Supporting Information

for

Size-selected Fe_3O_4–Au hybrid nanomaterials for improved magnetism-based theranostics

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Additional experimental information

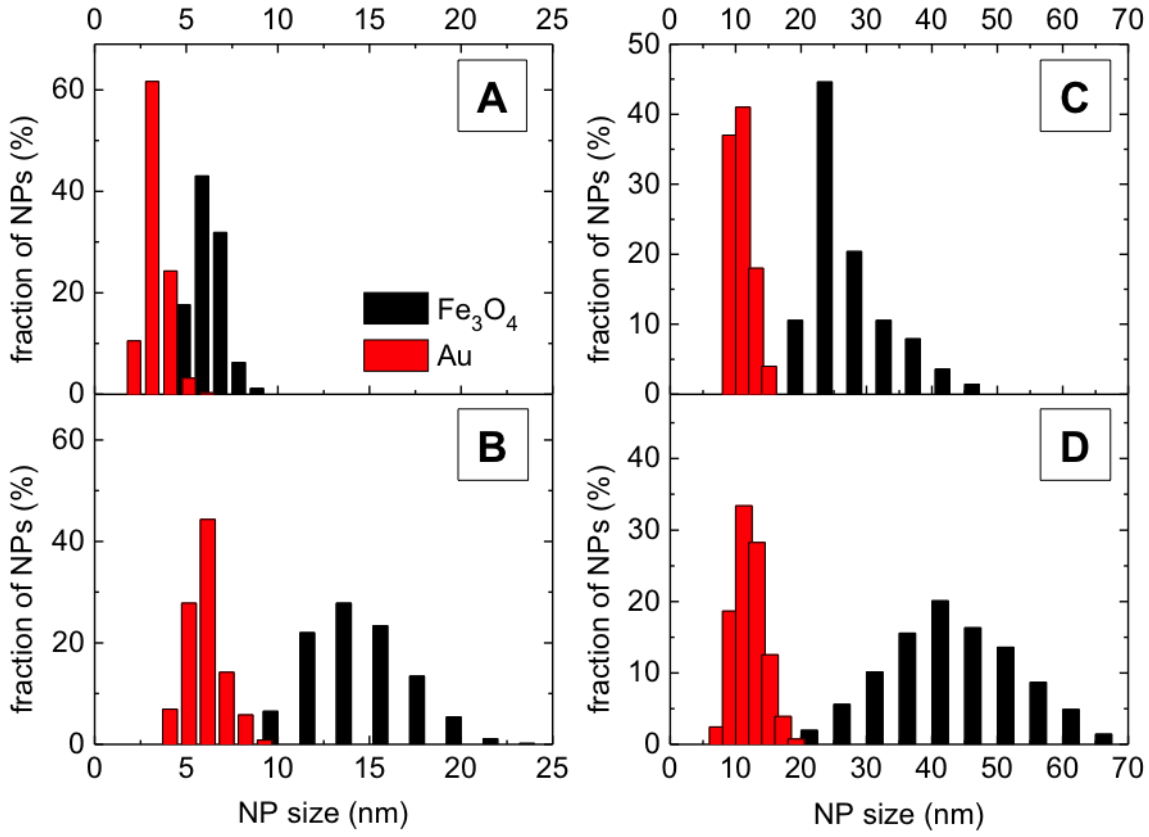

Figure S1: Size distributions calculated from TEM images of magnetite (black) and gold (red) parts of NPs for the samples with in situ synthesized Au seeds: A) MNP-6; B) MNP-15; and with pre-synthesized Au seeds: C) MNP-25; D) MNP-44.

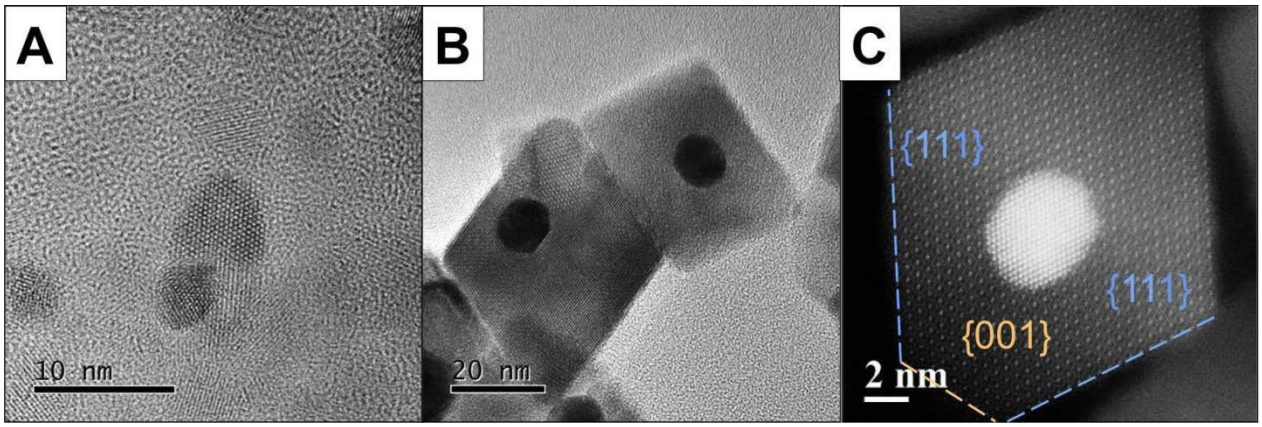

Figure S2: HRTEM images of size-selected magnetite-gold NPs: MNP-6 (A), MNP-44 (B), MNP-25 (C).

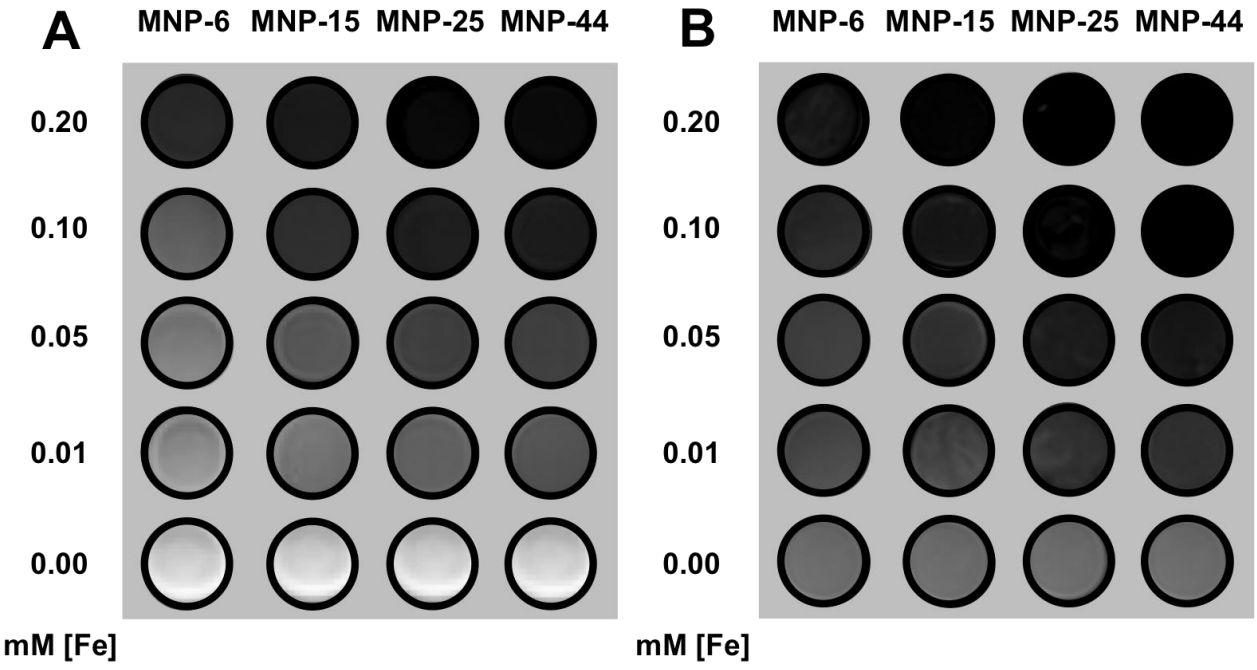

Figure S3: Series of T_2-weighted images of NPs solutions in water (A) and 2% agarose (B) acquired at TE = 48 ms and concentrations ranging from 0.00 to 0.20 mM Fe.

Table S1: Hydrodynamic size of hybrid Fe_3O_4-Au NPs, stabilized in water by DSPE-PEG-COOH determined by dynamic light scattering. Results are shown as means ± standard deviation.

Sample	Hydrodynamic diameter, nm
MNP-6	95 ± 2
MNP-15	112 ± 3
MNP-25	121 ± 5
MNP-44	160 ± 9
MNP-25 in RPMI	123 ± 7

Table S2: A cell viability study (MTS assay) of 4T1 cells after 15 min and 30 min of incubation: “RPMI” – culture medium without NPs at 37 °C; “NPs + no AMF” – in the presence of MNP-25 in cell medium at 37 °C; “NPs + AMF” – in the presence of MNP-25 in the cell medium heated up to 46 ± 1 °C in 261–393 kHz, 25 mT AMF.

Sample	15 min	30 min
RPMI	100 ± 3	100 ± 2
NPs + no AMF	97 ± 5	91 ± 2
NPs + AMF	78 ± 1	21 ± 9

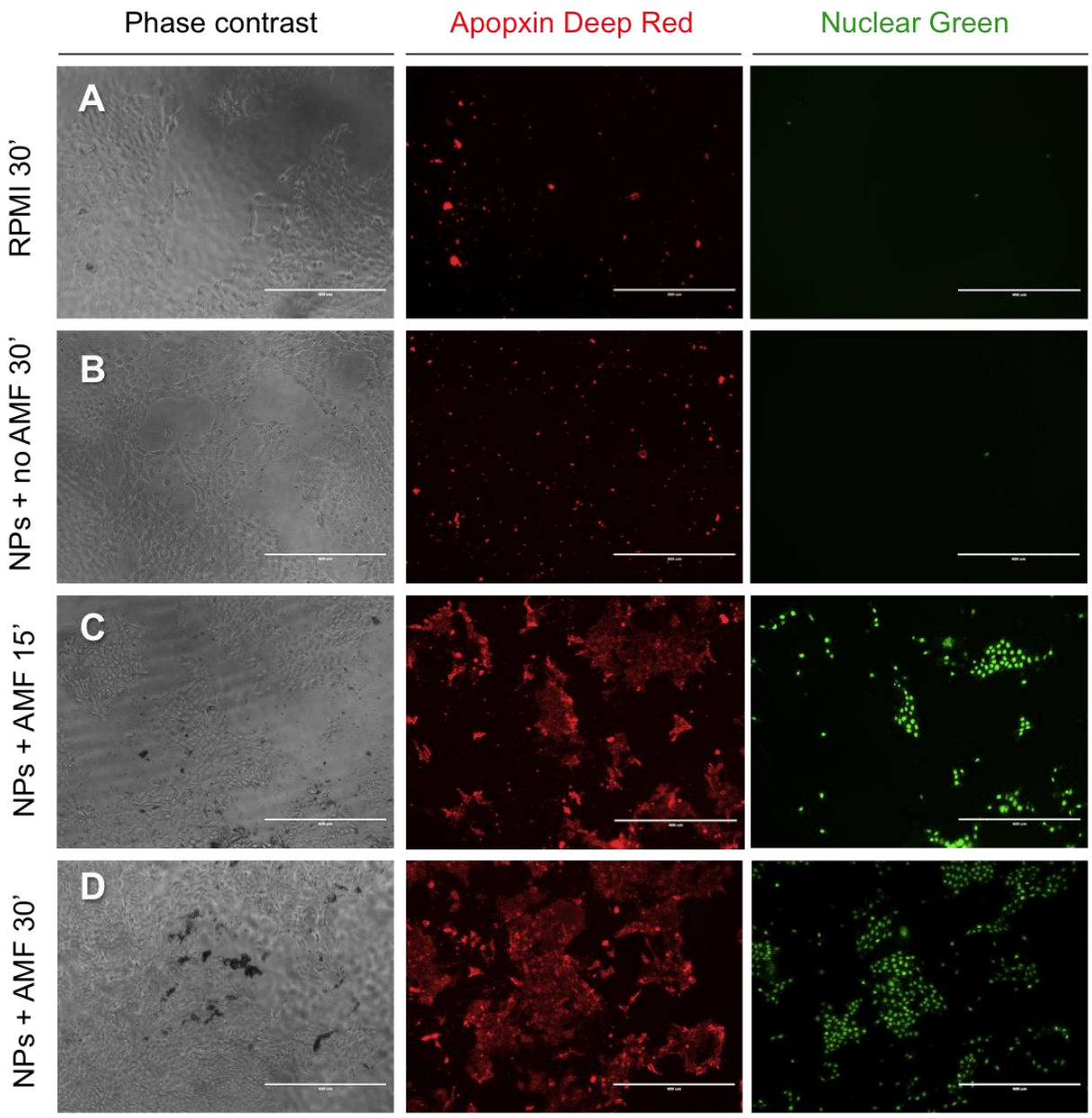

Figure S4: Apoptosis/necrosis activation in 4T1 cells, cultivated with polymer-stabilized MNP-25 NPs (3.6 mg·mL^{-1} Fe), phase contrast (left column) and fluorescent microscopy, intravital staining with Apopxin Deep Red (middle column) and Nuclear Green (right column). A) Control cells without NPs; B) cells incubated with NPs at 37 °C in zero AMF; C) cells incubated with NPs in 261–393 kHz, 25 mT AMF (46 ± 1 °C) during 15 min; D) cells incubated with NPs in 261–393 kHz, 25 mT AMF (46 ± 1 °C) during 30 min. Scale bar corresponds to 400 μm.

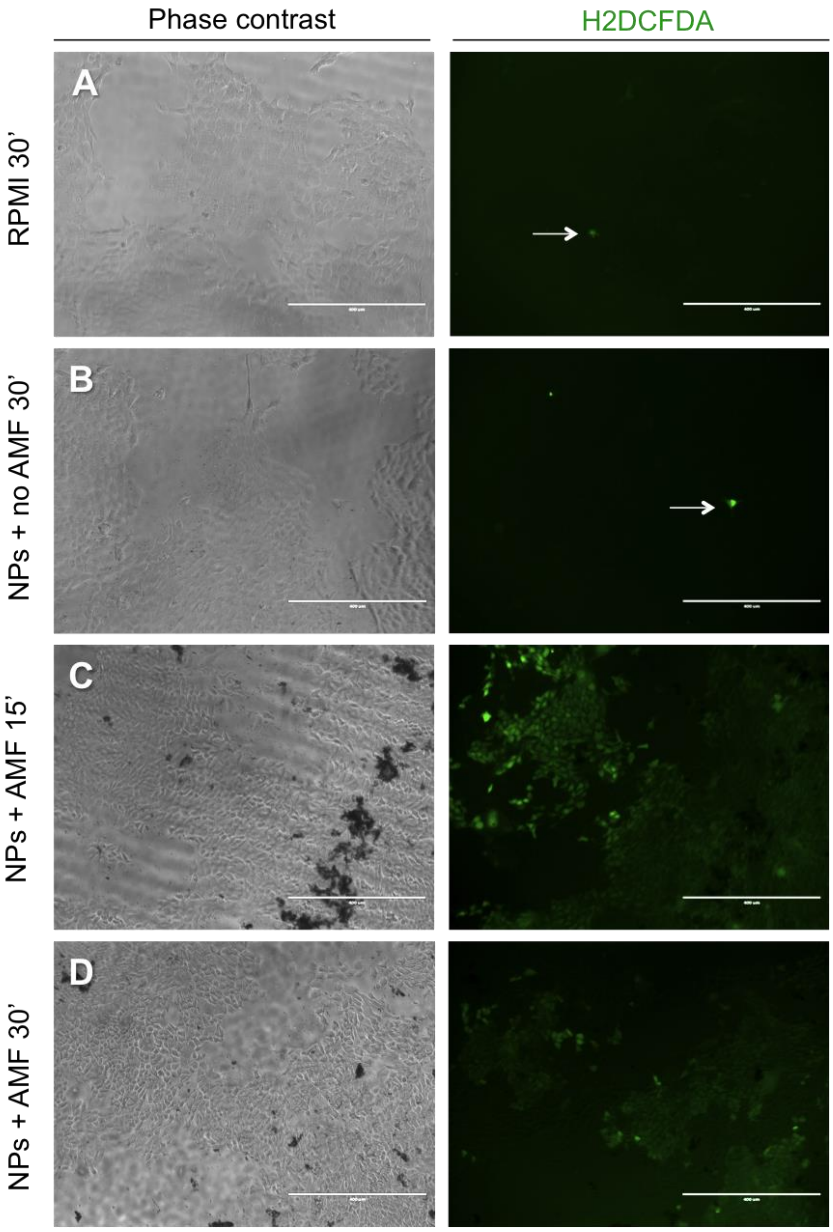

Figure S5: Reactive oxygen species (ROS) production by 4T1 cells, cultivated with polymer-stabilized MNP-25 NPs (3.6 mg·mL^{-1} Fe), phase contrast (left column) and fluorescent microscopy, intravital staining with H2DCFDA (right column). A) Control cells without NPs; B) cells incubated with NPs at 37 °C in zero AMF; C) cells incubated with NPs in 261–393 kHz, 25 mT AMF (46 ± 1 °C) during 15 min; D) cells incubated with NPs in 261–393 kHz, 25 mT AMF (46 ± 1 °C) during 30 min. White arrows show single cells with increased level of ROS production. Scale bar corresponds to 400 µm.

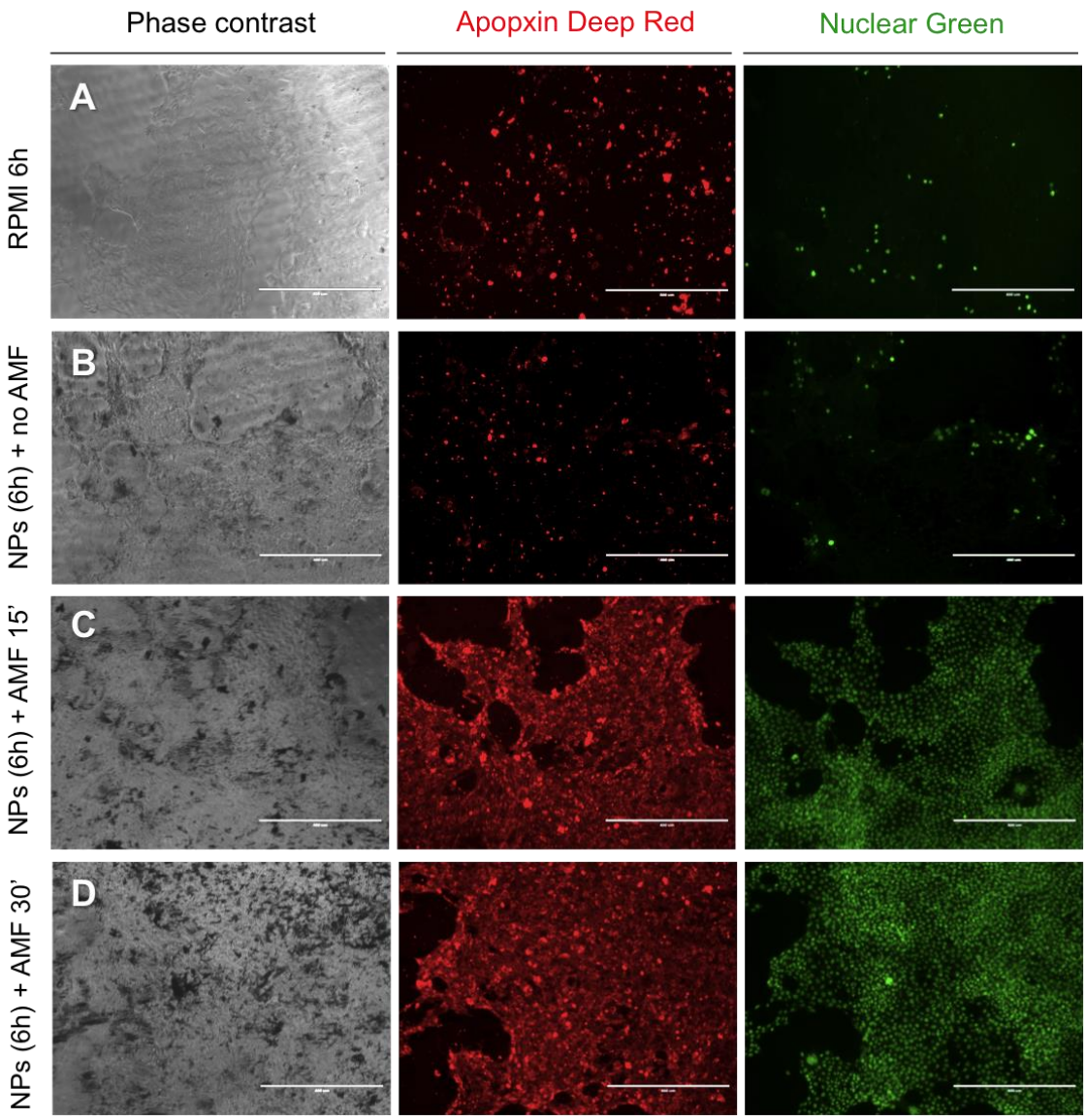

Figure S6: Apoptosis/necrosis activation in 4T1 cells, preliminary cultivated with polymer-stabilized MNP-25 NPs (3.6 mg·mL^{-1} Fe) during 6 h, phase contrast (left column) and fluorescent microscopy, intravital staining with Apopxin Deep Red (middle column) and Nuclear Green (right column). A) Control cells without NPs; B) cells incubated with NPs at 37 °C in zero AMF; C) cells incubated with NPs in 261–393 kHz, 25 mT AMF (46 ± 1 °C) during 15 min; D) cells incubated with NPs in 261–393 kHz, 25 mT AMF (46 ± 1 °C) during 30 min. Scale bar corresponds to 400 μm.

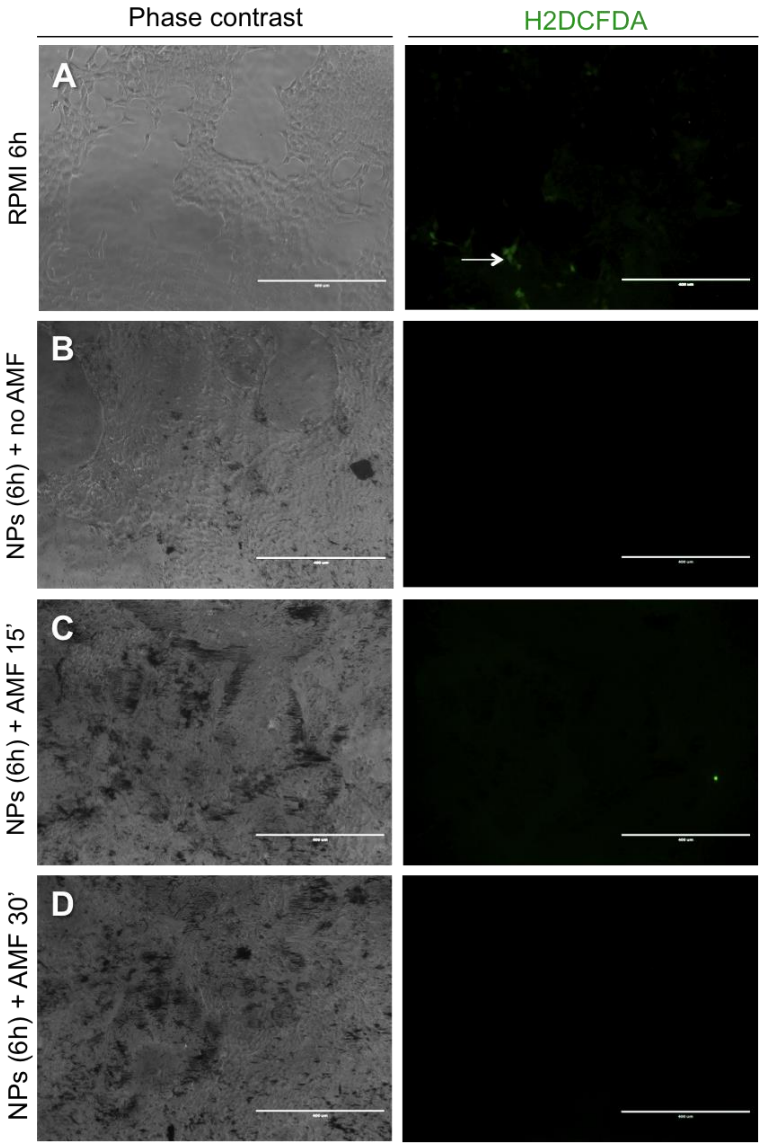

Figure S7: Reactive oxygen species (ROS) production by 4T1 cells, preliminary cultivated with polymer-stabilized MNP-25 NPs (3.6 mg·mL^{-1} Fe) during 6 h, phase contrast (left column) and fluorescent microscopy, intravital staining with H2DCFDA (right column). A) Control cells without NPs; B) cells incubated with NPs at 37 °C in zero AMF; C) cells incubated with NPs in 261–393 kHz, 25 mT AMF (46 ± 1 °C) during 15 min; D) cells incubated with NPs in 261–393 kHz, 25 mT AMF (46 ± 1 °C) during 30 min. White arrows show single cells with increased level of ROS production. Scale bar corresponds to 400 µm.