Biosynthesis of magnetic nanoparticles by human mesenchymal stem cells following transfection with the magnetotactic bacterial gene mms6.

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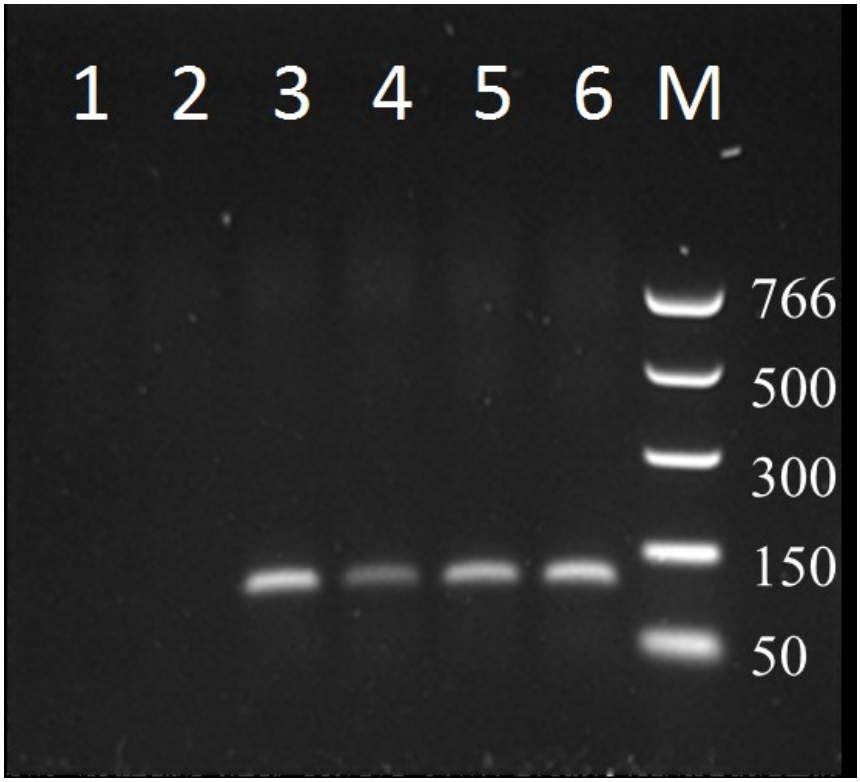
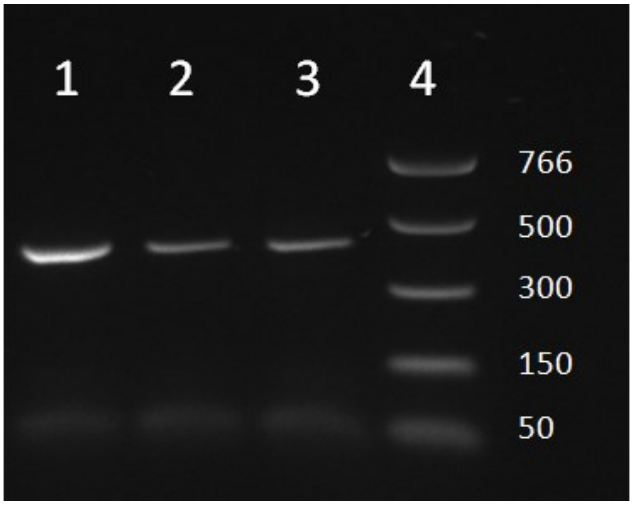


Supplementary Figure 1. Expression of the synthetic *mms6* gene by human MSCs.

1) Untransfected MSCs, 2) MSCs transfected with empty pcDNA3.1 vector 3) *mms6*-pcDNA3.1 vector, 4) MSCs transfected with *mms6* construct - day10, 5) MSCs transfected with *mms6* construct day 15 and 6) MSCs transfected with *mms6* construct day 21. M = DNA ladder (bp). Internal primer pair Forward: GTGGGGCCCGATTATTCT, Reverse: TCACGCAGTTCCACTTCTTC. Amplicon product length = 108 bp

A



B

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Ref.mms6:       ATGGGCGAAATGGAACGTGAAGGCGCGGCGGCGAAAGCGGGCGCGGCGAAAACCGGCGCG
PCR Day10       ATGGGCGAAATGGAACGTGAAGGCGCGGCGGCGAAAGCGGGCGCGGCGAAAACCGGCGCG
PCR Day15       ATGGGCGAAATGGAACGTGAAGGCGCGGCGGCGAAAGCGGGCGCGGCGAAAACCGGCGCG
PCR Day21       ATGGGCGAAATGGAACGTGAAGGCGCGGCGGCGAAAGCGGGCGCGGCGAAAACCGGCGCG
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Ref.mms6        GCGAAAACCGGCGCGGTGGCGAAAACCGGCATTGCGGCGAAAACCGGCGTGGCGACCGCG
PCR Day10       GCGAAAACCGGCGCGGTGGCGAAAACCGGCATTGCGGCGAAAACCGGCGTGGCGACCGCG
PCR Day15       GCGAAAACCGGCGCGGTGGCGAAAACCGGCATTGCGGCGAAAACCGGCGTGGCGACCGCG
PCR Day21       GCGAAAACCGGCGCGGTGGCGAAAACCGGCATTGCGGCGAAAACCGGCGTGGCGACCGCG
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Ref.mms6        GTGGCGGCGCCGGCGGCGCCGGCGAACGTGGCGGCGGCGCAGGGCGCGGGCACCAAAGTG
PCR Day10       GTGGCGGCGCCGGCGGCGCCGGCGAACGTGGCGGCGGCGCAGGGCGCGGGCACCAAAGTG
PCR Day15       GTGGCGGCGCCGGCGGCGCCGGCGAACGTGGCGGCGGCGCAGGGCGCGGGCACCAAAGTG
PCR Day21       GTGGCGGCGCCGGCGGCGCCGGCGAACGTGGCGGCGGCGCAGGGCGCGGGCACCAAAGTG
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Ref.mms6        GCGCTGGGCGCGGGCAAAGCGGCGGCGGGCGCGAAAGTGGTGGGCGGCACCATTTGGACC
PCR Day10       GCGCTGGGCGCGGGCAAAGCGGCGGCGGGCGCGAAAGTGGTGGGCGGCACCATTTGGACC
PCR Day15       GCGCTGGGCGCGGGCAAAGCGGCGGCGGGCGCGAAAGTGGTGGGCGGCACCATTTGGACC
PCR Day21       GCGCTGGGCGCGGGCAAAGCGGCGGCGGGCGCGAAAGTGGTGGGCGGCACCATTTGGACC
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Ref.mms6        GGCAAAGGCCTGGGCCTGGGCCTGGGCCTGGGCCTGGGCGCGTGGGGCCCGATTATTCTG
PCR Day10       GGCAAAGGCCTGGGCCTGGGCCTGGGCCTGGGCCTGGGCGCGTGGGGCCCGATTATTCTG
PCR Day15       GGCAAAGGCCTGGGCCTGGGCCTGGGCCTGGGCCTGGGCGCGTGGGGCCCGATTATTCTG
PCR Day21       GGCAAAGGCCTGGGCCTGGGCCTGGGCCTGGGCCTGGGCGCGTGGGGCCCGATTATTCTG
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Ref.mms6        GGCGTGGTGGGCGCGGGCGCGGTGTATGCGTATATGAAAAGCCGTGATATTGAAAGCGCG
PCR Day10       GGCGTGGTGGGCGCGGGCGCGGTGTATGCGTATATGAAAAGCCGTGATATTGAAAGCGCG
PCR Day15       GGCGTGGTGGGCGCGGGCGCGGTGTATGCGTATATGAAAAGCCGTGATATTGAAAGCGCG
PCR Day21       GGCGTGGTGGGCGCGGGCGCGGTGTATGCGTATATGAAAAGCCGTGATATTGAAAGCGCG
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Ref.mms6        CAGAGCGATGAAGAAGTGGAACTGCGTGATGCGCTGGCGCCACCAATG
PCR Day10       CAGAGCGATGAAGAAGTGGAACTGCGTGATGCGCTGGCGCCACCAATG
PCR Day15       CAGAGCGATGAAGAAGTGGAACTGCGTGATGCGCTGGCGCCACCAATG
PCR Day21       CAGAGCGATGAAGAAGTGGAACTGCGTGATGCGCTGGCGCCACCAATG
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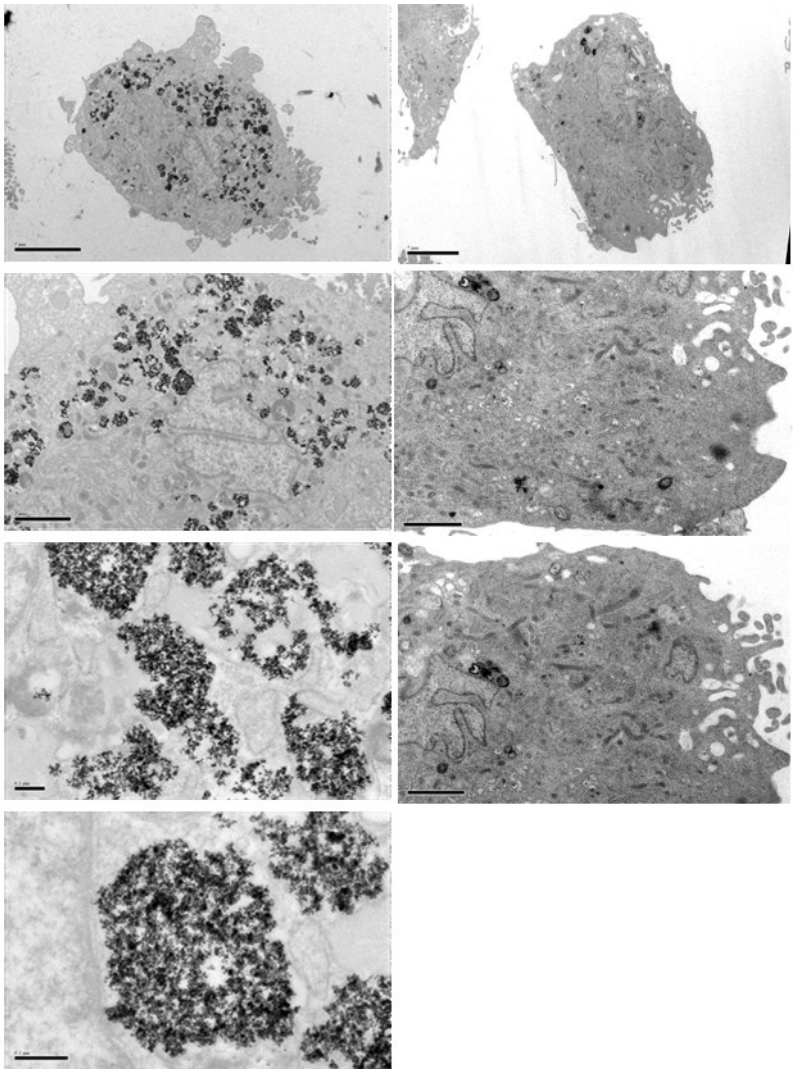
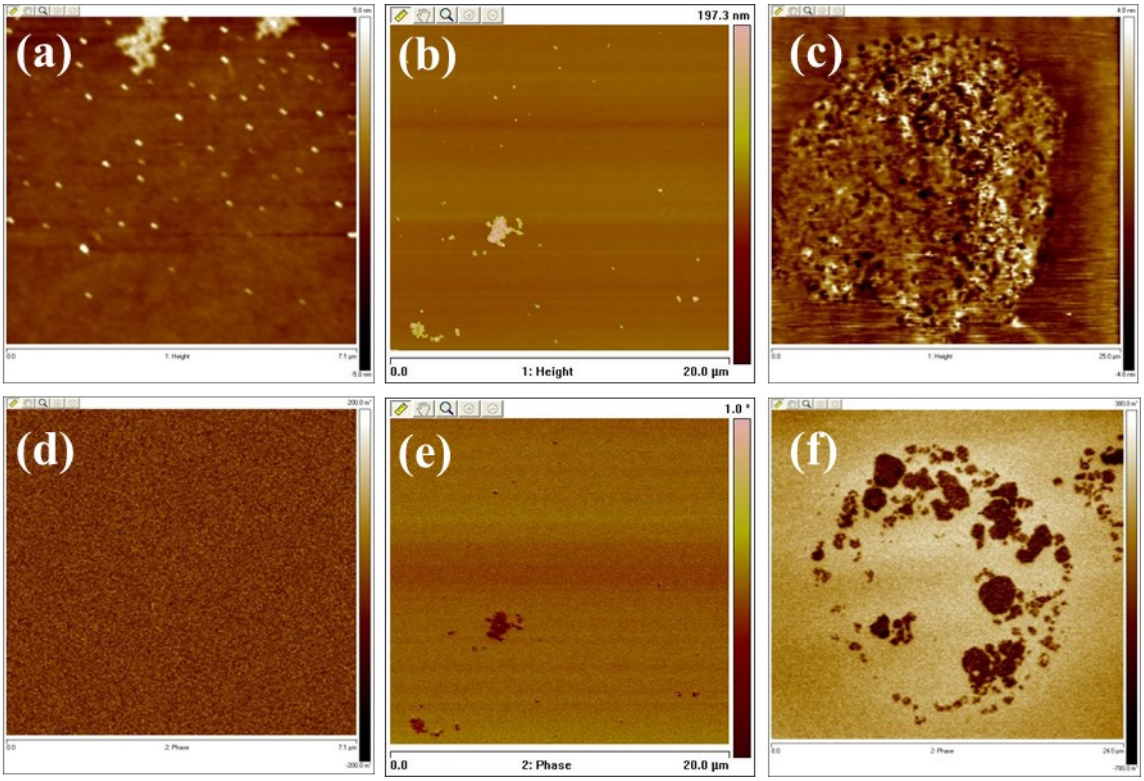
Supplementary Figure 2. Expression (A) and sequence (B) of the synthetic *mms6* gene following transfection of MSCs and *in vitro* culture.

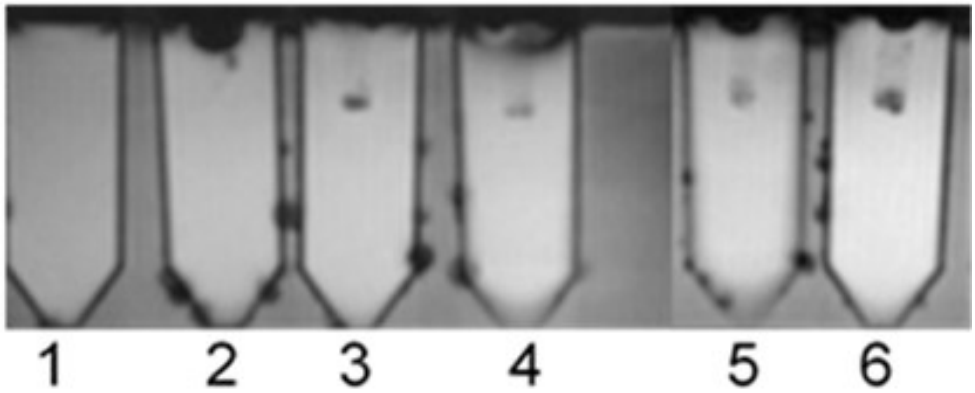
MSCs were transfected with the *mms6*-pcDNA3.1 vector and cultured for - lane 1) 10 days; lane 2) 15 days and lane 3) 21 days. Lane 4) = 766 bp markers. Following gel electrophoresis the PCR products were subjected to DNA Sanger sequencing and compared to the sequence of the initial synthetic *mms6* inserted within the pcDNA3.1 vector used for transfection. Sanger sequencing data confirmed 100% identity of the DNA sequence of the initial synthetic *mms6* gene and the gene expressed after 10, 15 and 21 days of transfection. CLUSTAL O (1.2.3) multiple sequence alignment compared with reference synthetic *mms6* gene sequence (Ref.*mms6*).



Supplementary Figure 3. Control TEM studies
TEM images of untransfected MSCs either loaded (left panel) or not loaded (right panel) with Fluid Mag DXS magnetic nanoparticles. Scale bars of images (from upper to lower): left panel - 5 μm, 2 μm, 0.2 μm, 0.2 μm; right panel 5 μm, 2 μm, 2 μm

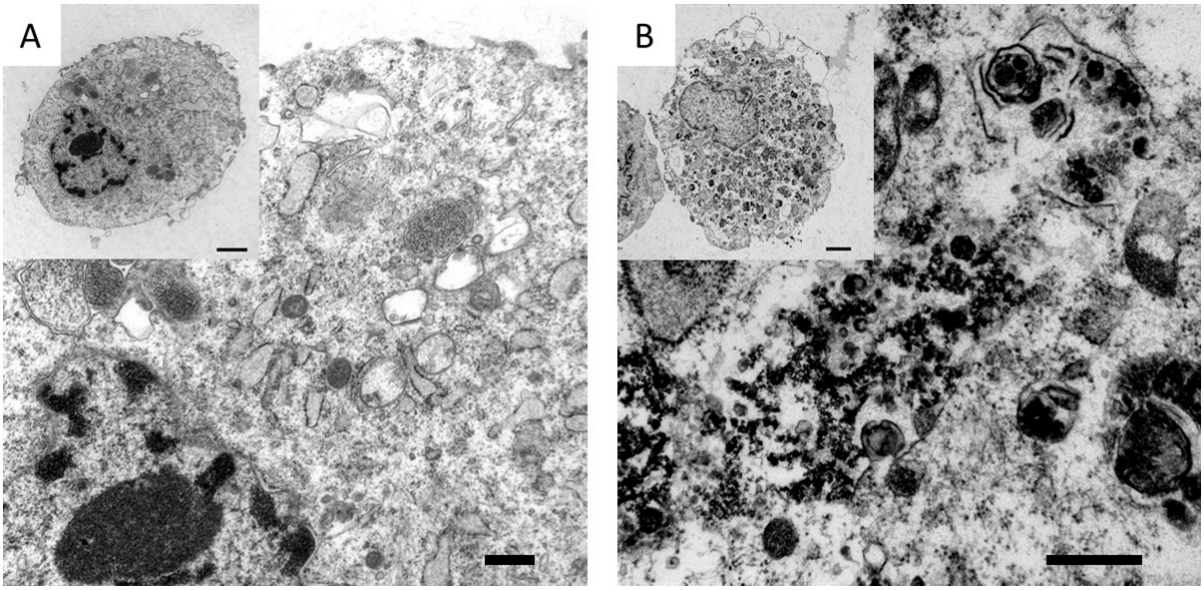


Supplementary Figure 4. Control AFM/MFM studies.
AFM / MFM of $FeCl_3$ powder (a, d): Fluid Mag DXS nanoparticles (b, e): MSCs incubated with Fluid Mag DXS (c, f). The upper row demonstrates topographic images whilst the lower demonstrates the equivalent MFM images. Magnetic particles are clearly identified in the MFM images as clusters of black spots due to attractive forces, showing the capability of MFM to detect magnetic paticles inside the cells. The absence of any features on (d) indicates the non-magnetic nature of $FeCl_3$ powder. Image widths: left panels 7.1 µm, centre 20 µm, right 25 µm (upper) 24 µm (lower).



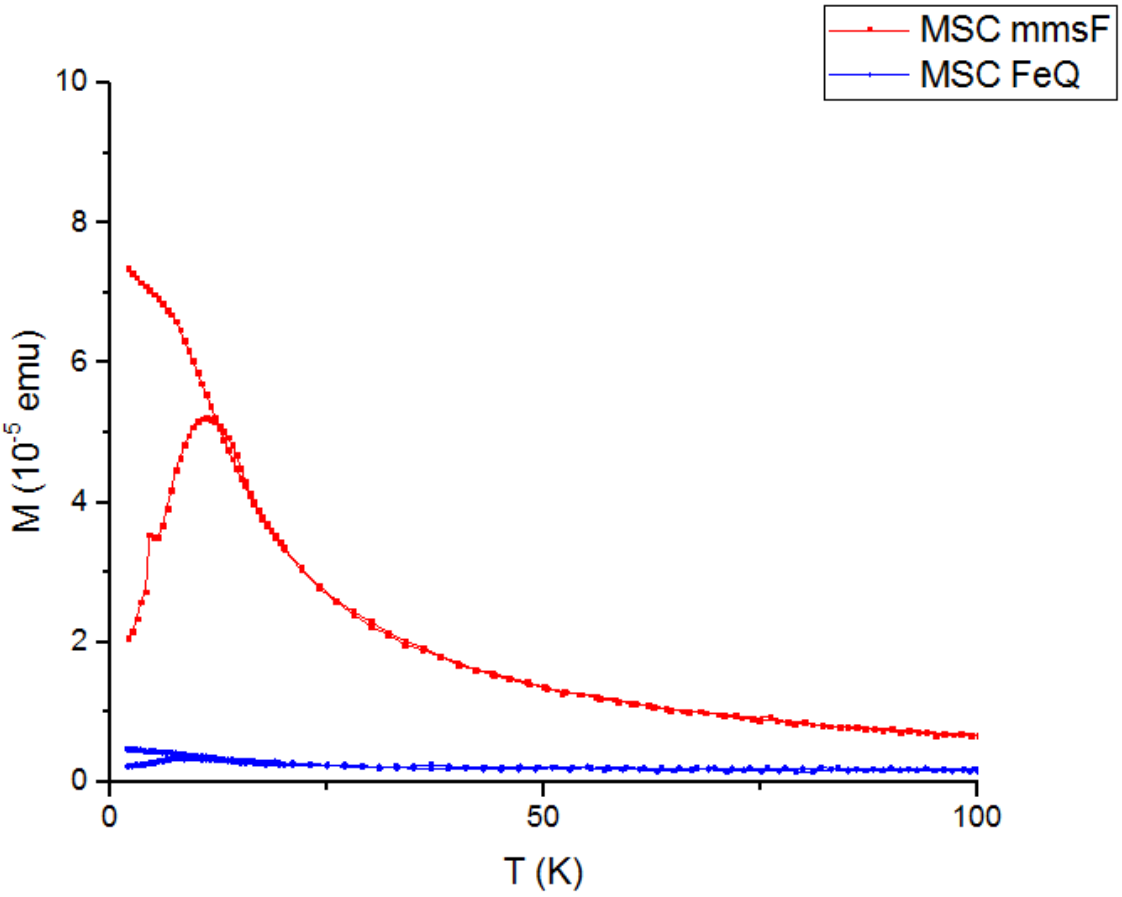
Supplementary Figure 5. MR images of Agarose Phantoms.

1. Agarose only, 2. 10^6 untransfected MSCs, 3. $2x10^4$ *mms6* transfected MSCs, 4. 10^5 *mms*6 transfected MSCs, 5. 10^6 *mms6* transfected MSCs, 6. 10^6 FluidMag DXS magnetic nanoparticle loaded MSCs



Supplementary Figure 6. TEM of *mmsF* transfected human embryonal bone marrow derived stem cells.

In contrast to untransfected MSCs (A), *mmsF* transfected MSCs (B) contain electron dense nanoparticles. MSCs were cultured in media containing 34mM ferric quinate. Bars in main figures = 500 nm; bars in inset = 2μm.



Supplementary Figure 7. SQUID Magnetometry of *mmsF* transfected human embryonal bone marrow derived stem cells.

mmsF transfected cells (MSC mmsF), cultured for 10 days, show magnetic behaviour as assessed by SQUID magnetometry whilst untransfected cells, cultured for 10 days in an identical 34mM ferric quinate containing media (MSC FeQ) show no magnetic behaviour.