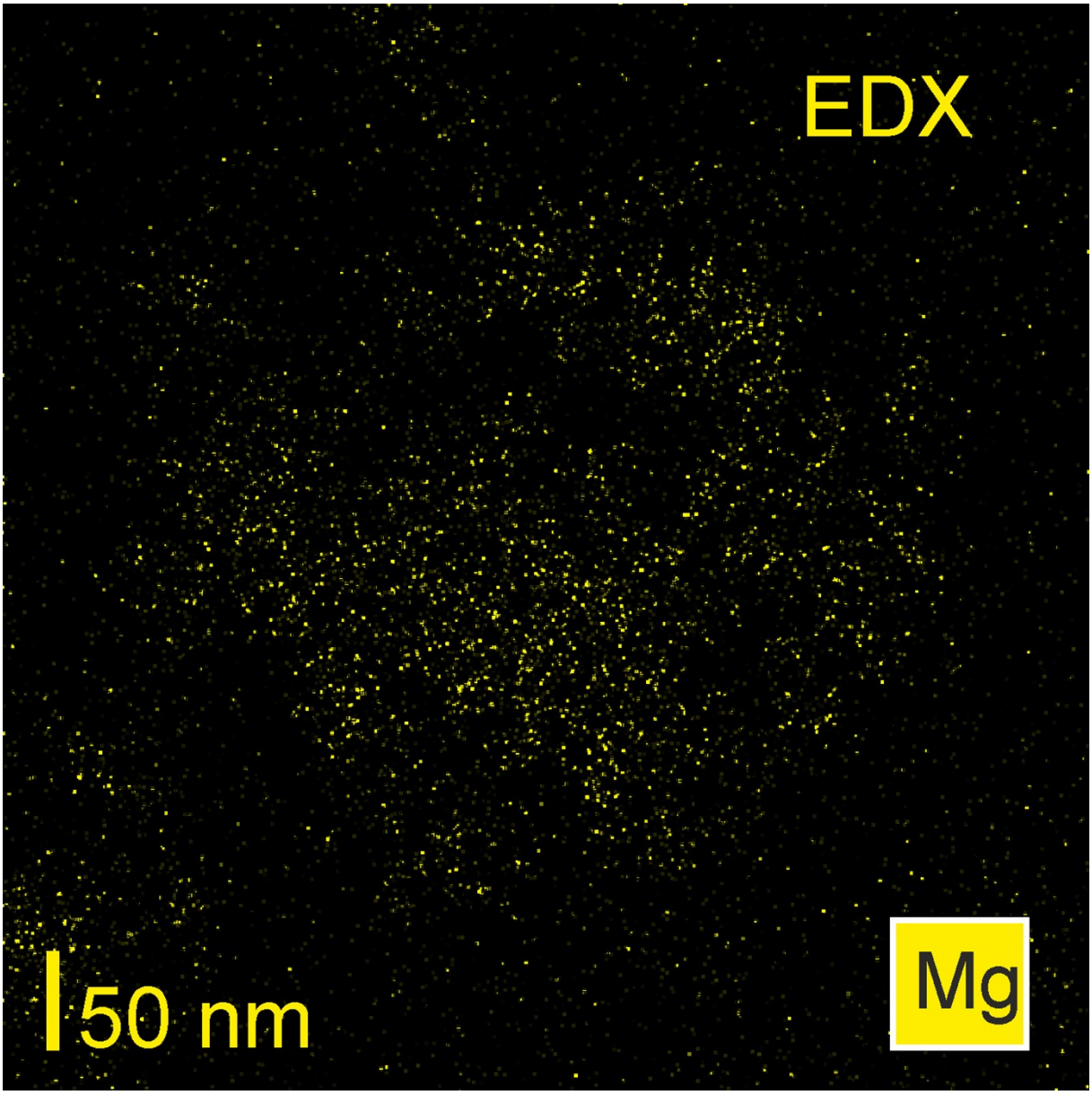


**Figure S4.** Representative high-angle annular dark-field (HAADF)-STEM image and energy dispersive X-ray (EDX) distribution map of magnesium (Mg) in iron(III) oxide nanoparticles doped with magnesium ($Mg_{0.1}$-γ-$Fe_2O_3$(mPEG-silane)$_{0.5}$).

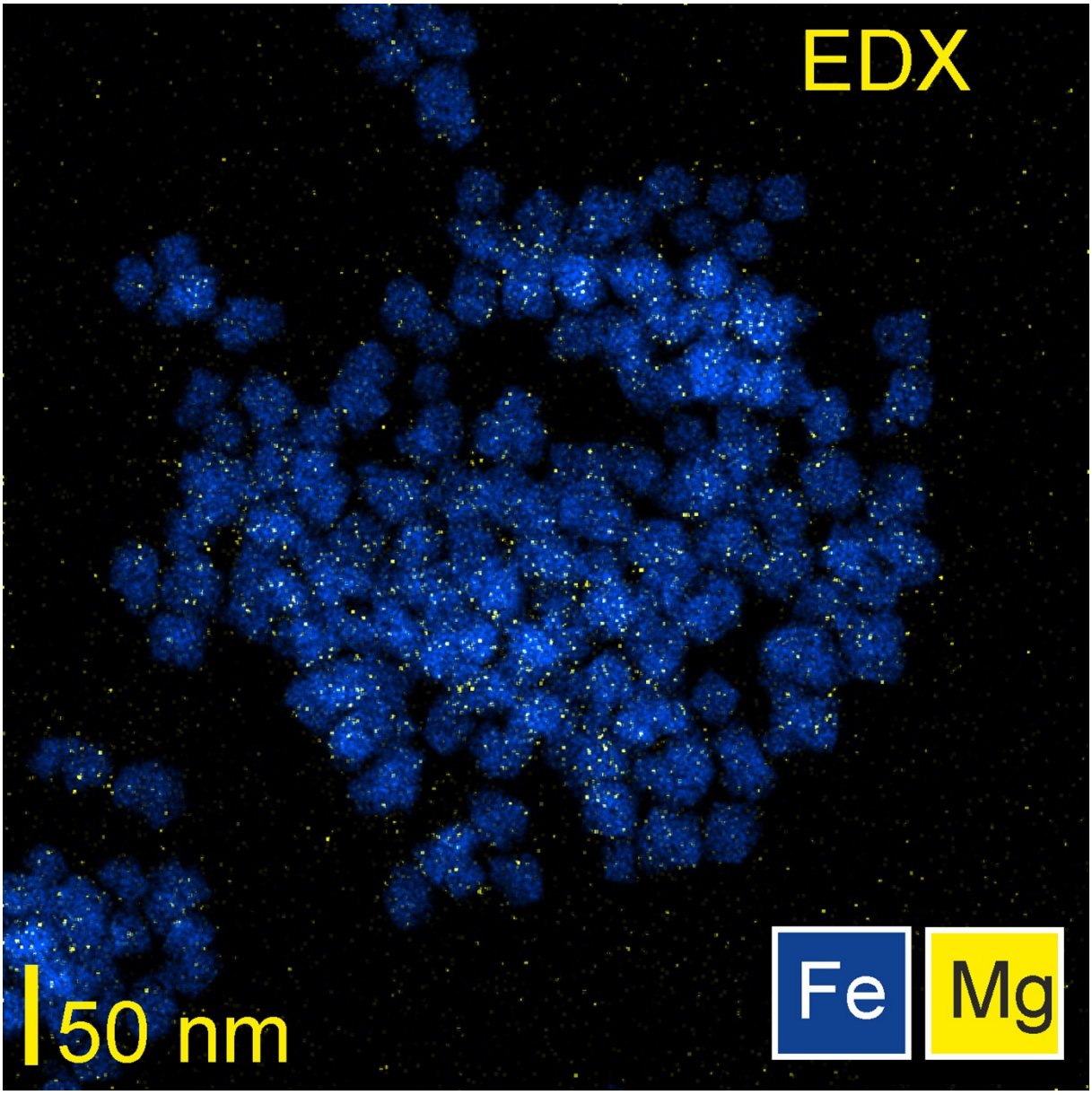


**Figure S5.** Representative high-angle annular dark-field (HAADF)-STEM image and energy dispersive X-ray (EDX) distribution map of iron (Fe) and magnesium (Mg) in iron(III) oxide nanoparticles doped with magnesium ($Mg_{0.1}$-γ-$Fe_2O_3$(mPEG-silane)$_{0.5}$).

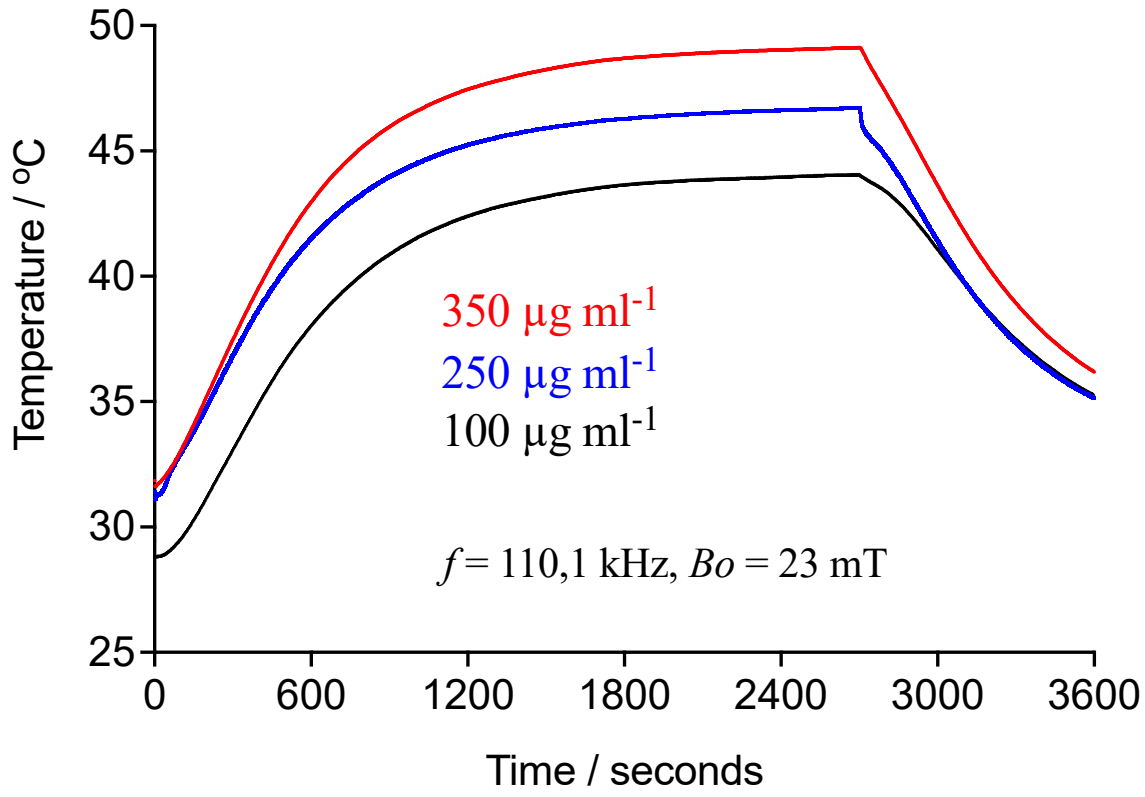


**Figure S6.** Thermal curves obtained in the cell model. Magnetic fluid hyperthermia heating properties of iron(III) oxide nanoparticles doped with magnesium ($Mg_{0.1}$-γ-$Fe_2O_3$(mPEG-silane)$_{0.5}$) were studied at 100, 250 and 350 µg·mL$^{-1}$.

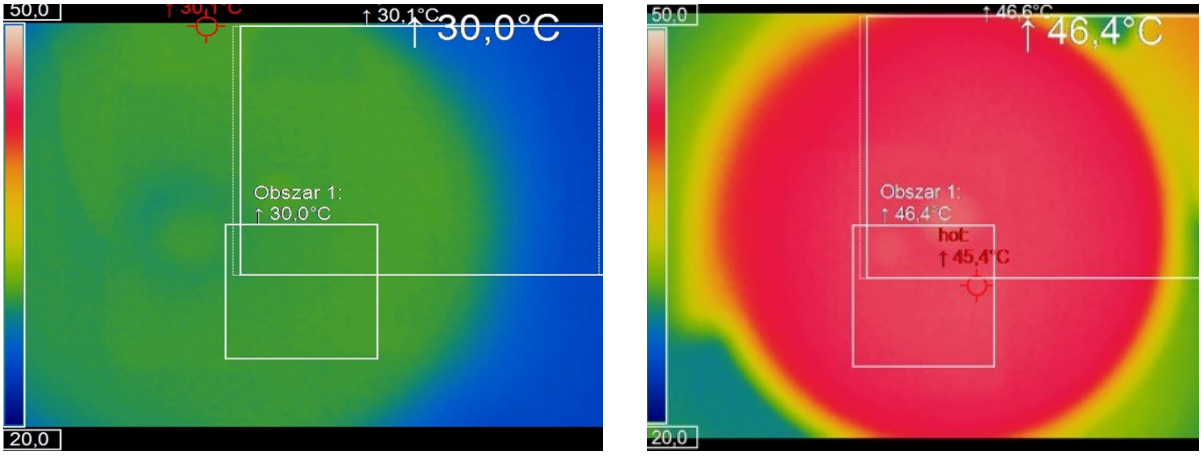


**Figure S7.** Representative IR thermal images of the A549 cells cultured on Petri dish and treated with iron(III) oxide nanoparticles doped with magnesium ($Mg_{0.1}$-γ-$Fe_2O_3$(mPEG-silane)$_{0.5}$) (250 μg·mL$^{-1}$) and subjected to AMF for 45 min. $B$ = 21 mT, $H_o$ = 16.7 kA·m$^{-1}$, $f$ = 109.96 kHz, capacitor 200 mF. Left image before AMF (0 min); Right image after AMF (45 min).

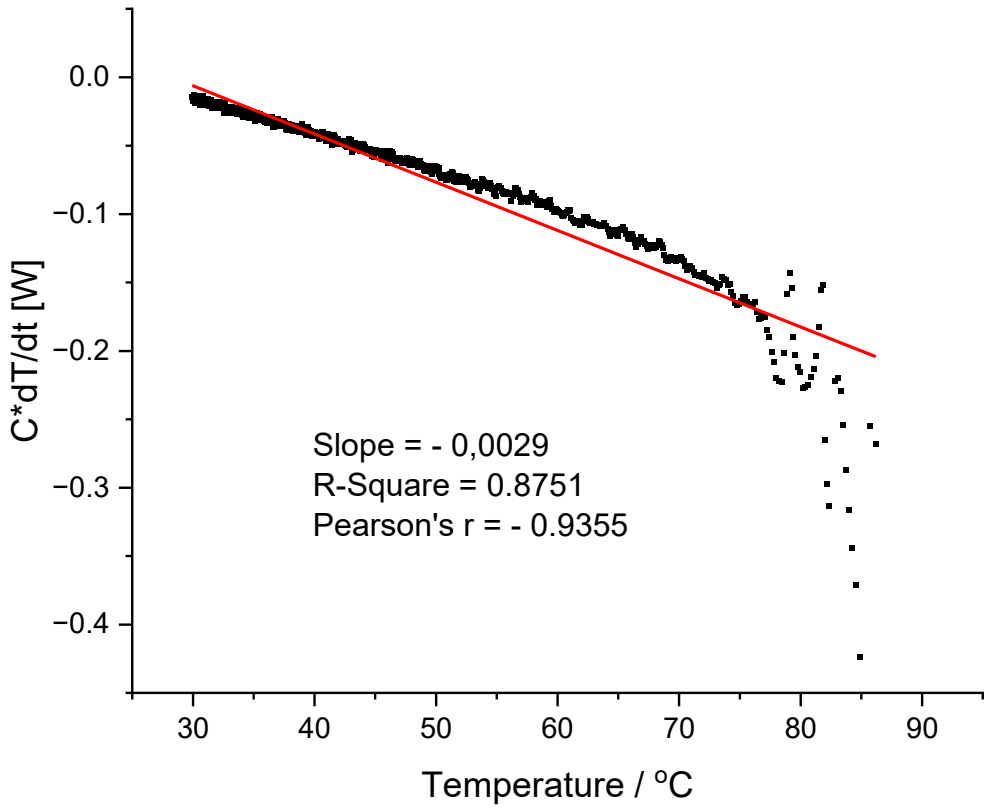


**Figure S8**. Representative linear fitting plot for the decay (cooling) part of the thermal curve due to AMF set for the "off" mode in the phantom model (MNP = 3 mg ·mL$^{-1}$). The calculated slope was used for SAR measurements based on the Corrected Slope Method (CSM). Red line – the linear fit, black dots – experimental data.

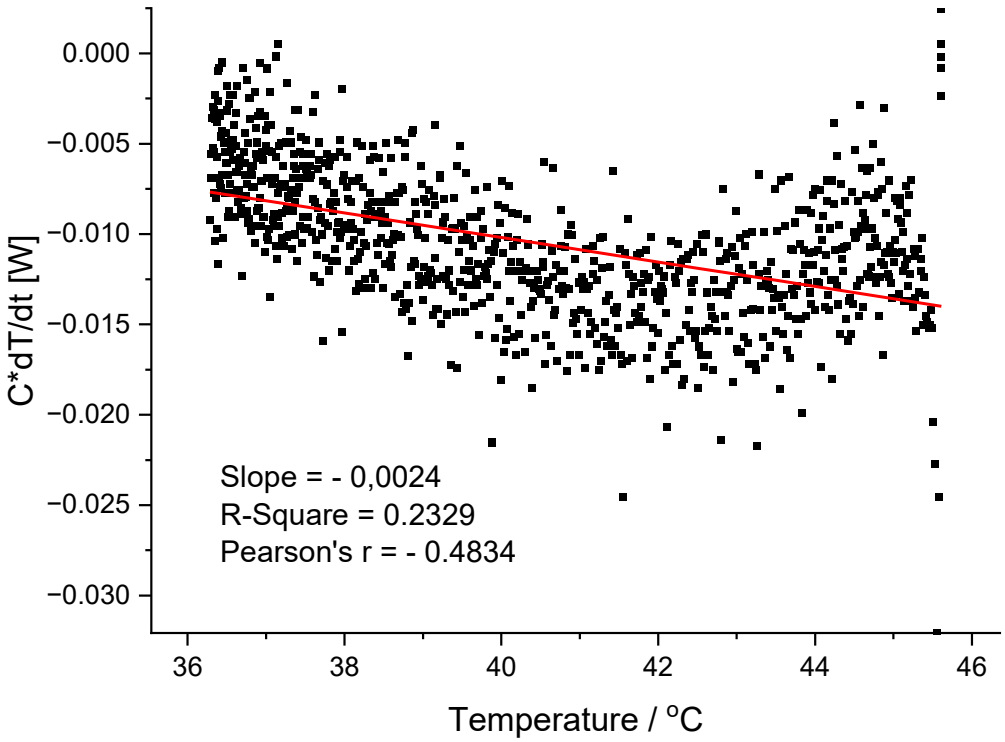


**Figure S9**. Representative linear fitting plot for the decay (cooling) part of the thermal curve due to AMF set for the "off" mode in the cell model (MNP = 250 μg ·mL$^{-1}$). The calculated slope was used for SAR measurements based on the Corrected Slope Method (CSM). Red line – the linear fit, black dots – experimental data.

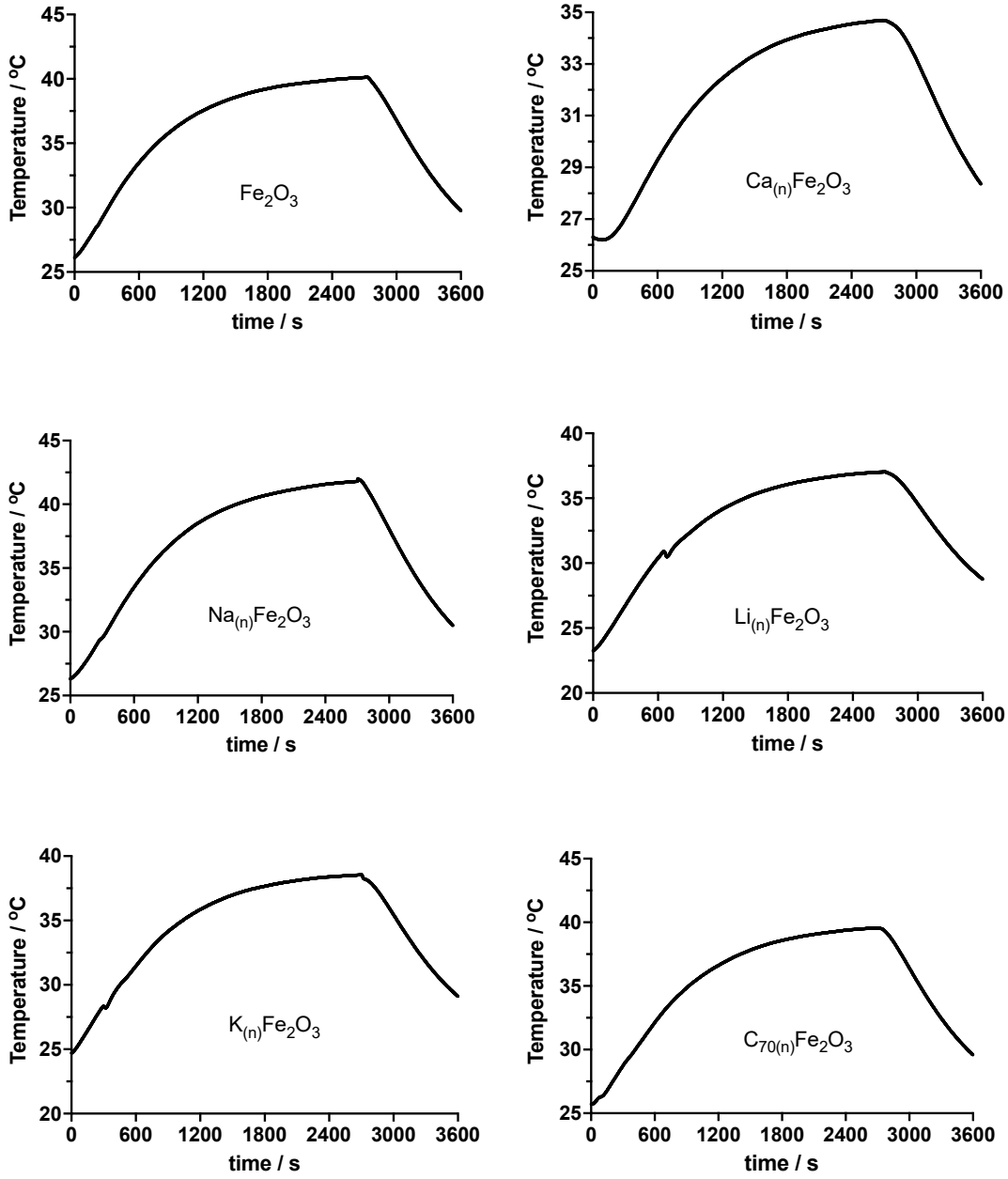


**Figure S10.** Thermal curves obtained in the phantom model. Iron(III) oxide nanoparticles doped without and with such elements as Ca, Na, K, Li and C70 fullerene were tested at the concentration of 3 mg/ml (water fluid dispersions) for magnetic fluid hyperthermia under AMF ($B$ = 23 mT, $H_o$ = 18.3 kA·m$^{-1}$, $f$ =109.96 kHz, capacitor 200 mF).

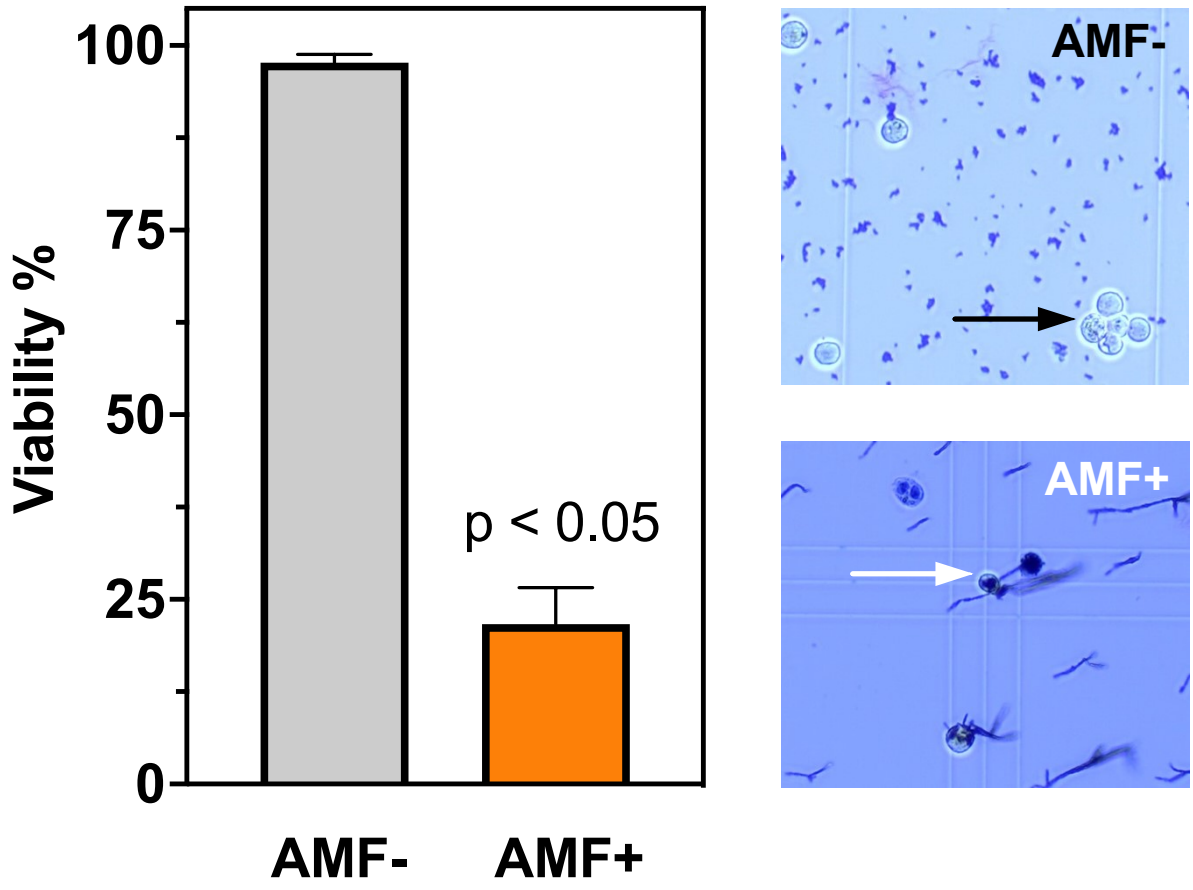


**Figure S11**. Cytotoxic effects of the water ferrofluid (250 μg·mL$^{-1}$) composed of iron(III) oxide nanoparticles doped with magnesium ($Mg_{0.1}$-γ-$Fe_2O_3$(mPEG-silane)$_{0.5}$) on adenocarcinomic human alveolar basal epithelial cells (A549) treated with an alternating magnetic field (AMF+) and without AMF-. AMF: $B$ = 21 mT, $H_o$ = 16.7 kA·m$^{-1}$, $f$ = 109.96 kHz, capacitor 200 mF. Data are mean ± SD from three independent experiments ($n$ = 18). TrypanBlue assay was applied in the studies. To data, the A549 cells were seeded into 3 cm-Petri dishes at a density of 250 000 cells per dish and incubated for 24 h. After 24 h of cell adhesion, the A549 cells were exposed to magnetic nanoparticles (0.25 mg/ml) and further magnetized for 45 minutes with alternative magnetic fields. After 24 h of incubation the medium from cells was collected, and the cells were washed two times with PBS and trypsinized. The cells were harvested, combined with the collected medium and centrifuged. Pellet was resuspended in 500 μl of culture media. 10 μl of Trypan blue solution was added to a 10 μl of cell suspension. The viable (unstained) and nonviable (blue-stained) cells were counted manually with a Bürker chamber. Representative microscopy images (Mag. 10x) of viable (AMF-) and nonviable (AMF+) cells.

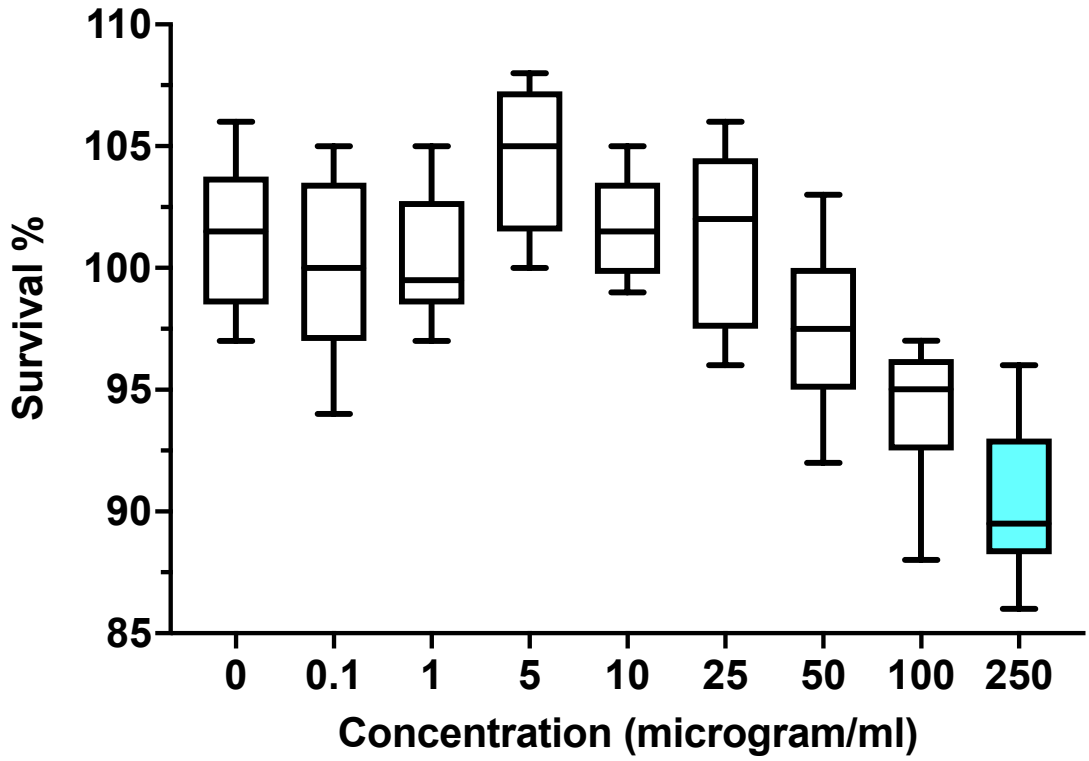


**Figure S12**. Cytotoxic effects of iron(III) oxide nanoparticles doped with magnesium ($Mg_{0.1}$-γ-$Fe_2O_3$(mPEG-silane)$_{0.5}$) tested as water ferrofluid (0.1 - 250 μg·mL$^{-1}$) on adenocarcinomic human alveolar basal epithelial cells (A549) treated for 24 hours. Data are mean ± SD from three independent experiments ($n$ = 18). AlamarBlue assay was applied in the studies. No AMF was used.

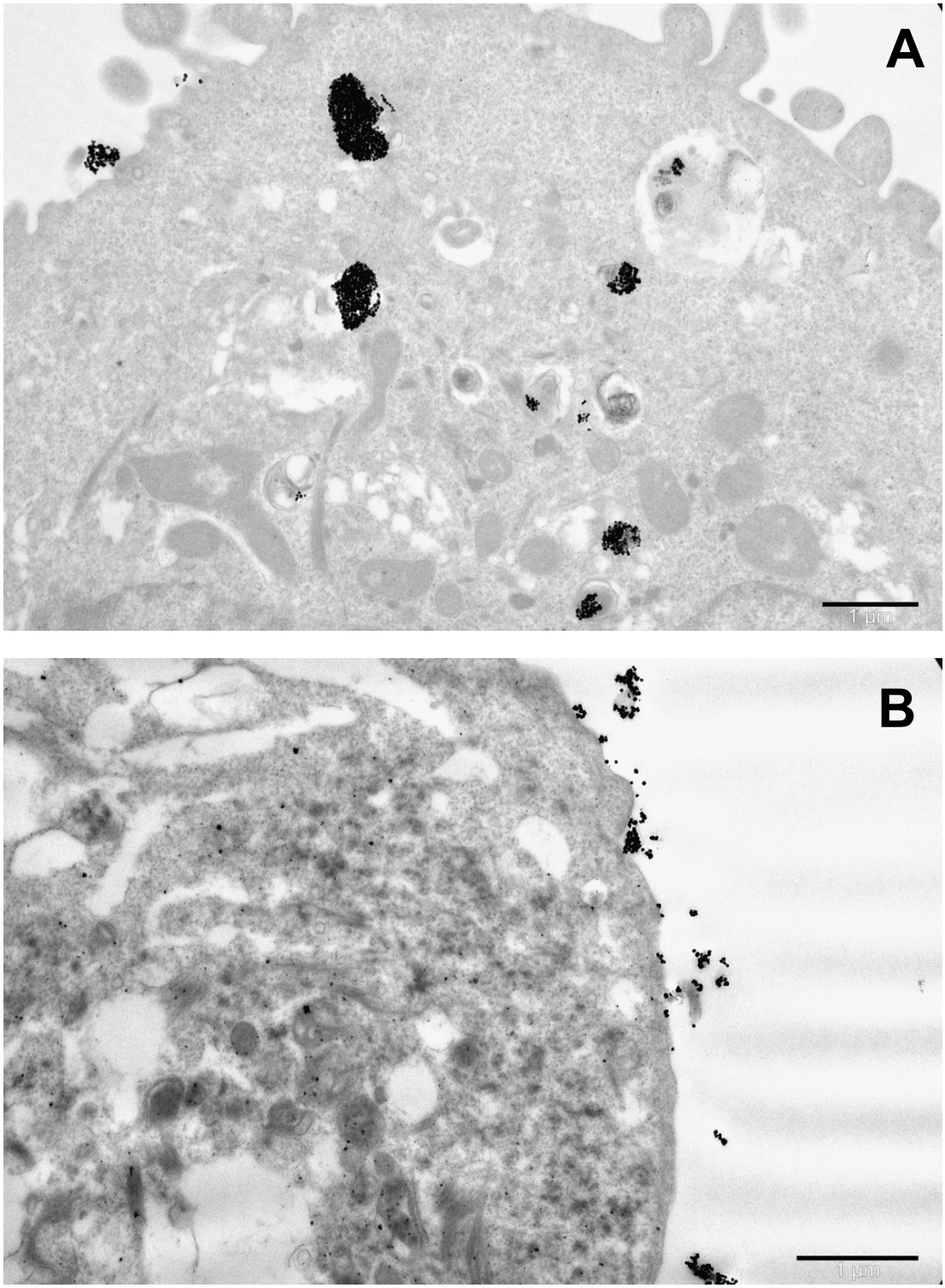


**Figure S13**. Representative TEM images of adenocarcinomic human alveolar basal epithelial cells (A549) subjected to the water ferrofluid (250 µg·mL$^{-1}$) composed of iron(III) oxide nanoparticles doped with magnesium ($Mg_{0.1}$-γ-$Fe_2O_3$(mPEG-silane)$_{0.5}$) and exposed without (A) and with alternating magnetic field (B). AMF: $B$ = 21 mT, $H_o$ = 16.7 kA·m$^{-1}$, $f$ = 109.96 kHz, capacitor 200 mF. Dark spots located in the cell and cell membrane as circular clusters are nanoparticles.