Supplementary Information

Translating Magnetic Fluid Hyperthermia toward Lung Cancer Treatment

Malgorzata Sikorska [*a], Magdalena Bamburowicz-Klimkowska [a], Monika Ruzycka-Ayoush [a], Anna M. Nowicka [b], Agata Kowalczyk [b], Artur Kasprzak [c], Grzegorz Domanski [d], Barbara Wagner [e], Monika Prochorec-Sobieszek [f], Marzena Cabaj [f], Anna Szumera-Cieckiewicz [g], Maciej Glogowski [h], Andrzej Cieszanowski [i] and Ireneusz Piotr Grudzinski [*a]

[a] Department of Toxicology and Food Science, Faculty of Pharmacy, Medical University of Warsaw, 1 Banacha St., PL-02-097 Warsaw, Poland

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

[c] Department of Organic Chemistry, Faculty of Chemistry, Warsaw University of Technology, 3 Noakowskiego St., PL-00-664 Warsaw, Poland

[d] Institute of Radioelectronics and Multimedia Technology, Faculty of Electronics and Information Technology, Warsaw University of Technology, 15/19 Nowowiejska St., PL-00-665 Warsaw, Poland

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

[f] Department of Hematology Diagnostics, Institute of Hematology and Transfusiology, 14 Indiry Gandhi St., PL-02-776 Warsaw, Poland

[g] Department of Cancer Pathomorphology, Maria Sklodowska-Curie National Research Institute of Oncology, 5 Roentgena St., PL-02-781 Warsaw, Poland

[h] Department of Lung Cancer and Chest Tumors, Maria Sklodowska-Curie National Research Institute of Oncology, 5 Roentgena St., PL-02-781 Warsaw, Poland

[i] Department of Clinical Radiology, Medical University of Warsaw, 1 Banacha St., PL-02-097 Warsaw, Poland

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

Table S1 Summary of physicochemical characterizations and biological evaluations performed with mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium.

Parameter / Method	**Result**	**Ref.**
Shape (SEM/SEM)	Hexagonal and/or rhombohedral	[1]
Size (TEM)	27 nm	[1]
Magnetic saturation	70 emu·g^{-1} (remnant magnetization: 1.6 emu·g^{-1})	[1]
Zeta Potential	-17.054±2.717 mV	[1]
Polydispersity Index	0.083-0.119	[1]
FT-IR	Absorption bands observed in the range 400–1000 cm^{-1}: - 580 cm (vibration of γ-Fe-O), - 450, 632 687, 798, 900, 940 cm^{-1} (pure maghemite) - 1090, 1246, 1403, 1532, 2878, 3100-3500 (mPEG-silane)	[1]
XRD	Peaks at (220), (311), (400), (422), (511), and (440) characteristic of γ -Fe_2O_3)	[1]
Raman	Bands at 380, 600, 740; 900-1800 cm^{-1}	[1]
TEM	mean particle size ≈ 27 nm	[1]
HR-TEM	Well-formed faces	[1]
EDS (quantitative analysis)	Stoichiometric composition of nanoparticles confirmed	[1]
ICP-MS	58.5 ± 0.05 wt%	[1]
Heating capabilities	A ferrofluid concentration of 3 mg·mL^{-1} reached >90 °C within 10 minutes under a field strength of ~18.3 kA·m^{-1} at 110.1 kHz	[1]
SAR	429 to 596 W·g^{-1}	[1]
Cytotoxicity (Alamar Blue assay)	Approximately 15–20% cell viability	[1]
Dynamic Light Scattering (DLS)	Nanoparticle aggregation occurred only in nutrient-rich media and at higher nanoparticle concentrations	[2]
Nephelometry measurements	Precipitation/sedimentation processes were moderate; estimated reaction rate: 0.127 h^{-1}	[2]
Neutral Red Assay (NRU)	Slight reduction in survival (~15%) observed only at high concentrations of NPs; no overall reduction in cell viability	[2]

Flow cytometry	A slight increase in apoptotic cells and a decrease in necrotic cells were observed at the highest concentration of NPs	[2]
Uptake / binding	Higher concentrations of NPs correlated with increased uptake by A549 cells	[2]
TEM (cell studies)	A dosage of 250 $\mu g \cdot mL^{-1}$ of NPs did not affect cell viability during 24 h incubation	[2]
SC-ICP-MS analysis	Demonstrated a significant increase in cellular iron and magnesium content after incubation with NPs; Treated A549 cells exhibited ~20-fold increase in Mg content and >200-fold increase in Fe content	[2]
ROS	NPs did not generate reactive oxygen species	[2]
Comet assay	No induction of DNA strand breaks was detected in any of the tested cell lines following exposure	[2]
Electrical impedance measurements	NPs did not appear to affect the growth curve of A549 cells at the tested concentrations. In contrast, the NPs altered the growth curve of BEAS-2B cells in a concentration-dependent manner.	[2]
Scratch assay	NPs had no significant effect on the migration rate of A549 cells, regardless of dosage.	[2]
Activation Energy measurements	349 $kJ \cdot mol^{-1}$ (A549 cells treated NPS and exposed to magnetization for 30 min at 14.4 $kA \cdot mol^{-1}$)	[3]
CEM43 (cumulative number of equivalent minutes at 43 °C)	9.6 min (BALB/c NUDE mice bearing A549 xenografts; treatment: 18.3 $kA \cdot mol^{-1}$, 0.2 mL NPs (3 $mg \cdot mL^{-1}$), 30 min exposure)	[3]

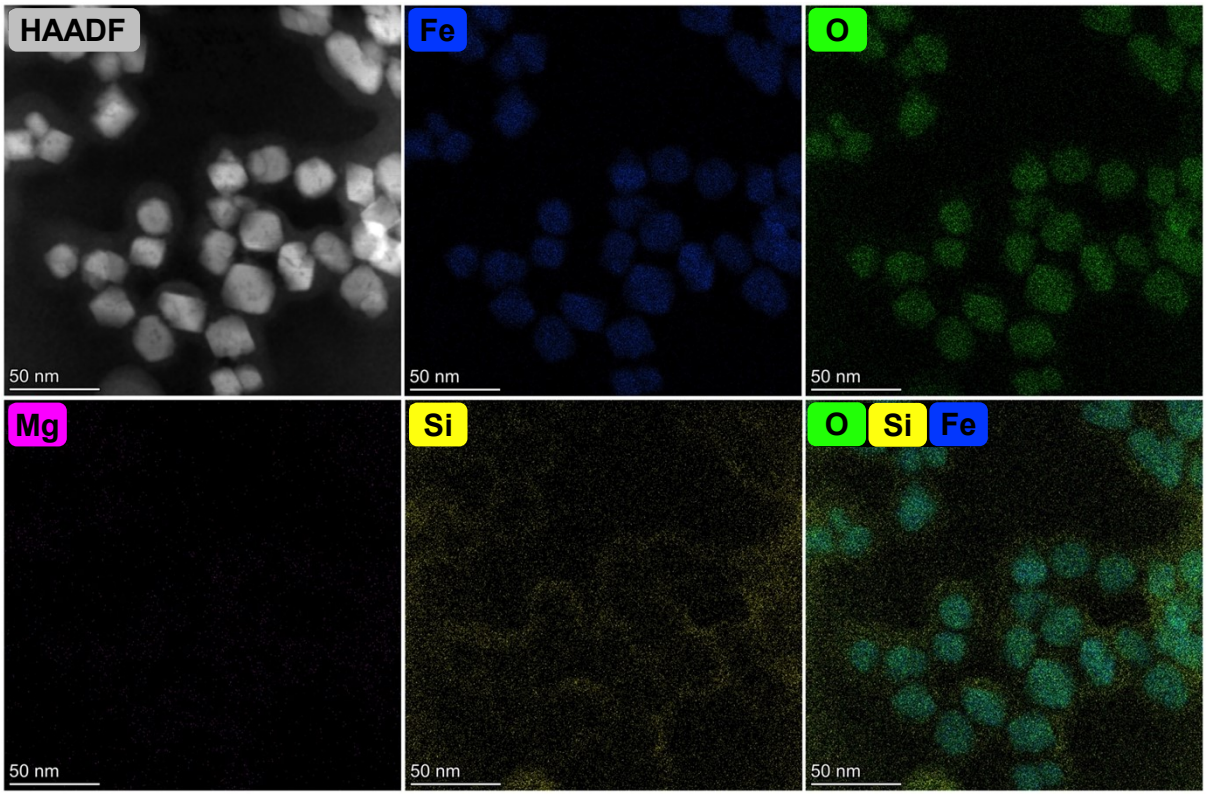

Fig. S1 Representative high-angle annular dark-field (HAADF)-STEM image and energy dispersive X-ray spectroscopy (EDS) map of iron (Fe), oxygen (O), magnesium (Mg) and silicon (Si) elements in mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium.

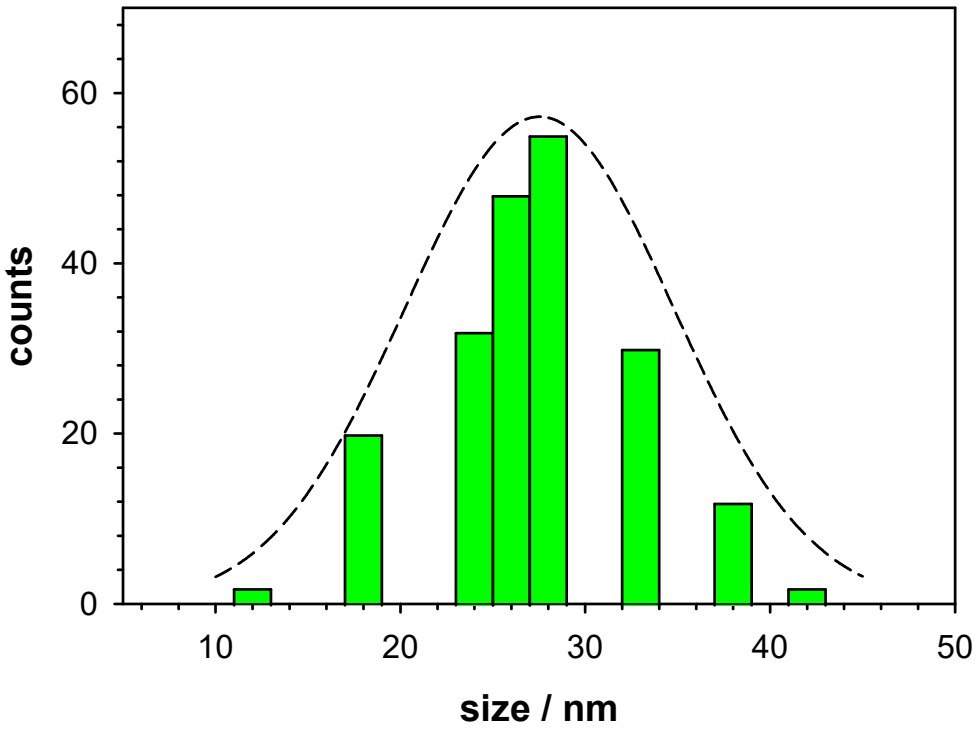

Fig. S2 Size of mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium.

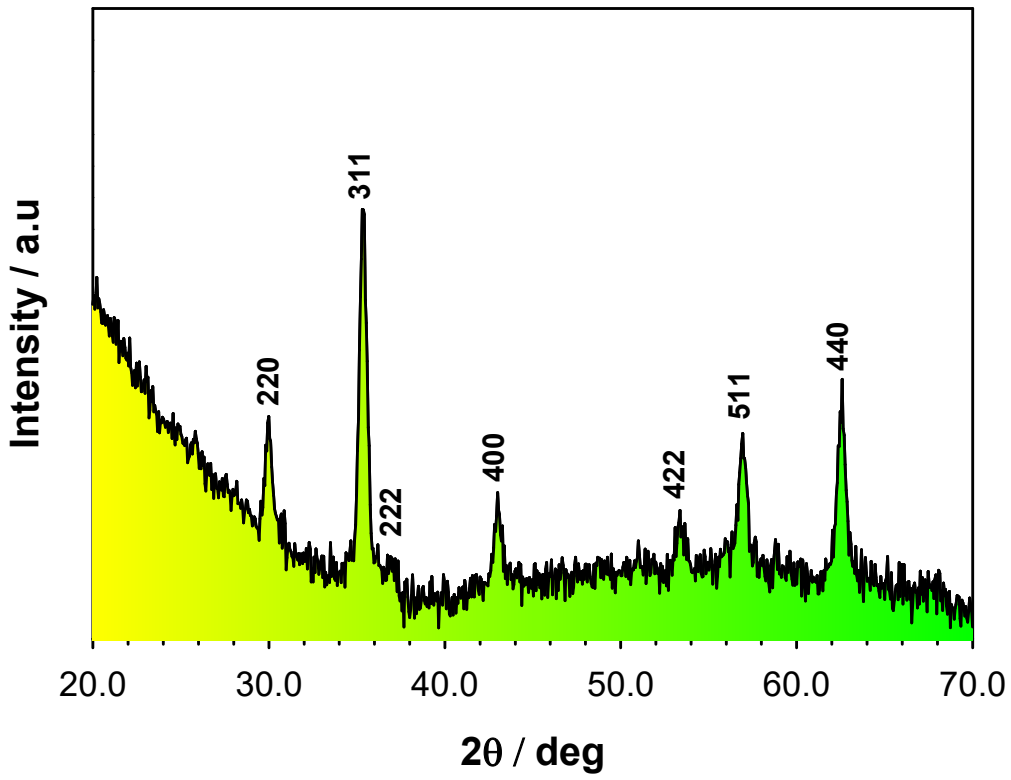

Fig. S3 Powder X-ray diffraction analysis pattern of mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium.

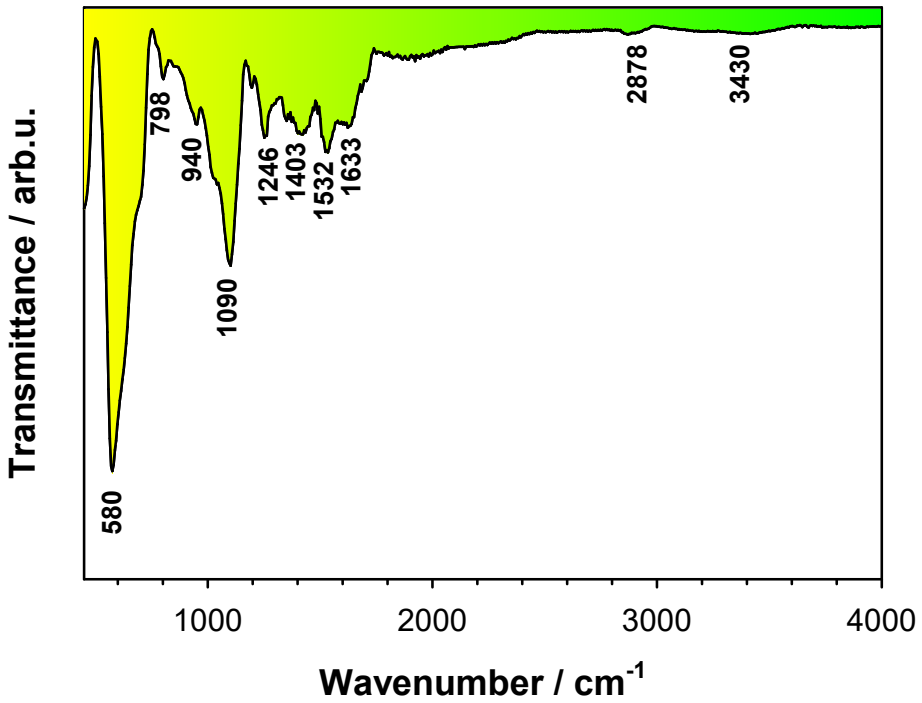

Fig. S4 Fourier-transform infrared spectroscopy of mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium.

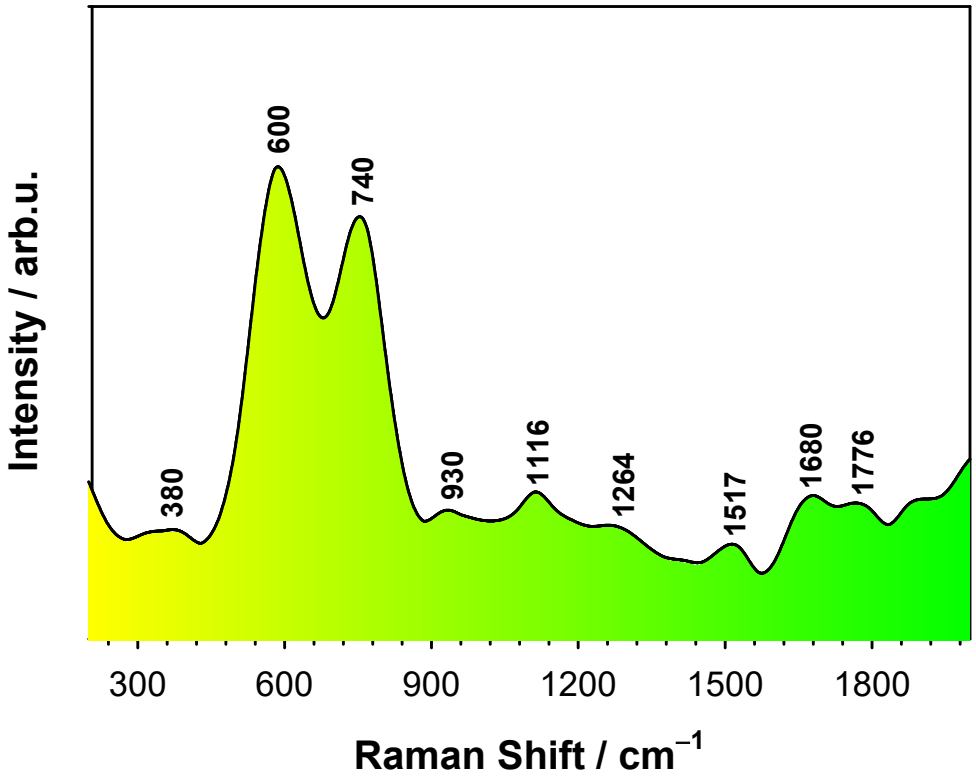

Fig. S5 Raman spectra of mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium.

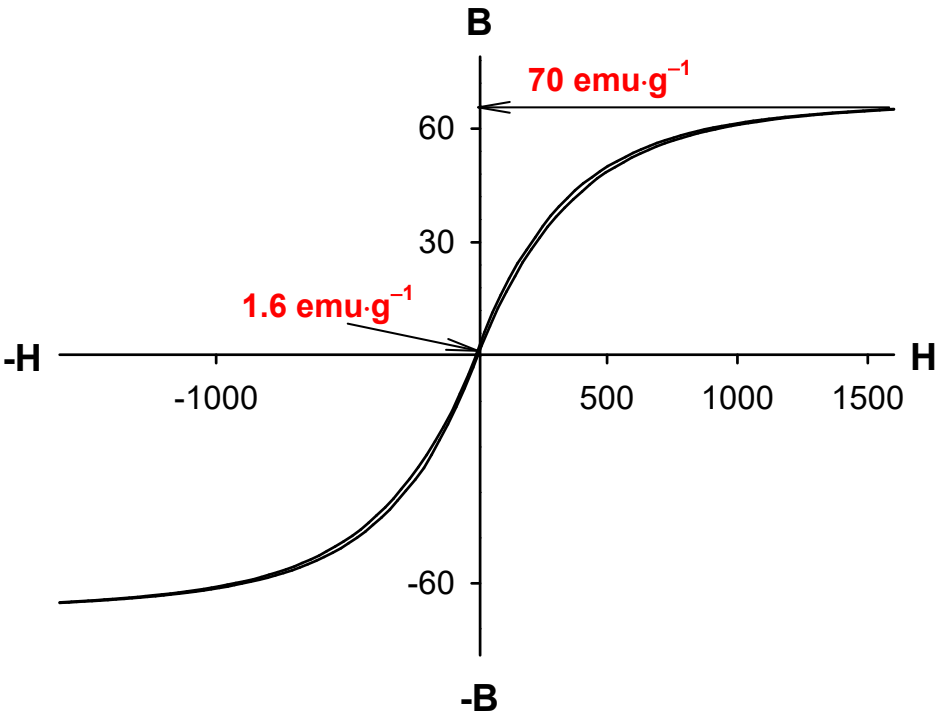

Fig. S6 Magnetic hysteresis of mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium.

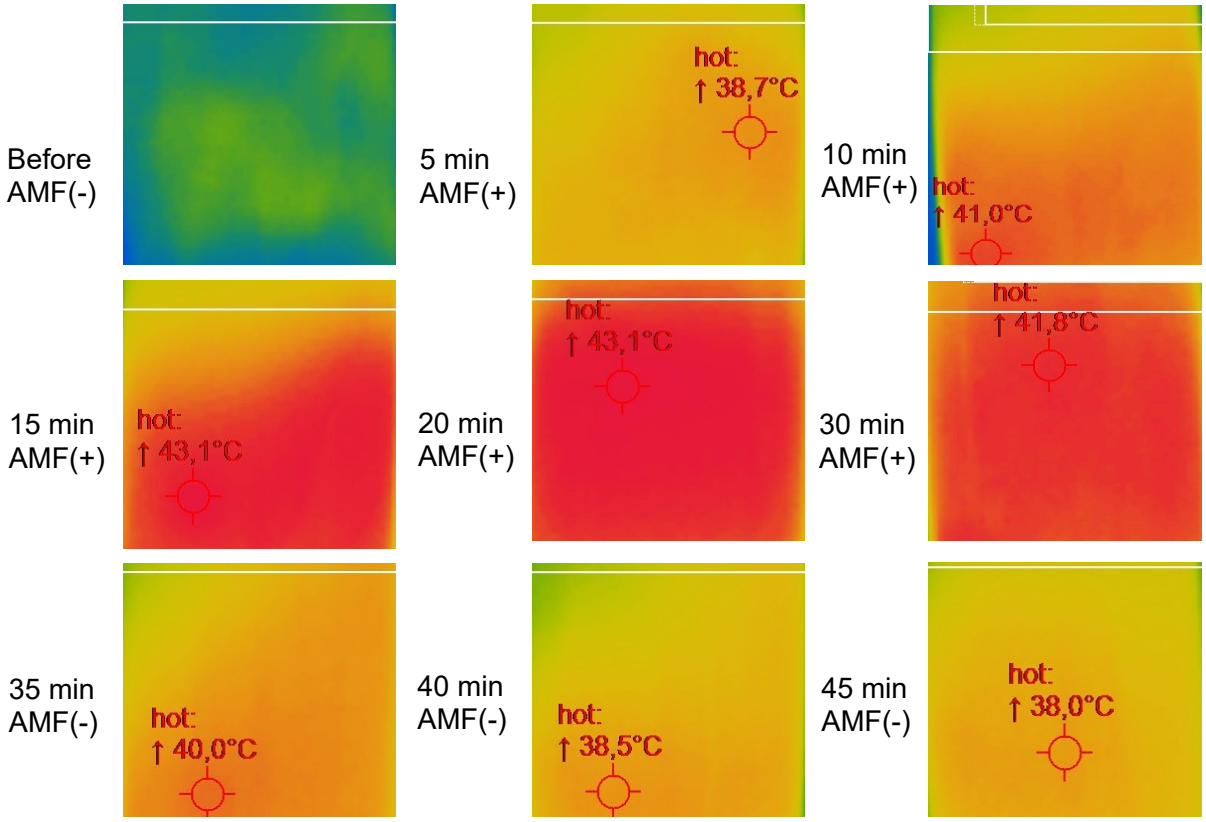

Fig. S7 Representative 2D infrared (IR) thermal images of the H22/987 tumor in a randomly selected NUDE BALB/c mouse treated with mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium - $Mg_{0.1}$-γ-Fe_2O_3(mPEG-silane)$_{0.5}$ (3 mg·mL^{-1}) and subjected to an AMF for 30 minutes (B = 23 mT, H_O = 18.3 kA·m^{-1}, f = 110.10 kHz, capacitor 200 mF) following 15 minutes (30-45 min) without AMF (off). AMF (-) – no alternating magnetic field; AMF (+) – alternating magnetic field.

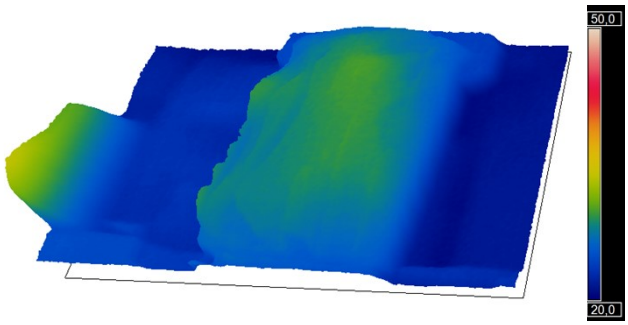

Fig. S8 Representative 3D infrared (IR) thermal image of the H22/987 tumor in a randomly selected NUDE BALB/c mouse treated with mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium - $Mg_{0.1}$-γ-Fe_2O_3(mPEG-silane)$_{0.5}$ (3 mg·mL^{-1}) and subjected to an AMF for 30 minutes. B = 23 mT, H_O = 18.3 kA·m^{-1}, f = 110.10 kHz, capacitor 200 mF. The image was taken prior AMF. Thermal bar in colors (deg C) on the right.

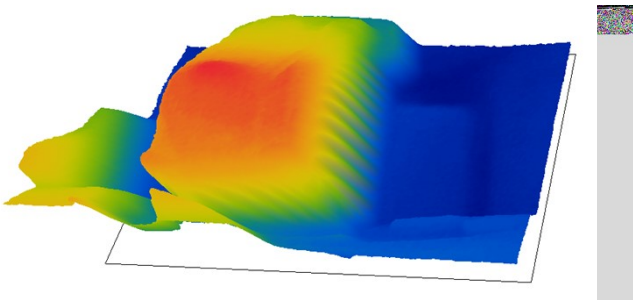

Fig. S9 Representative 3D infrared (IR) thermal image of the H22/987 tumor a randomly selected NUDE BALB/c mouse treated with mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium - $Mg_{0.1}$-γ-Fe_2O_3(mPEG-silane)$_{0.5}$ nanoparticles (3 mg·mL^{-1}) and subjected to an AMF for 30 minutes. B = 23 mT, H_O = 18.3 kA·m^{-1}, f = 110.10 kHz, capacitor 200 mF. The image was taken at 15 minutes AMF. Thermal bar in colors (deg C) on the right.

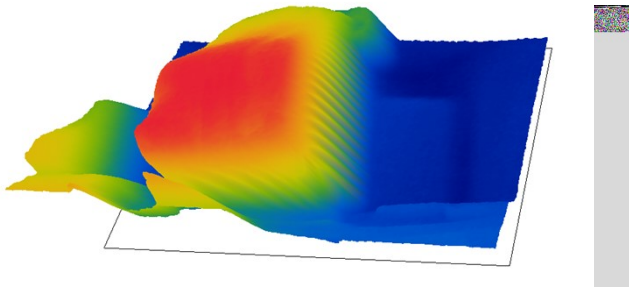

Fig. S10 Representative 3D infrared (IR) thermal image of the H22/987 tumor a randomly selected NUDE BALB/c mouse treated with mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium - $Mg_{0.1}$-γ-Fe_2O_3(mPEG-silane)$_{0.5}$ nanoparticles (3 mg·mL^{-1}) and subjected to an AMF for 30 minutes. B = 23 mT, H_O = 18.3 kA·m^{-1}, f = 110.10 kHz, capacitor 200 mF. The image was taken at 30 minutes AMF. Thermal bar in colors (deg C) on the right.

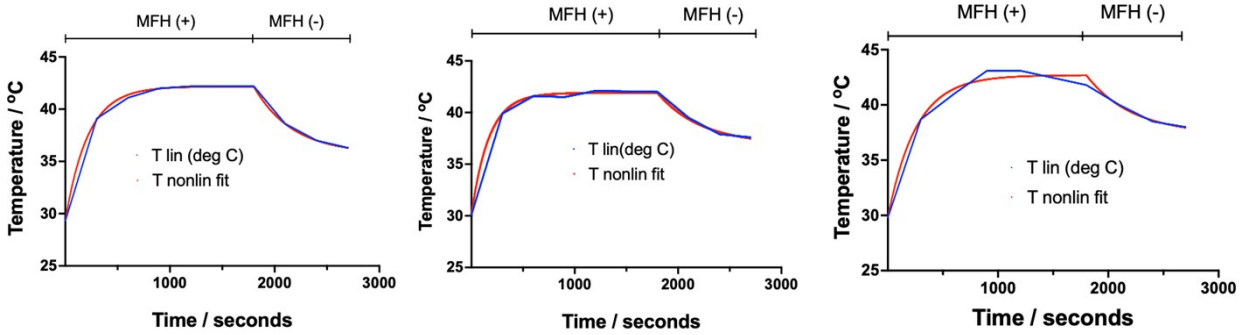

Fig. S11 Thermal curves of the H22/987 tumor measured in three randomly selected NUDE BALB/c mice treated with mPEG-silane-coated iron(III) oxide nanoparticles doped with magnesium - $Mg_{0.1}$-γ-Fe_2O_3(mPEG-silane)$_{0.5}$ nanoparticles (3 mg·mL^{-1}) and subjected to an AMF for 30 minutes (B = 23 mT, H_O = 18.3 kA·m^{-1}, f = 110.10 kHz, capacitor 200 mF) following 15 minutes (30-45 min) without AMF (off). Magnetic fluid hyperthermia (MFH).

References

[1] A. M. Nowicka, M. Ruzycka-Ayoush, A. Kasprzak, A. Kowalczyk, M. Bamburowicz-Klimkowska, M. Sikorska, K. Sobczak, M. Donten, A. Ruszczynska, J. Nowakowska and I. P. Grudzinski, Application of biocompatible and ultrastable superparamagnetic iron(iii) oxide nanoparticles doped with magnesium for efficient magnetic fluid hyperthermia in lung cancer cells, *Journal of Materials Chemistry B*, 2023, **11**, 4028-4041.

[2] M. Sikorska, M. Ruzycka-Ayoush, I. Rios-Mondragon, E. M. Longhin, S. Meczynska-Wielgosz, M. Wojewodzka, A. Kowalczyk, A. Kasprzak, J. Nowakowska, K. Sobczak, M. Muszynska, M. R. Cimpan, E. Runden-Pran, S. Shaposhnikov, M. Kruszewski, M. Dusinska, A. M. Nowicka and I. P. Grudzinski, Lack of cytotoxic and genotoxic effects of mPEG-silane coated iron(III) oxide nanoparticles doped with magnesium despite cellular uptake in cancerous and noncancerous lung cells, *Toxicology in Vitro*, 2024, **99**, 105850.

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