Supplementary Information

Bifunctional Magnetite-Gold Nanoparticles for Magneto-Mechanical Actuation and Cancer Cell Destruction

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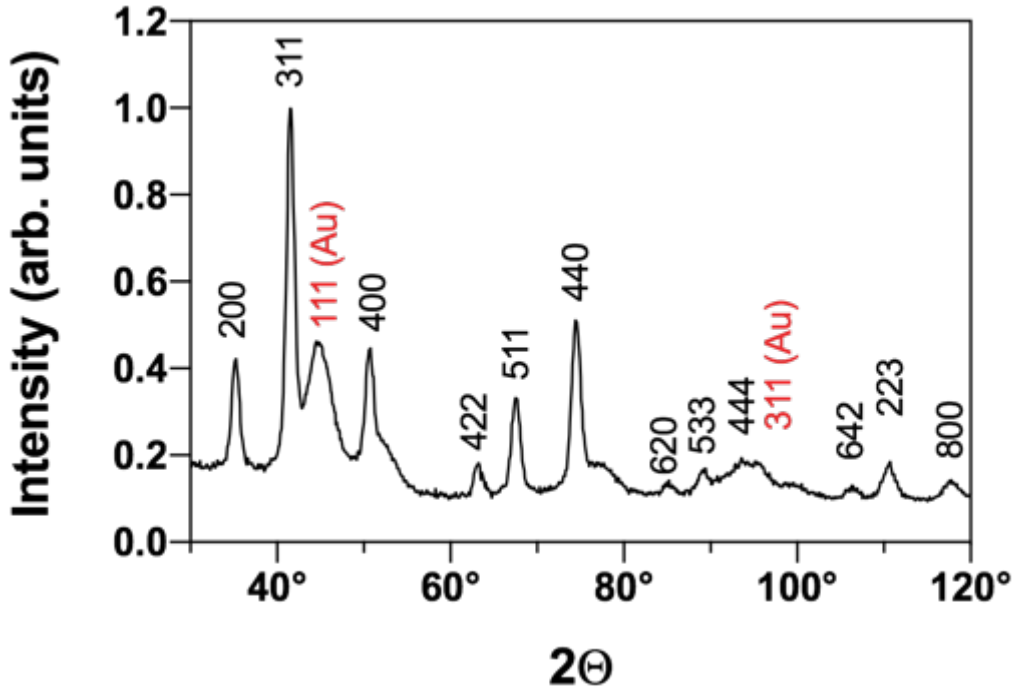

Figure S1. Experimental X-Ray diffraction pattern of Fe_3O_4-Au MNPs. Black and red Miller indices correspond to Fe_3O_4 and Au phases, respectively. The intensity is normalized to the strongest peak, i.e. the Fe_3O_4 (311).

Table S1. Results of the MNPs' structural characterization by XRD. The volume fraction of Fe_3O_4 and Au, crystallite size and lattice parameter (a) are listed.

Volume fraction, %		Crystallite diameter, nm		Lattice parameter, nm	
Fe_3O_4	Au	Fe_3O_4	Au	Fe_3O_4	Au
96.0	4.0	12.0	3.0	0.8363	0.4055

For detailed investigation of MNP-Cy5 localization relative to the cells 2 h after their co-incubation we stained LNCaP and PC-3 cells with anti-β-catenin antibodies and made Z-stack images of these cells (Figure S2). Orthogonal views demonstrated that MNP-Cy5 conglomerates interacted with cells in two ways – NPs located inside the cell, predominantly in the perinuclear area (Figure S2a, S2c), or contacted with cell plasma membrane (Figure S2b, S2d). Probably, in the latter case, such MNP-Cy5 position preceded their internalization by cells.

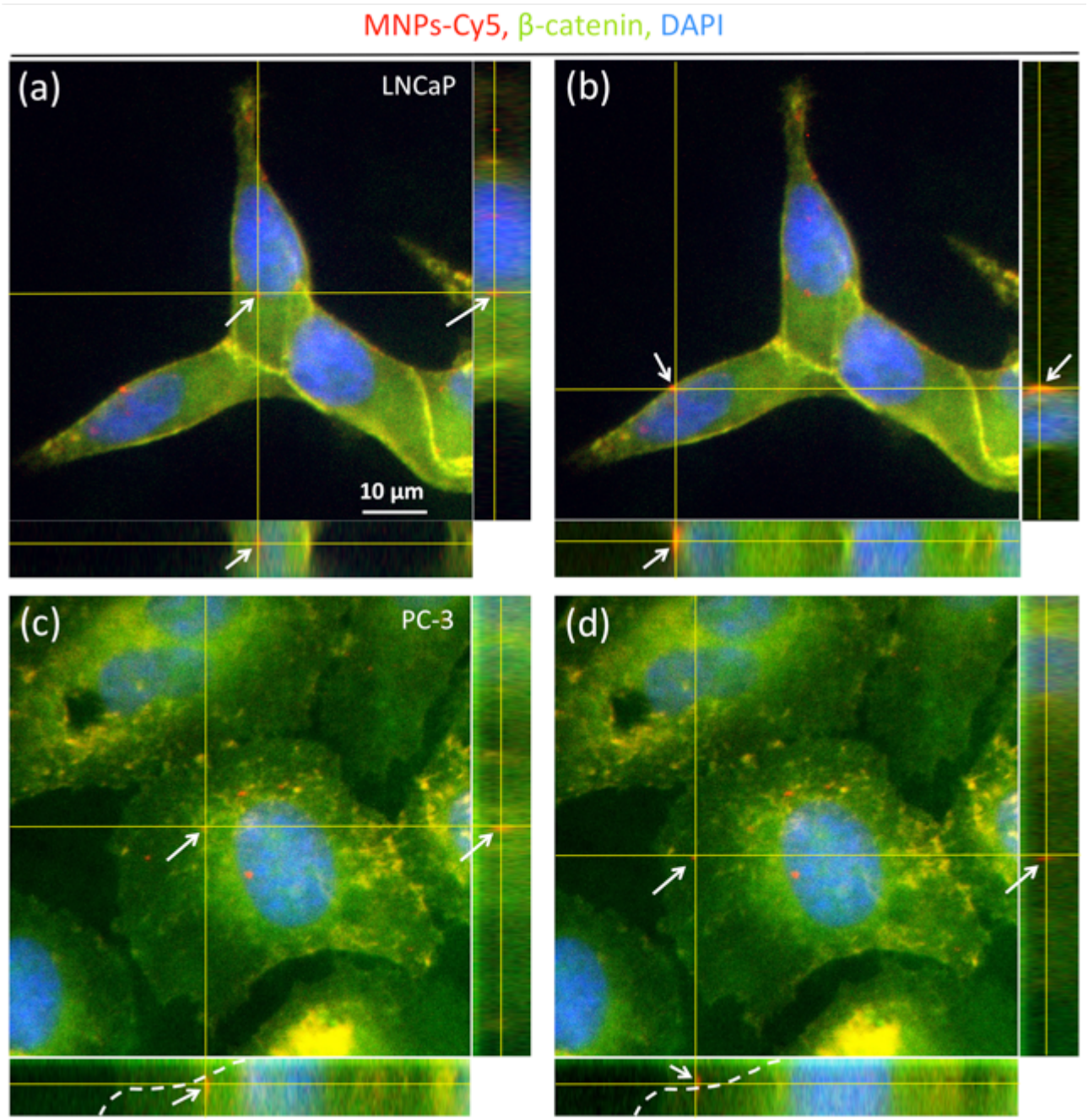

Figure S2. XY-, XZ- and YZ-projections of LNCaP (a,b) and PC-3 (c,d) cells Z-stacks (8 steps, 500 nm each) after 2 h of co-incubation with MNPs-Cy5 (30 µg mL^{-1} Fe_3O_4, 5 µg mL^{-1} Au, 1 µg mL^{-1} Cy5). (**a,c**) Intracellular localization of NPs; (**b,d**) Extracellular localization of NPs on the cell plasma membrane. White arrows indicate MNPs-Cy5 of the interest in three orthogonal projections. Dashed lines indicate cell profiles. Fluorescent staining of cells with anti-β-catenin antibodies and DAPI. Laser scanning confocal microscope Nikon C2.

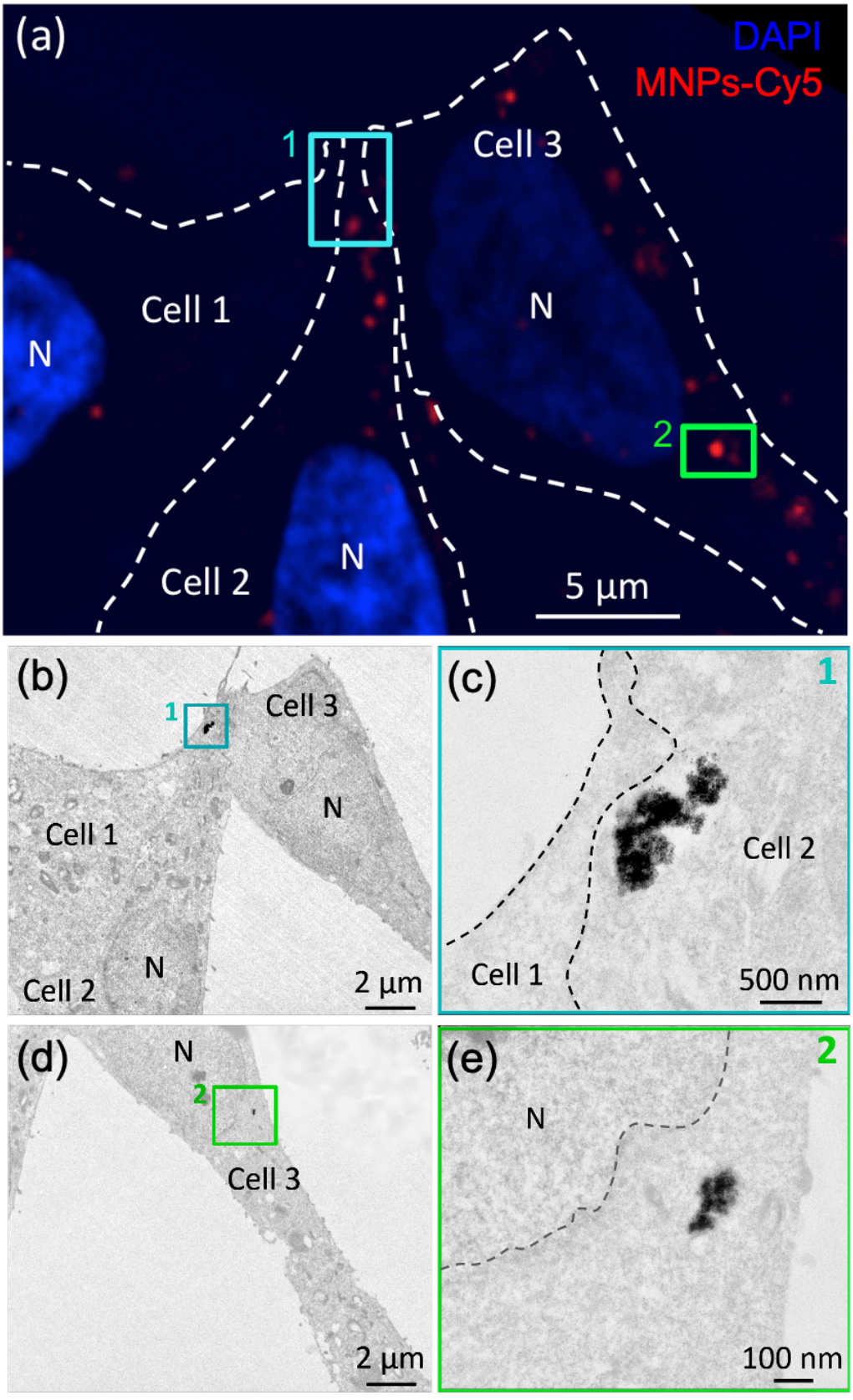

Figure S3. Correlative light-electron microscopy of LNCaP cells co-incubated with MNPs-Cy5 (30 µg mL^{-1} Fe_3O_4, 5 µg mL^{-1} Au, 1 µg mL^{-1} Cy5) for 2 h. (**a**) Laser scanning confocal microscopy of three cells with MNPs-Cy5 inside (fluorescent staining of cells with DAPI); (**b-e**) TEM of the same cells. Blue (1) and green (2) rectangles highlight areas of interest with MNPs-Cy5 inside the cells. Panels (**c,e**) are the magnified views of the areas indicated in panels (**b,d**), respectively. Dashed lines on panels (**a,c**) indicate cell profiles, on panel (**e**) – nucleus profile. N – nucleus.

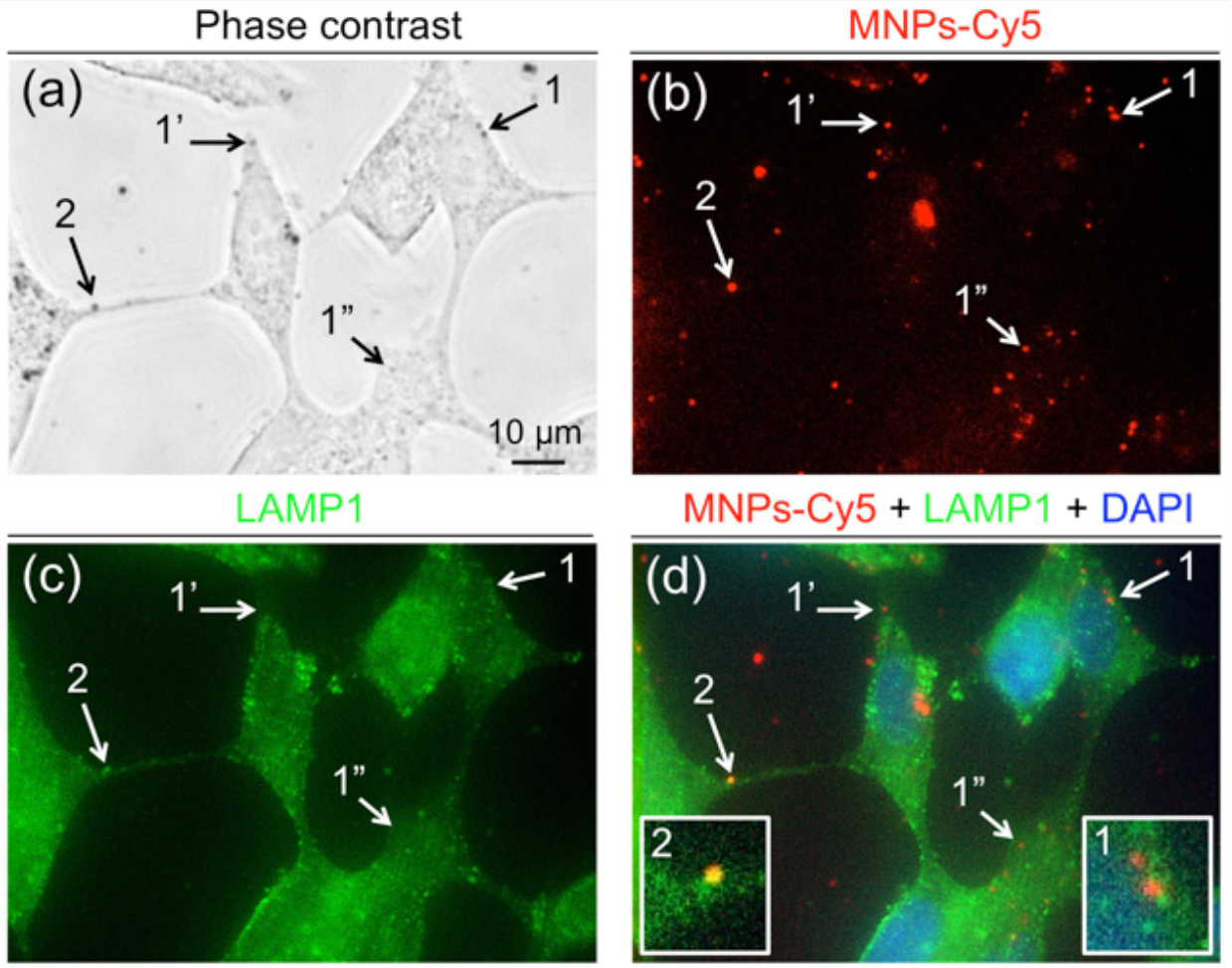

Figure S4. MNP-Cy5 localization inside LNCaP cells after 2 h of co-incubation (30 μg mL^{-1} Fe_3O_4, 5 μg mL^{-1} Au, 1 μg mL^{-1} Cy5). Arrows with indices 1, 1', and 1'' point to examples of MNPs-Cy5 located not in acid vesicular compartment of the cells. Arrows with index 2 point to an example of MNPs-Cy5 located in lysosome. White rectangles with indices 1 and 2 on the panel (**d**) highlight the magnified views of areas, which are indicated by arrows with corresponding indices. Fluorescent staining of cells with anti-LAMP1 antibodies and DAPI. Fluorescent microscope EVOS.

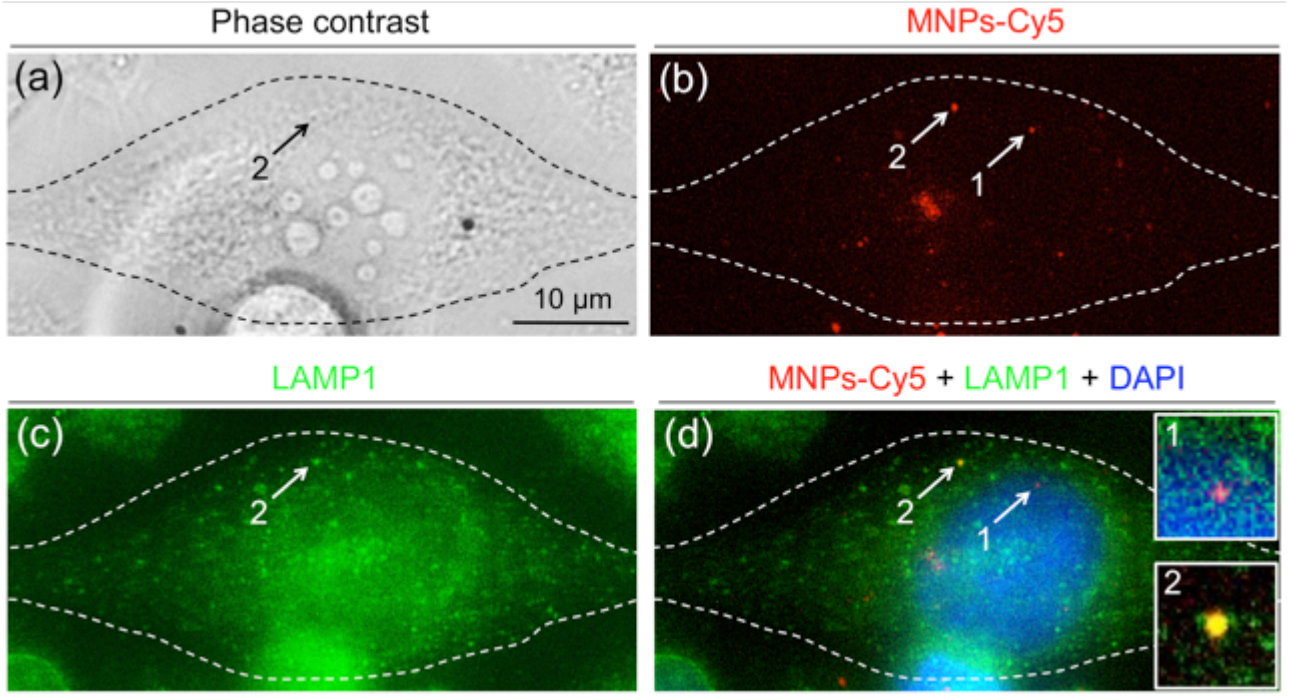

Figure S5. MNP-Cy5 localization inside PC-3 cells after 2 h of co-incubation (30 μg mL^{-1} Fe_3O_4, 5 μg mL^{-1} Au, 1 μg mL^{-1} Cy5). Arrows with index 1 point to an example of MNPs-Cy5 located not in acid vesicular compartment of the cell. Arrows with index 2 point to an example of MNPs-Cy5 located in lysosome. White rectangles with indices 1 and 2 on the panel (**d**) highlight the magnified views of areas, which are indicated by arrows with corresponding indices. Dashed lines indicate cell profiles. Fluorescent staining of cells with anti-LAMP1 antibodies and DAPI. Fluorescent microscope EVOS.

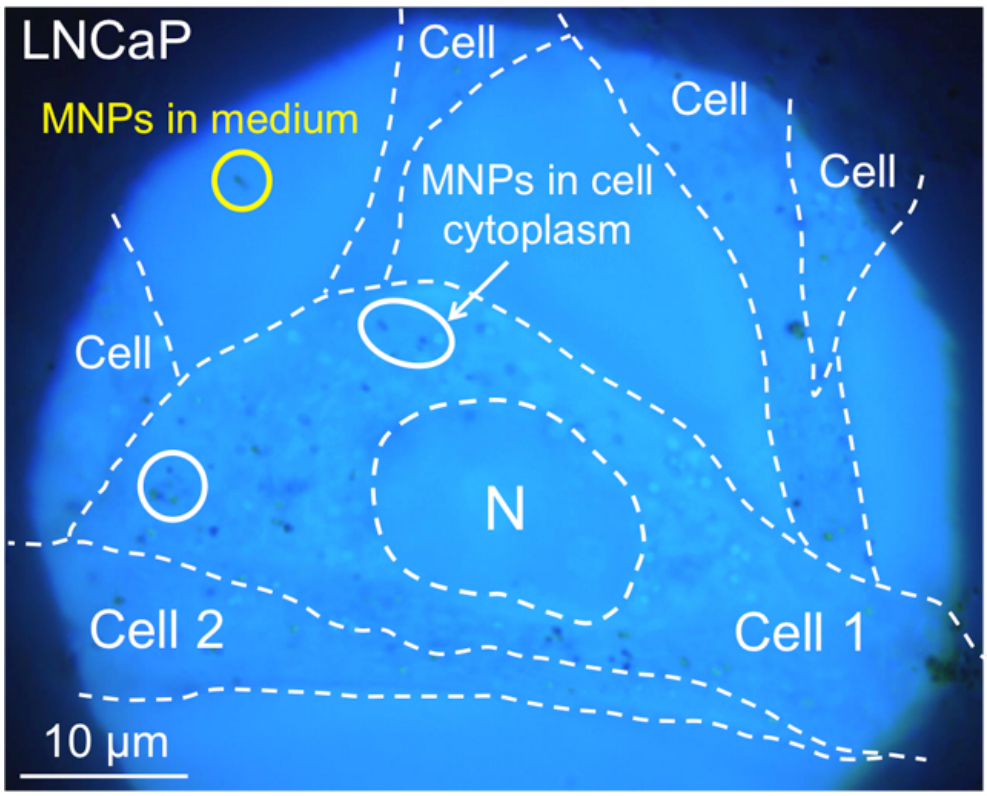

Figure S6. Micrograph of LNCaP cells after 24 h of co-incubation with MNPs-Cy5 (30 μg mL^{-1} Fe_3O_4, 5 μg mL^{-1} Au, 1 μg mL^{-1} Cy5) and subsequent placement in a rotation magnetic field (1 Hz, 7 mT) in magneto-optic system. Dashed lines indicate cells and nucleus profiles. N – nucleus. Explanatory figure for Video S5, S6.

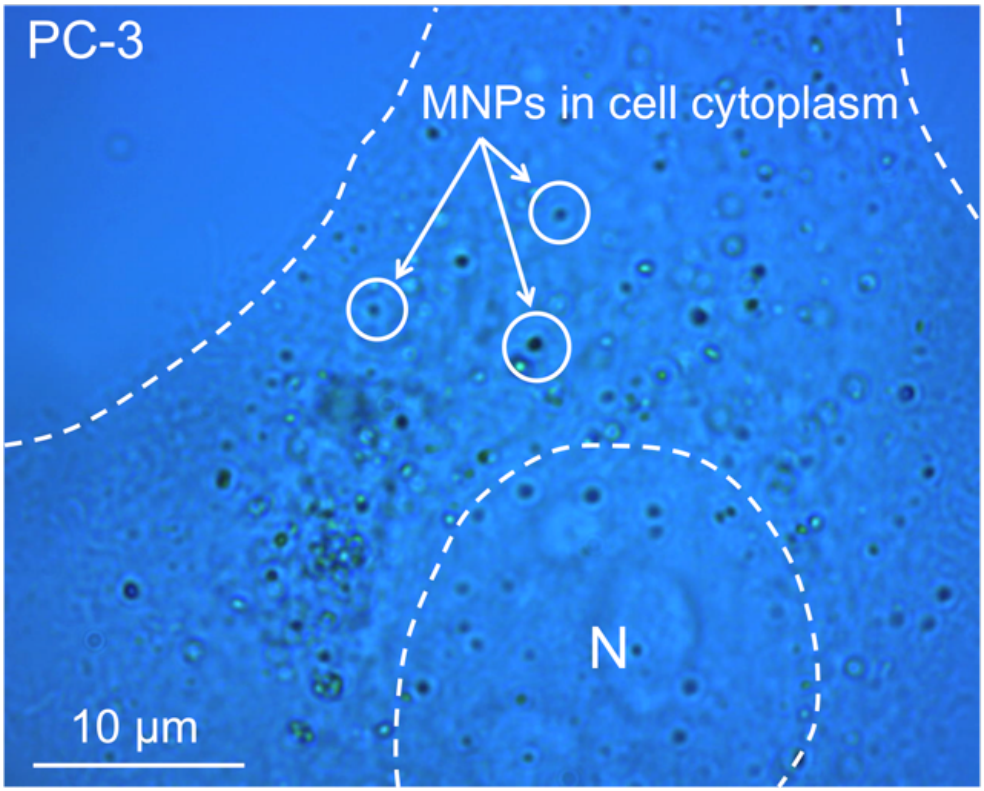

Figure S7. Micrograph of PC-3 cell after 24 h of co-incubation with MNPs-Cy5 (30 μg mL^{-1} Fe_3O_4, 5 μg mL^{-1} Au, 1 μg mL^{-1} Cy5) and subsequent placement in a rotation magnetic field (1 Hz, 7 mT) in magneto-optic system. Dashed lines indicate cell and nucleus profiles. N – nucleus. Explanatory figure for Video S7.

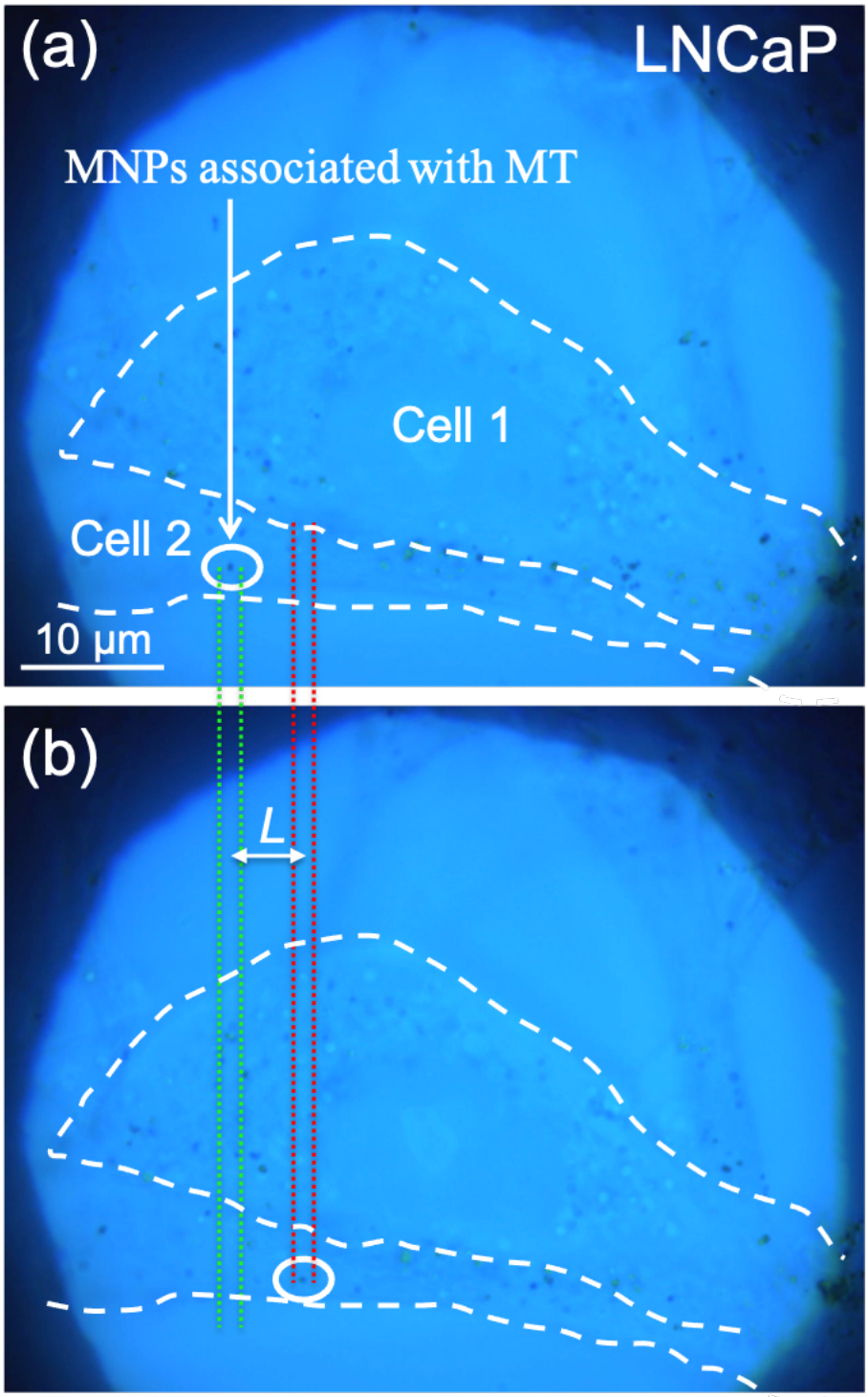

Figure S8. Micrographs of LNCaP cells after 24 h of co-incubation with MNPs-Cy5 (30 μg mL^{-1} Fe_3O_4, 5 μg mL^{-1} Au, 1 μg mL^{-1} Cy5) and subsequent placement in a rotation magnetic field (1 Hz, 7 mT) in magneto-optic system. Dashed wight lines indicate cells profiles, green and red lines show the localization of MNPs conglomerate at different time points. MT – microtubules, L – distance traveled by MNPs in a time between frames presented on panels (**a**) and (**b**). Explanatory figure for Video S8.

Video S1. Real-time observation of magnetite–gold dumbbell MNP-Cy5 behavior in water solution without magnetic field application.

Video S2. Real-time observation of magnetite–gold dumbbell MNP-Cy5 behavior in water solution under low-frequency rotation field (1 Hz, 7 mT).

Video S3. Real-time observation of magnetite–gold dumbbell MNP-Cy5 behavior in water solution under low-frequency constant field (7 mT).

Video S4. Real-time observation of magnetite–gold dumbbell MNP-Cy5 behavior in water solution during alternating current field exposure (1 Hz, 7 mT) and after its termination.

Video S5. Real-time observation of magnetite–gold dumbbell MNP-Cy5 behavior inside LNCaP cell ("Cell 1" in Figure S6) after 24 h of co-incubation and following placement in a rotation magnetic field (1 Hz, 7 mT) in magneto-optic system.

Video S6. Real-time observation of magnetite–gold dumbbell MNP-Cy5 behavior in medium and inside LNCaP cell ("Cell 1" in Figure S6) after 24 h of co-incubation and following placement in a rotation magnetic field (1 Hz, 7 mT) in magneto-optic system.

Video S7. Real-time observation of magnetite–gold dumbbell MNP-Cy5 behavior inside PC-3 cell after 24 h of co-incubation and following placement in a rotation magnetic field (1 Hz, 7 mT) in magneto-optic system.

Video S8. Real-time observation of magnetite–gold dumbbell MNP-Cy5 behavior inside LNCaP cell ("Cell 2" in Figure S8) after 24 h of co-incubation and following placement in a rotation magnetic field (1 Hz, 7 mT) in magneto-optic system.