Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting Information

Biocompatibility, uptake and subcellular localization of bacterial magnetosomes in mammalian cells

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Supplementary Methods

Fluorescence microscopy

500,000 FaDu cells were seeded in 6-well plates and cultivated overnight. Subconfluent cell layers were incubated with fluorescent, DyLight488-labeled WT magnetosomes (25 µg cm^{-2}, corresponding to 97.2 µg mL^{-1} Fe) for 24 h. Cells were detached by trypsin-EDTA treatment and applied to magnetic cell separation. Magnetically labelled cells were diluted 1:5 in PE (2 mM EDTA in PBS) and transferred into a 96-well plate. After 2 h the wells were analyzed with a CellCelector™ device (Automated Lab Solutions (ALS), Jena, Germany). Bright-field and fluorescence images (excitation 490 nm) were recorded with the device software. Particle uptake was documented by merging bright-field image and the corresponding fluorescence image.

Supplementary Figures

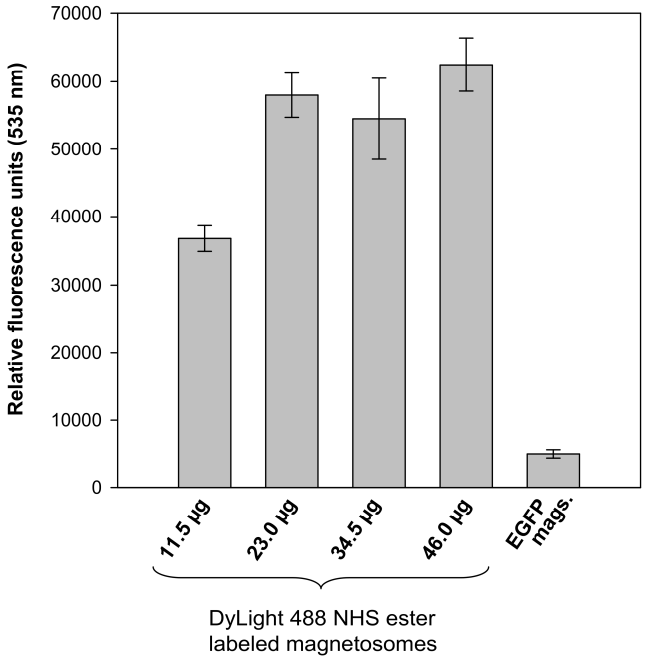

Fig. S1 Relative fluorescence of DyLight 488 labeled magnetosomes. Wildtype (WT) magnetosomes isolated from *M. gryphiswaldense* (24 µg Fe in 50 mM $NaHCO_3$, pH 9.0) were incubated with the indicated amounts of DyLight 488 NHS ester for 2 h at 16 °C. After removal of excess dye by extensive washing steps particles were resuspended in 10 mM HEPES + 1 mM EDTA pH 7.2 and subjected to fluorescence microscopy analyses. Relative fluorescence units indicate the saturation of the particle surface for a DyLight 488 amount of 23 µg. Compared to EGFP magnetosomes (used as an additional control because they had been shown to display 80-200 EGFP copies on the surface; Borg et al. 2014), DyLight 488 labeled particles displayed an up to 12-fold increased fluorescence. Error bars represent standard deviations calculated from at least three independent experiments.

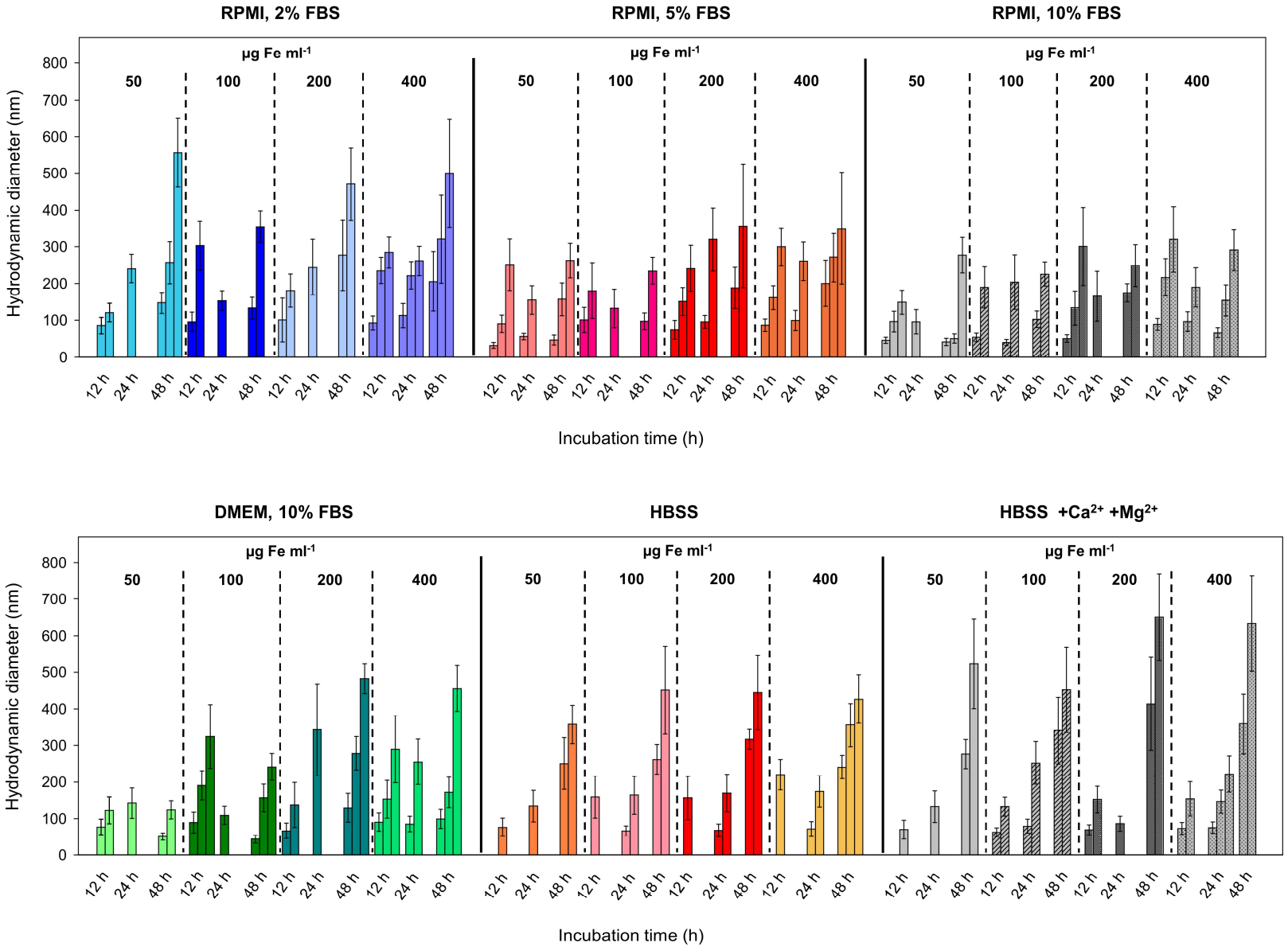
RPMI, 2% FBS
RPMI, 5% FBS
RPMI, 10% FBS
DMEM, 10% FBS
HBSS
HBSS +Ca2+ +Mg2+
µg Fe ml-1
50
100
200
400
Hydrodynamic diameter (nm)
0
100
200
300
400
500
600
700
800
12 h
24 h
48 h
Incubation time (h)

Fig. S2 Hydrodynamic diameters of WT magnetosomes and particle agglomerates formed upon incubation in different cell culture media and buffer solutions. Isolated particles were suspended in the indicated media or buffer compositions (final concentration 50, 100, 200 or 400 µg Fe mL^{-1}). DLS was used to determine the hydrodynamic diameters / sizes after 12, 24 and 48 h of incubation and to monitor the formation of potential aggregates. Measurements were performed on three biological replicates, and each replicate was measured in quintuplicates (n_{total} = 15; error bars correspond to the standard deviations). For the different conditions investigated, several size "classes" of particles or agglomerates were detected (represented as bars). Depending on the tested condition and incubation time, a different number of such size "classes" was partially observed (reflected by varying bar numbers). RPMI, Roswell Park Memorial Institute Medium; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; HBSS, Hank's Balanced Salt Solution.

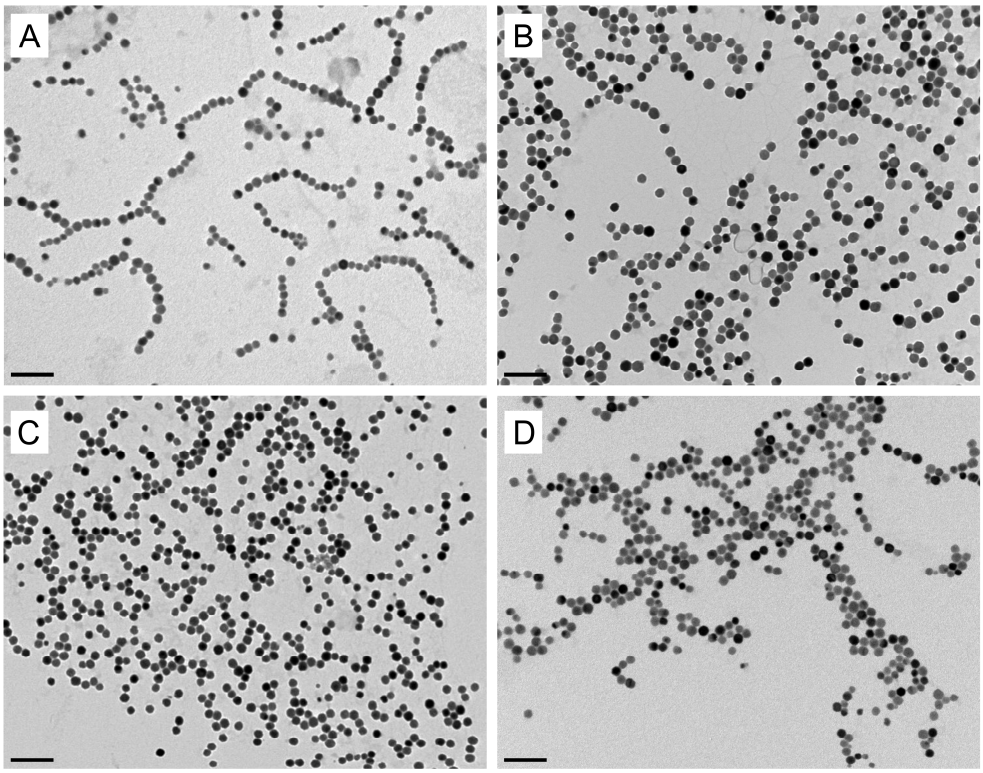

Fig. S3 Transmission electron microscopy images of isolated magnetosomes (400 µg mL^{-1} Fe) incubated in different cell culture media for 48 h. Isolated particles were pelleted by centrifugation (4000 *g*, 4 °C, 1 h) and resuspended in RPMI1640 + GlutaMAX-I supplemented with 2% (A), 5% (B) or 10% (C) FBS, or DMEM + GlutamMAX-I supplemented with 10% FBS (D). TEM analysis was performed after 48 h of incubation and indicated the presence of well-dispersed particles that tended to form smaller chains and aggregates. Scale bar 200 nm.

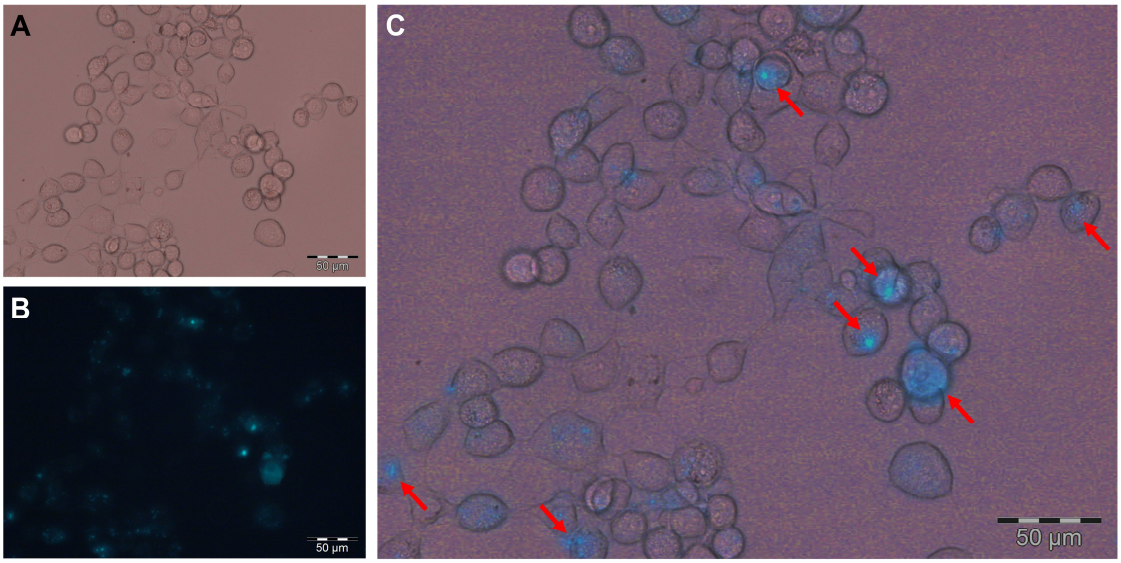

Fig. S4 Fluorescence microscopy analysis of magnetosome internalization by FaDu cells. Subconfluent FaDu cell layers were incubated with fluorescent, DyLight488-labeled WT magnetosomes (25 µg cm^{-2}) for 24 h. Particle uptake was investigated by merging bright-field (A) and fluorescence images (B). In the composite image (C) fluorescent signals clearly co-localize with the cells (indicated by red arrows), suggesting particle internalization; scale bar 50 µm.

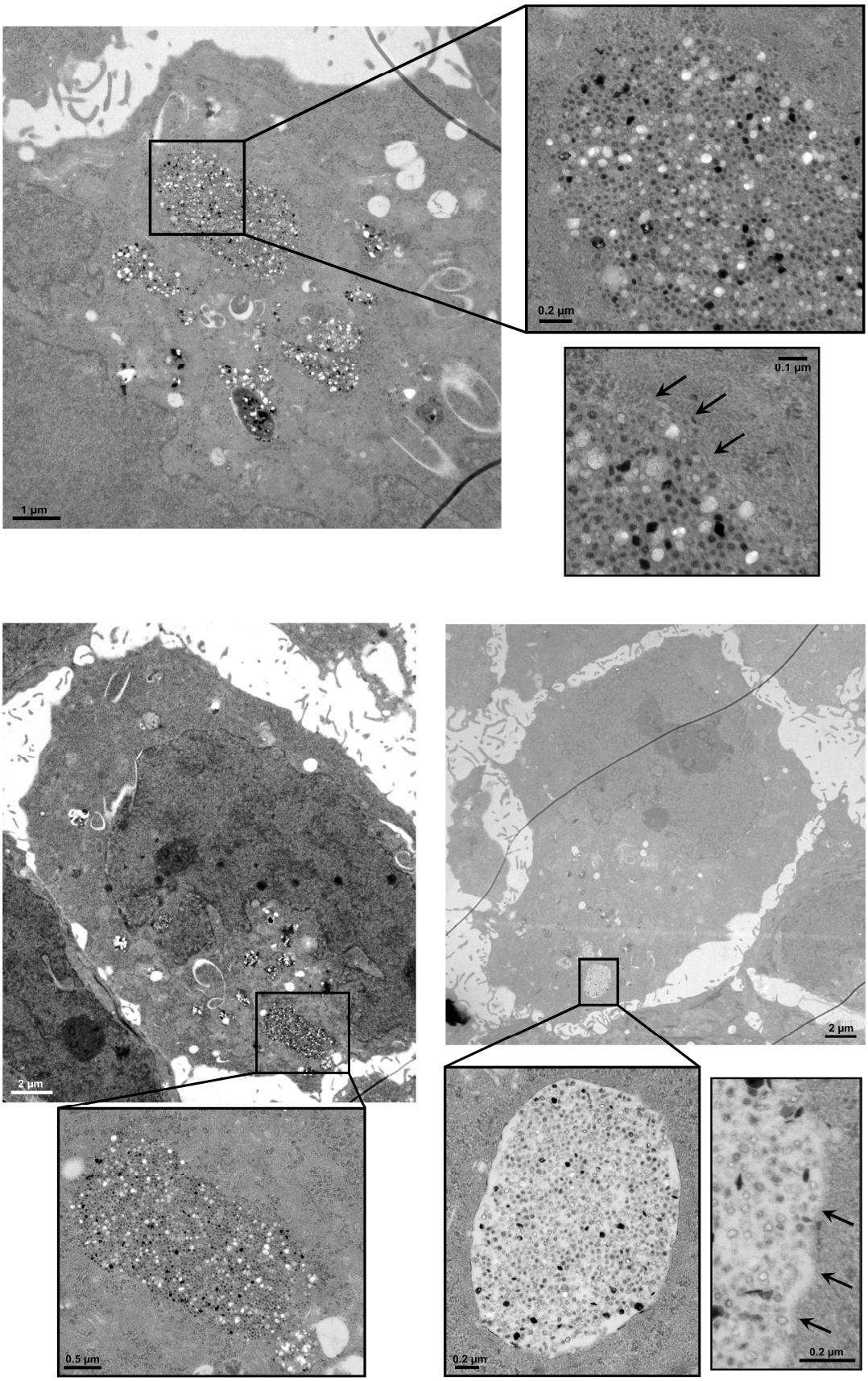

Fig. S5 Transmission electron micrographs of FaDu cells with internalized magnetosomes. FaDu cells were incubated with isolated magnetosome suspensions for 24 h. The magnifications show high amounts of electron-dense particles internalized in vesicle-like structures. The latter are surrounded by a distinct membrane, indicated by arrows.

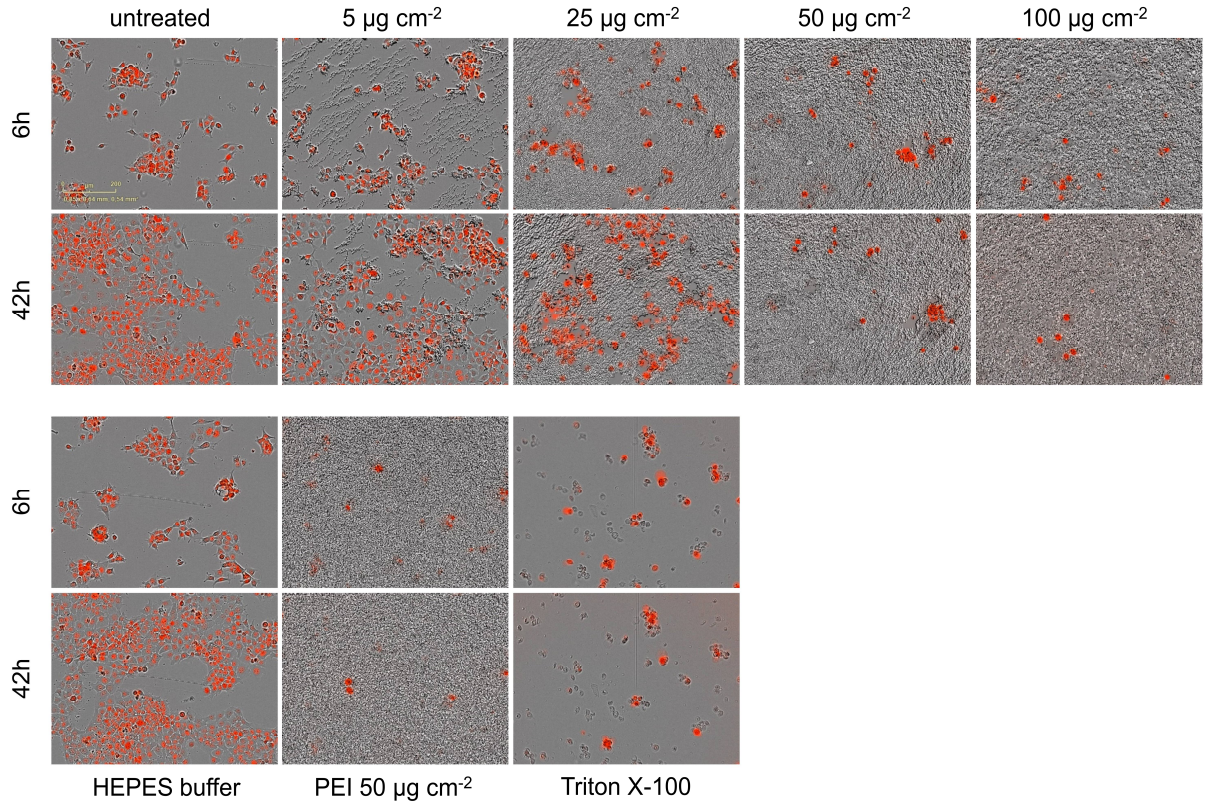

Fig. S6 Microscopic analysis of FaDu-NR cells incubated with different amounts of isolated magnetosomes. In the course of magnetosome-treatment, a concentration-dependent effect on the proliferation rates was observed (see Fig. 6). However, microscopy analyses of FaDu-NR cells incubated with 5, 25, 50 or 100 µg cm^{-2} magnetosomes indicate the presence of intact cells, suggesting impaired cell division. Display windows from the IncuCyte Zoom image collection representing the same area of FaDu-NR cell cultures after 6 h and 42 h incubation time under treatment conditions as indicated are presented. For control, FaDu cells were treated with HEPES buffer (solvent of the magnetosomes, final concentration 2 mM), 50 µg cm^{-2} PEI-coated iron oxide nanoparticles or 0.1% Triton X-100.

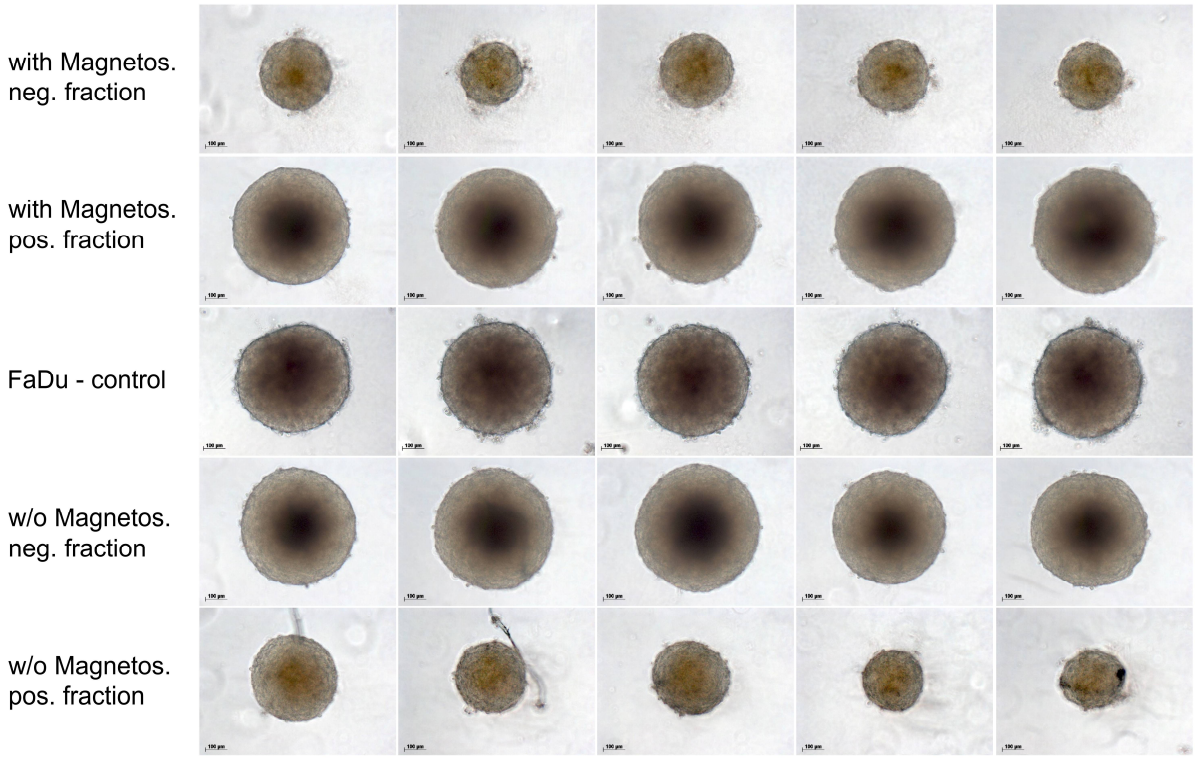

Fig. S7 Spheroid formation of FaDu cells after magnetosome-labelling. 150,000 FaDu-NR cells were subjected to magnetic separation (MACS MS columns), either directly (i.e. without magnetosome-treatment) or after incubation with 25 µg cm^{-2} magnetosomes for 24 h. Whereas the majority of the untreated cells directly passed the column ("w/o Magnetos. neg. fraction") and only a low number of cells was retained within the matrix ("w/o Magnetos. pos. fraction"), a high portion of the magnetosome-incubated cells were sufficiently magnetized to retain in the column ("with Magnetos. pos. fraction"). Thus, only few cells were directly eluted ("with Magnetos. neg. fraction"). Afterwards, the formation of spheroids was investigated as indicator for adequate cell behavior. For that purpose, the eluted FaDu-NR cells were seeded in U-bottom shaped 96-well plates and monitored in the IncuCyte system for 96 h.
Please note, that the number of cells seeded for spheroid formation differed depending on fraction and cell yield that was eluted from the column. Thus, for the magnetically enriched, magnetosome-treated FaDu-NR cells ("with Magnetos. pos. fraction") and the untreated cells that directly passed the column ("w/o Magnetos. neg. fraction"), an amount of 5,000 cells was seeded for subsequent spheroid formation. For fractions "with Magnetos. neg. fraction" and "w/o Magnetos. pos. fraction" (both yielding only low cell numbers) 2,000 cells were seeded. Differences in the size of the formed spheroids can therefore be explained by varying cell amounts. Furthermore, spheroid formation was compared to untreated FaDu-NR cells that were neither magnetosome-incubated nor subjected to magnetic separation (5,000 cells seeded). Scale bar: 100 µm

Supplementary Tables

Table S1 Bacterial strains and mammalian cell lines used in this study.

Strain	Description	Source or reference
Bacterial strains		
Magnetospirillum gryphiswaldense MSR-1 R3/S1	Rif^R, Sm^R spontaneous mutant, lab strain	Schultheiss and Schüler, 2003
Mammalian cell lines		
FaDu	squamous hypopharyngeal carcinoma cell line, adherent, ATCC HTB-43	Rangan 1972
FaDu-NR	transduced FaDu cell line	
BeWo	trophoblast cell line, adherent, DSMZ ACC 458	Pattillo and Gey 1968
HCC78	non-small cell lung carcinoma cell line, adherent, DSMZ ACC 563	Virmani et al. 1998
hPC-PL	primary human placental pericytes, adherent, mesenchymal-like cells	PromoCell GmbH, Heidelberg, Germany

(See table on the next page.)

Table S2 Statistical analysis on PrestoBlue cell viability data. Different mammalian cell lines (BeWo, FaDu, HCC78, hPC-PL) were incubated with the indicated magnetosome amounts (5-100 µg cm^{-2}) for 24 or 48 h. Cell viability was normalized to an untreated sample (negative control), HEPES and 0.02% Triton X-100 treated cells served as further controls. Provided *p*-values were calculated by one-way ANOVA tests with 95% confidence intervals followed by a multiple comparison test and correction according to Tukey (1949) (SPSS Statistics software version 26).

BeWo 24h	untreated	Triton	5 µg	25 µg	50 µg	100 µg	HEPES
untreated		0.000	0.854	1.000	0.999	1.000	0.934
Triton			0.000	0.000	0.000	0.000	0.000
5 µg				0.903	0.582	0.784	1.000
25 µg					0.996	1.000	0.963
50 µg						0.996	1.000
100 µg							0.887
HEPES							

BeWo 48h	untreated	Triton	5 µg	25 µg	50 µg	100 µg	HEPES
untreated		0.000	0.981	0.907	0.712	0.006	0.457
Triton			0.000	0.000	0.000	0.008	0.000
5 µg				1.000	0.986	0.026	0.884
25 µg					0.999	0.048	0.973
50 µg						0.099	0.999
100 µg							0.204
HEPES							

FaDu 24h	untreated	Triton	5 µg	25 µg	50 µg	100 µg	HEPES
untreated		0.006	0.999	0.987	0.995	0.938	0.994
Triton			0.017	0.032	0.025	0.059	0.001
5 µg				1.000	1.000	0.997	0.916
25 µg					1.000	1.000	0.795
50 µg						1.000	0.852
100 µg							0.628
HEPES							

FaDu 48h	untreated	Triton	5 µg	25 µg	50 µg	100 µg	HEPES
untreated		0.010	0.991	1.000	1.000	0.999	0.989
Triton			0.002	0.006	0.009	0.026	0.002
5 µg				0.998	0.994	0.904	1.000
25 µg					1.000	0.995	0.997
50 µg						0.999	0.991
100 µg							0.890
HEPES							

HCC78 24h	untreated	Triton	5 µg	25 µg	50 µg	100 µg	HEPES
untreated		0.000	1.000	1.000	0.980	0.999	0.851
Triton			0.000	0.000	0.000	0.000	0.000
5 µg				0.999	0.897	0.976	0.962
25 µg					0.992	1.000	0.783
50 µg						1.000	0.370
100 µg							0.563
HEPES							

HCC78 48h	untreated	Triton	5 µg	25 µg	50 µg	100 µg	HEPES
untreated		0.000	0.965	0.004	0.001	0.000	0.145
Triton			0.000	0.000	0.001	0.005	0.000
5 µg				0.021	0.005	0.000	0.518
25 µg					0.978	0.387	0.446
50 µg						0.838	0.134
100 µg							0.013
HEPES							

hPC-PL 24h	untreated	Triton	5 µg	25 µg	50 µg	100 µg	HEPES
untreated		0.000	0.905	0.593	0.067	0.049	0.279
Triton			0.000	0.000	0.000	0.000	0.000
5 µg				0.997	0.506	0.419	0.026
25 µg					0.849	0.776	0.006
50 µg						1.000	0.000
100 µg							0.000
HEPES							

hPC-PL 48h	untreated	Triton	5 µg	25 µg	50 µg	100 µg	HEPES
untreated		0.002	1.000	0.325	0.899	0.321	0.999
Triton			0.004	0.119	0.017	0.122	0.001
5 µg				0.525	0.984	0.519	0.976
25 µg					0.916	1.000	0.164
50 µg						0.913	0.678
100 µg							0.161
HEPES							

Table S3 Cell death rates for different mammalian cell lines (BeWo, FaDu, HCC78 and hPC-PL) incubated with isolated magnetosomes (100 µg cm^{-2}) for 24 h or 48 h. Values were determined using the SYTOX™ assay followed by flow cytometry analysis. Incubation with Triton X-100 (positive control) resulted in cell death rates ≥ 90%. For the magnetosome-treated cells ("w M"), the number of cell death events was comparable to the untreated fraction ("w/o M") for 24 h incubation, and prolonged treatment up to 48 h resulted in only slightly increased cell death rates. Data are presented as mean ± standard deviation, $n \geq 2$.

Cell line	24 h			48 h		
	w/o M [%]	w M [%]	Triton X-100 [%]	w/o M [%]	w M [%]	Triton X-100 [%]
		100 µg cm^{-2}			100 µg cm^{-2}	
BeWo	10.28 ± 0.15	19.27 ± 0.01	91.50 ± 2.21	11.54 ± 1.10	15.85 ± 0.03	89.46 ± 2.35
FaDu	7.63 ± 0.02	5.50 ± 0.02	95.29 ± 0.09	7.32 ± 0.04	10.92 ± 0.12	100.00 ± 0.00
HCC78	3.55 ± 0.20	2.40 ± 0.01	100.00 ± 0.00	11.74 ± 0.07	15.00 ± 0.11	100.00 ± 0.00
hPC-PL	10.87 ± 0.19	10.77 ± 0.01	96.34 ± 0.55	18.70 ± 0.13	28.25 ± 0.04	98.18 ± 1.10

Table S4 Statistical analysis on the cell death rates of magnetosome-treated BeWo, FaDu, HCC78, and hPC-PL cells. SPSS Statistics software (version 26) was used for one-way ANOVA tests with 95% confidence intervals followed by a multiple comparison test and correction according to Tukey (1949). Differences considered as statistically significant are specified as follows: $p < 0.05$ (∗), $p < 0.01$ (∗∗), or $p < 0.001$ (∗∗∗).

BeWo	w/o M 24h	w M 24h	Triton X-100 24h	w/o M 48h	w M 48h	Triton X-100 48h
w/o M 24h		0.004	0.000	0.916		
w M 24h			0.000		0.230	
Triton X-100 24h						0.651
w/o M 48h					0.107	0.000
w M 48h						0.000
Triton X-100 48h						

FaDu	w/o M 24h	w M 24h	Triton X-100 24h	w/o M 48h	w M 48h	Triton X-100 48h
w/o M 24h		0.995	0.000	1.000		
w M 24h			0.000		0.856	
Triton X-100 24h						0.949
w/o M 48h					0.989	0.000
w M 48h						0.000
Triton X-100 48h						

HCC78	w/o M 24h	w M 24h	Triton X-100 24h	w/o M 48h	w M 48h	Triton X-100 48h
w/o M 24h		1.000	0.000	0.778		
w M 24h			0.000		0.344	
Triton X-100 24h						1.000
w/o M 48h					0.990	0.000
w M 48h						0.000
Triton X-100 48h						

hPC-PL	w/o M 24h	w M 24h	Triton X-100 24h	w/o M 48h	w M 48h	Triton X-100 48h
w/o M 24h		1.000	0.000	0.019		
w M 24h			0.000		0.000	
Triton X-100 24h						0.843
w/o M 48h					0.007	0.000
w M 48h						0.000
Triton X-100 48h						

References Supporting Information

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