Electronic Supplementary Information for Chemical Communications

Self-assembling magnetic resonance beacon for detection of microRNA-1

Jonghwan Lee[a,b],‡ Hyo Jin Kang[a,b],‡ Yong Seung Lee[a,b],‡ Hyejung Heo[a,b], Ha-Na Gu[a,b], Sujeong Cho[a,b] and Soonhag Kim[a,b,*]

[a]*Institute for Bio-Medical Convergence, College of Medicine, Catholic Kwandong University, Gangneung-si, Gangwon-do, 270-701, Republic of Korea*

[b]*Catholic Kwandong University International St. Mary's Hospital, Incheon Metropolitan City, 404-834, Republic of Korea*

‡ These authors contributed equally to this work

***Corresponding author:**

Soonhag Kim, PhD

[a]Institute for Bio-Medical Convergence, College of Medicine, Catholic Kwandong University, Gangneung-si, Gangwon-do 270-701, Republic of Korea

[b]Catholic Kwandong University International St. Mary's Hospital, Incheon Metropolitan City, 404-834, Republic of Korea

E-mail: kimsoonhag@empal.com; Telephone: +82-32-290-2771

Experimental procedures

Preparation of miR-1 MRBs

Cobalt ferrite magnetic fluorescence (MF) nanoparticles (50 nm, Biterials, Seoul, Korea) were prepared as previously described.[1] The surfaces of nanoparticles were functionalized with RITC (excitation/emission: 555/578 nm) for imaging analysis by confocal microscope. Oligomers including the 3'-adaptor (5'-TCACAGATGAGTAAAAAAAAAA-NH_2-3'), 5'-adaptor (5'-NH_2-TGTAAGGTCTCG-3'), miR-1 linker (5'-ACTCATCTGTGACGAGACCTT ACATTTCTTCATACATA-3', and miR-1 mt linker (5'-ACTCATCTGTGACGAGATTCCGT GACCTTACAATAGATGCCATGGT-3', were synthesized by Bioneer (Daejeon, Korea); the underlined regions show the miR-1 binding regions and mutated regions for the miR-1 and miR-1 mt linkers, respectively. The miR-1 binding regions were designed based on the mature miR-1 sequence reported by miRBase database (www.mirbase.org). The MF nanoparticles were conjugated with the 3'- or 5'- adaptor oligomer using N-(3-dimethylaminopropyl)-N-ethylcarbodimide (EDC, Sigma-Aldrich, USA) for 1 h at room temperature. Next, the 3a-MF and 5a-MF nanoparticles were further incubated with the miR-1 linker or miR-1 mt linker to construct the miR-1 MRB or miR mt MRB.

The miR-1 linker (38-mer) contains adaptor binding region and miR-1 binding region. A partial sequence (1 to 12 bp) of a 3'-adaptor oligomer (22-mer) is complementary to the sequence of the miR-1 linker oligomer (1 to 12 bp) and a full sequence of the 5'-adaptor oligomer (12-mer) containing a partial complementary sequence (8 bp) of miR-1 is complementary to the miR-1 linker oligomer sequence (13 to 24 bp). The miR-1 binding region of the miR-1 linker (17 to 38 bp) is complementary to the mature miR-1 (22-mer). Therefore, the miR-1 binding region is exposed to the outside of the self-assembled miR-1 MRBs. Thus, when the target miR-1 hybridized with the miR-1 binding region in the miR-1

linker, it forces the hybridization between miR-1 linker and 3'- or 5'-adaptor oligomers to physically separate. Finally it induces the miR-1 MRBs disassembly.

For confirmation of the MF nanoparticle self-assembly and disassembly of the miR-1 MRB or the miR-1 mt MRB, nanoparticles were kept in test tubes and incubated with different concentrations (0, 500 and 1000 pmol) of miR-1 at room temperature for 24 h to induce nanoparticle precipitation. To confirm the target specificity of the miR-1 MRB, miR-9 was added and incubated for 24 h. Fluorescence images were taken using the Maestro imaging system (CRi Inc., Woburn, MA, USA). The size distribution of the prepared MF, 3a-MF, 5a-MF, miR-1 MRB, and miR-1 mt MRBs with or without miR-1 were determined by TEM (JEM 1010 system, JEOL, Japan) and DLS (Zetasizer Nano ZS system, Malvern Instruments, Worcestershire, UK).

Cell culture and transfection

HeLa cells and C2C12 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific Hyclone, USA) with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (Gibco, Grand Island, NY, USA). For myogenic differentiation of C2C12 cells, cells were cultured in DMEM supplemented with 1% FBS. HeLa cells were transfected with precursor miR-1 (Ambion, Austin, TX, USA) using X-tremeGENE (Roche Diagnostics, TN, USA). Transfection of MF, 3a-MF, 5a-MF, miR-1 MRB, and miR-1 mt MRB into HeLa cells and C2C12 cells was performed using a magnetic plate (Chemicell, GmbH, Berlin, Germany). All transfection experiments were performed in triplicate.

Cytotoxicity testing

HeLa cells (5×10^3) were cultured in 96-well plates with 200 μl of culture medium. HeLa

cells were incubated with ten different amounts (0, 0.2, 0.5, 1, 1.5, 2, 5, 10, 15, and 20 μg) of the 3a-MF and 5a-MF nanoparticle mixtures without miR-1 linker for 24 h. Next, 20 μl of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, 1 mg/ml) solution was added to the cells and incubated for 4 h at 37°C. Finally, 200 μl of dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. A microplate reader (Microplate Reader 680, Bio-Rad, Hercules, CA, USA) was used to measure the optical density. All samples were analyzed in triplicate.

Confocal microscopy

Cells (1×10^5) were cultured on 13 mm-diameter glass cover slips placed in 24-well plates for 24 h at 37°C and 5% CO_2. Next, the cells were transfected with either 3a-MF, 5a-MF, miR-1 MRB, or miR-1 mt MRB with or without miR-1. Cells were fixed with a 4% formaldehyde solution (Sigma-Aldrich, USA) for 15 min at room temperature. After washing three times with phosphate buffered saline (PBS) for 10 min, the cover slips were mounted with an aqueous mounting solution containing 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) (Vector Laboratories, Inc., Burlingame, CA, USA) and imaged using a confocal laser scanning microscope (LSM 510; Carl Zeiss, Germany).

Quantification of miR-1 expression

For cDNA synthesis, small RNA was isolated from C2C12 cells using mirVana™ miRNA isolation kits (Ambion). To compare miR-1 expression levels, quantitative real-time PCR (qRT-PCR) was conducted using the mirVana™ qRT-PCR primer set, the mirVana™ qRT-PCR miRNA kit (Ambion), and SYBR Premix Ex Taq™ (2x; Takara, Japan). Reactions were performed in triplicate using an iCycler (Bio-Rad, USA) at 95°C for 15 min followed by 40 cycles of 95°C for 15 s and 64°C for 1 min. 5SeRNA was used to normalize relative amounts

of miR-1. Statistical significance was analyzed using Student's *t*-test, and p-values < 0.005 were considered significant.

Acquisition of T2-weighted MRI

T2-weighted phantom images of 3a-MF, 5a-MF, miR-1 MRB, and miR-1 mt MRB in test tubes, HeLa cells, and C2C12 cells were obtained using a 1.5T MR imaging system (GE Medical Systems, Milwaukee, WI, USA) with a micro-47 surface coil (Intera; Philips Medical Systems, Best, Netherlands). T2-weighted images were acquired using the Carr-Purcell-Meiboom-Gill (CPMG) sequence at room temperature. The parameters of T2-weighted image were as follows: TR = 1400 ms, TE = 55.8 ms, and slice thickness = 2.0 mm. For *in vivo* MRI, 1×10^7 C2C12 cells were harvested in 150 μl of PBS and incorporated within a Matrigel® (Corning, USA) scaffold. The C2C12-scaffold complex was subcutaneously implanted into both thighs of male Balb/c nude mice (7 weeks old; n = 6). For these animal experiments, the left thigh was treated to induce myogenesis while the right thigh was not treated and used as a control. *In vivo* T2-weighted imaging and T2 mapping were performed with a 9.4 T/160-mm small animal imaging system (Agilent Inc., Palo Alto, CA, USA) with a 30-mm millipede volume coil. For MRB scanning, all mice were anesthetized with mixture gas (1-2% isoflurane + air) delivered by nose cone and their respiratory rate, electrocardiogram, and rectal temperature were monitored. Axial T2-weighted fast spin echo (FSE) images were used to completely cover the injected regions. The parameters of T2-weighted image were as follows: TR = 4000 ms, kzero = 4, echo spacing (ESP) = 12 ms, 32 segments, echo train length (ETL) = 8, effective TE = 48 ms, 2 averages, matrix = 256×256, field of view (FOV) = 25×30 mm, slice thickness = 0.8 mm, and total scan time = 4 min 24 sec, respectively. The transverse relaxation rates (T2 values) were measured on T2 maps which were obtained using a multi-echo multi-slices (MEMS)

sequence as follows: TR = 4000 ms, TE range = 10-150 ms, total 15 echoes (10 ms echo space), average = 1, matrix = 128×128, slice thickness = 0.8 mm, and total scan time = 8 min 32 sec. The number and orientation of slices, FOV, and matrix were the same as for T2-weighted imaging. The mean T2 signal intensities of regions of interest (ROIs) were obtained and normalized to analyze changes in the brightness of T2-weighted images of miR-1 MRB in the left thighs and ratio of the T2 values of the left thigh to that of right thigh.

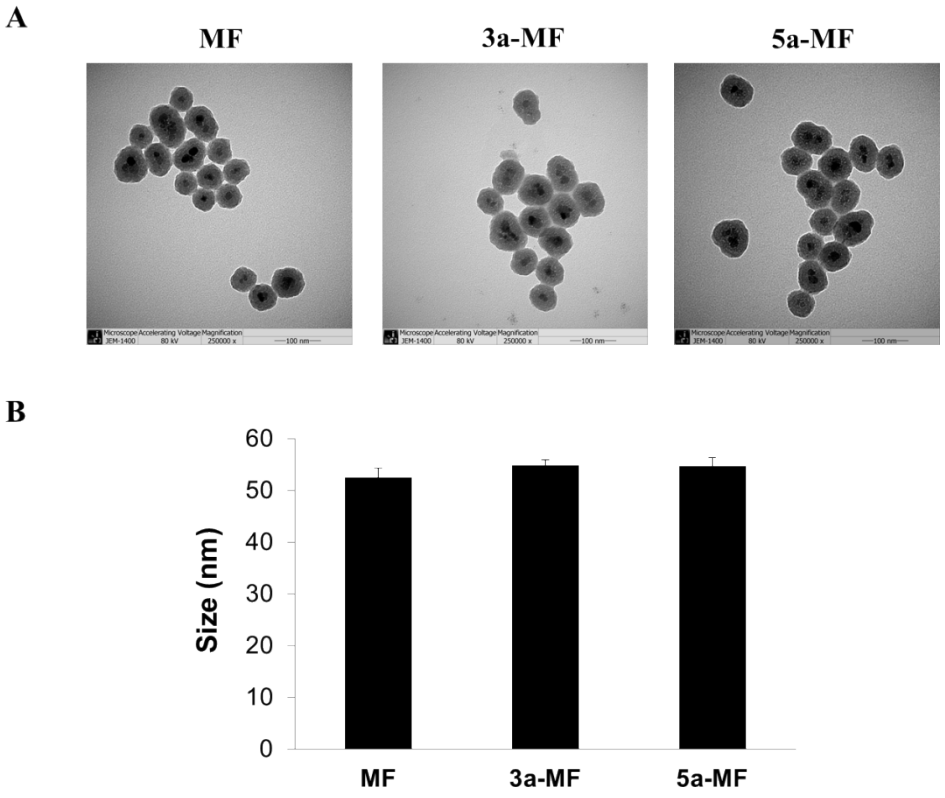

Figure S1. (a) TEM images and (b) DLS analysis of the MF, 3a-MF, and 5a-MF nanoparticles.

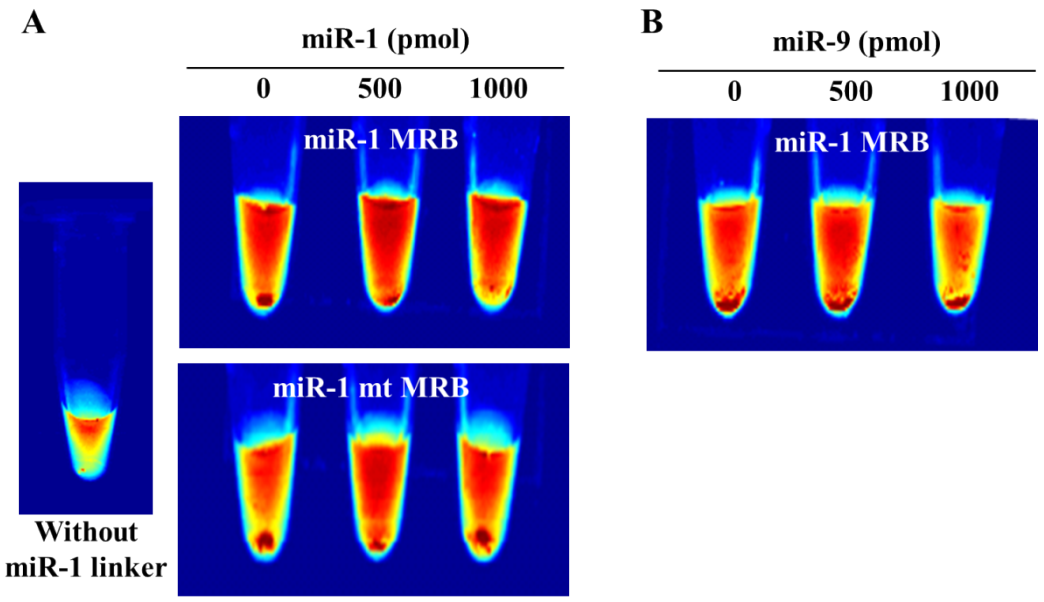

Figure S2. Fluorescence image of the prepared nanoparticles and the MRBs. (A) To compare the aggregated size-dependent precipitation, the miR-1 MRBs and the miR-1 mt MRBs were incubated with different concentrations of miR-1 at room temperature for 24 h. The 3a-MF and 5a-MF nanoparticles mixtures without the miR-1 linker were used as controls. (B) To confirm the target specificity of the miR-1 MRB, the miR-1 MRBs were incubated with different concentration of miR-9 at room temperature for 24 h.

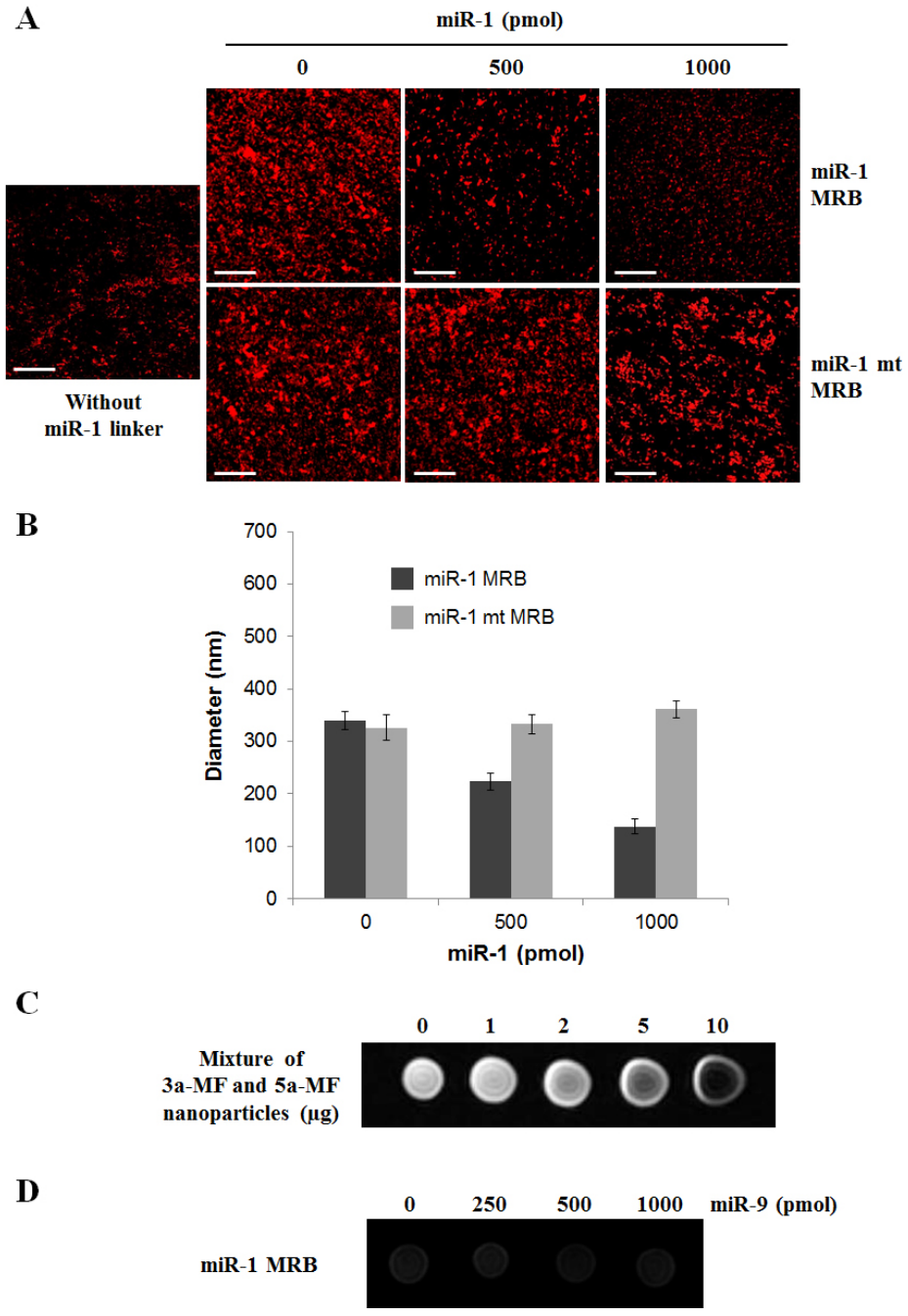

Figure S3. Characterization of the prepared nanoparticles and MR beacons. The 3a-MF and 5a-MF nanoparticle mixtures were analyzed in the absence of the miR-1 linker. The miR-1 MRBs and miR-1 mt MRBs were incubated with different concentrations of miR-1. (A) Confocal microscope images (scale bar, 20 μm). (B) DLS analysis. (C) T2-weighted MR image of 3a-MF and 5a-MF nanoparticles mixture without the miR-1 linker. (D) T2-weighted MR image of the miR-1 MRBs incubated with

different concentrations of miR-9.

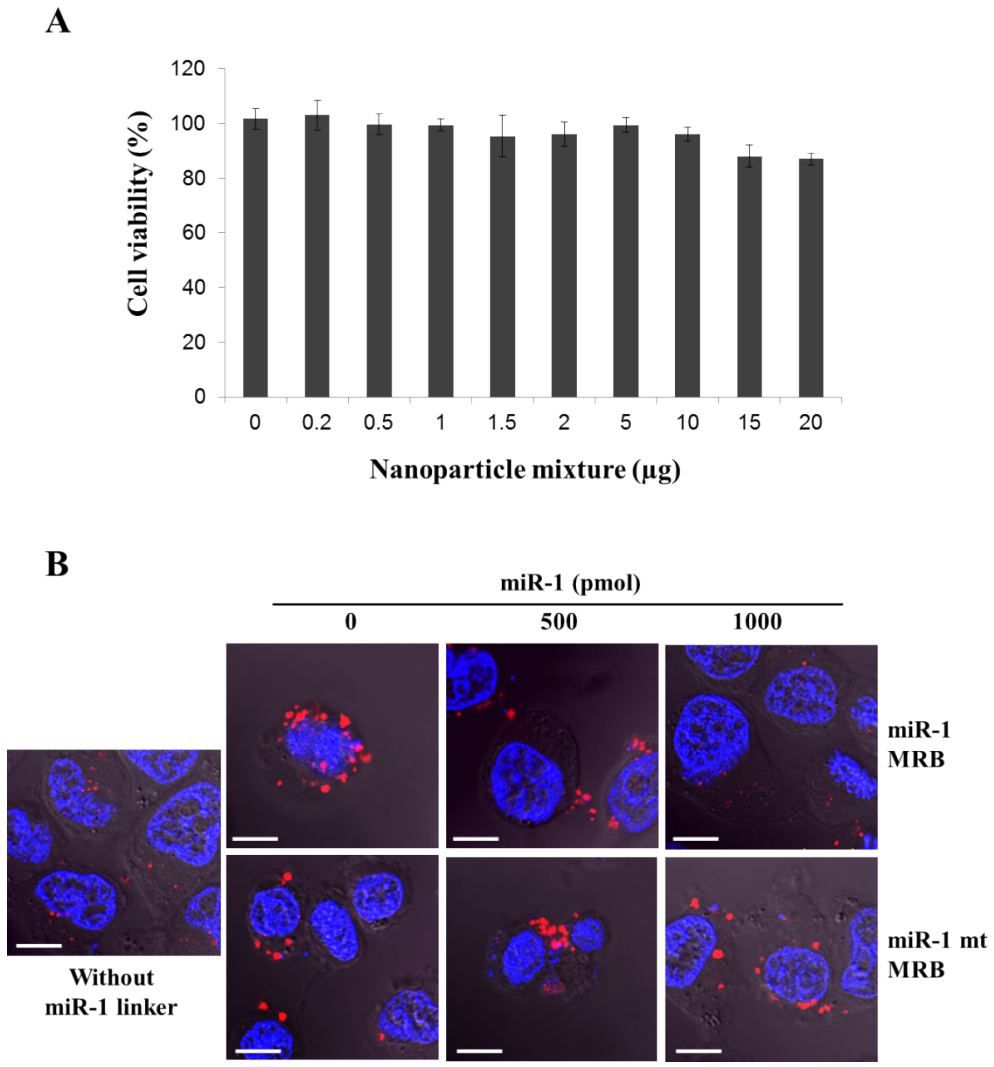

Figure S4. Exogenous miR-1 sensing specificity of miR-1 MRBs in HeLa cells. (a) Cytotoxicity analysis of the nanoparticle preparations. The 3a-MF and 5a-MF nanoparticles were transfected into HeLa cells with different concentrations for 24 h. Cell viability was evaluated by MTT assay. The data are presented as the mean ± standard error of triplicate samples. (b) Confocal microscope images (scale bar, 10 μm).

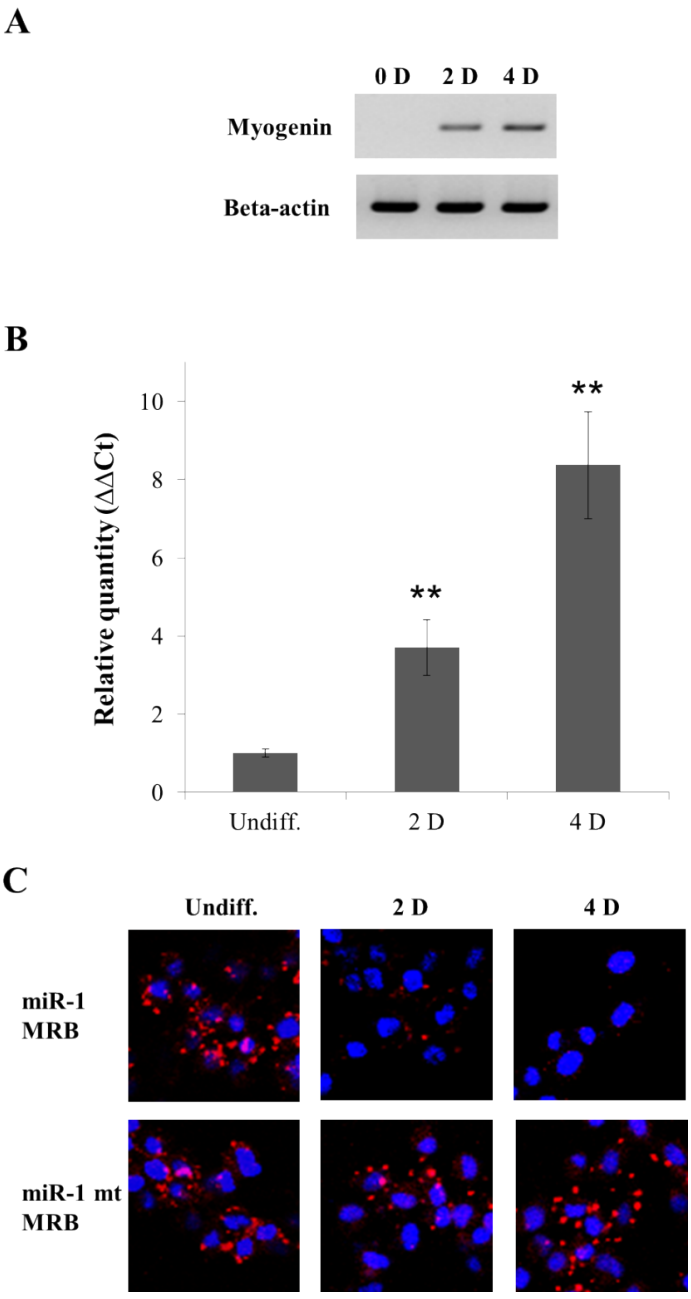

Figure S5. Characterization of C2C12 cells during myogenesis. Expression of (A) myogenin and (B) mature miR-1. Data represent the mean standard error of triplicate samples (**, P<0.005). (C) Confocal microscope images of the miR-1 MRB in an undifferentiated state (Undiff.) and up to 4 days after myogenic differentiation of C2C12 cells.

References

1. D. W. Hwang, I. C. Song, D. S. Lee and S. Kim, *Small*, 2010, **6**, 81-88.