

Supplementary Information

Msh homeobox 1 (Msx1)- and *Msx2*-overexpressing bone marrow–derived mesenchymal stem cells resemble blastema cells and enhance regeneration in mice

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Table 1 S: Description of mouse primer used in qRT-PCR.

Gene symbol	Sequences(5-3)	Accessionno.	Amplicon size (bp)
<i>Gapdh</i>	FOR: 5' ACTTCAACAGCAACTCCCAC3' REV: 5' TCCACCACCCTGTTGCTGTA3'	NM_008084	60 ⁰ C
<i>Fgf8</i>	FOR: 5' GGGGAAGCTAATTGCCAAGA3' REV: 5' CCTTGCGGGTAAAGGCCAT 3'	NM_001166361.1	60 ⁰ C
<i>Bmp4</i>	FOR: 5' GTCGTTTTATTATGCCAAGTC3' REV: 5' ATGCTGCTGAGGTTGAAGAG3'	NM_001316360.1	60 ⁰ C
<i>Msx1</i>	FOR: 5' CTGCTATGACTTCTTTGCC 3' REV: 5' CTCCTGTGATCGGCCAT 3'	NM_010835.2	60 ⁰ C
<i>Msx2</i>	FOR: 5' CACCACATCCCAGCTTCTA 3' REV: 5' GCAGTCTTTTCGCCTTAGC 3'	NM_013601.2	60 ⁰ C
<i>Runx2</i>	FOR: 5' CAGCATCCTATCAGTTCCCAA 3' REV: 5' CAGCGTCAACACCATCATT 3'	NM_001145920.2	60 ⁰ C
<i>Col1a1</i>	FOR: 5' CAAGAAGACATCCCTGAAGTC 3' REV: 5' ACAGTCCAGTTCTTCATTGC 3'	NM_007742.4	60 ⁰ C
<i>Bglap</i>	FOR: 5' AAGCAGGAGGGCAATAAGGT 3' REV: 5' CAGAGTTTGGCTTTAGGGCA 3'	NM_001032298.3	60 ⁰ C
<i>Alpl</i>	FOR: 5' GCCAGCAGGTTTCTCTCTTG 3' REV: 5' GGGATGGAGGAGAGAAGGTC 3'	NM_001287172.1	60 ⁰ C
<i>Pparg</i>	FOR: 5' GAGCACTTCACAAGAAATTACC 3' REV: 5' AATGCTGGAGAAATCAACTG 3'	NM_011146.3	60 ⁰ C
<i>Lpl</i>	FOR: 5' AATGCCATGACAAGTCTCTG 3' REV: 5' AAACCCACTTTCAAACACCC 3'	NM_008509.2	60 ⁰ C
<i>Adipoq</i>	FOR: 5' TGTTCTCTTAATCCTGCCCA 3' REV: 5' CCAACCTGCACAAGTTCCCTT 3'	NM_009605.4	60 ⁰ C
<i>Col II</i>	FOR: 5' ATGATCCGCCTCGGGGCTC 3' REV: 5' GGGCCTGTCTGCTTCTTGTA 3'	NM_001113515	60 ⁰ C
<i>Sox9</i>	FOR: 5' TGAATCTCCGGACCCCTTCATG 3' REV: 5' CACAGCTCACCAGACCCTGAG 3'	NM_011448.4	60 ⁰ C
<i>Aggrecan</i>	FOR: 5' ATGACCACTTTACTCTT 3' REV: 5' CCCAGCATGGCCCACTGA 3'	NM_007424.2	60 ⁰ C

Table 2 S: Whole mount analysis of regenerated digit tips.

Groups	Number of analyzed digits	Score 1	Score 2	Score 3	Score 4
Intact digit	30	0	0	0	30
Nrm Reg.	30	29	1	0	0
Sham	30	28	2	0	0
BICs	30	5	18	7	0
mBMSCs	30	3	17	10	0
MSX1	30	0	8	18	4
MSX2	30	0	6	16	8
MSX1/2	30	0	0	3	27

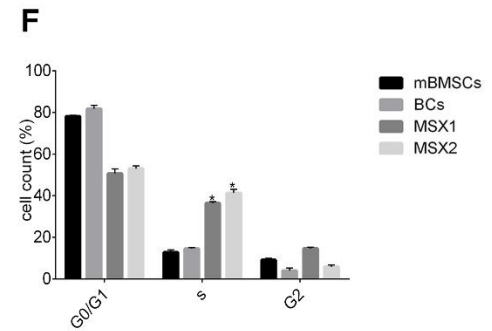
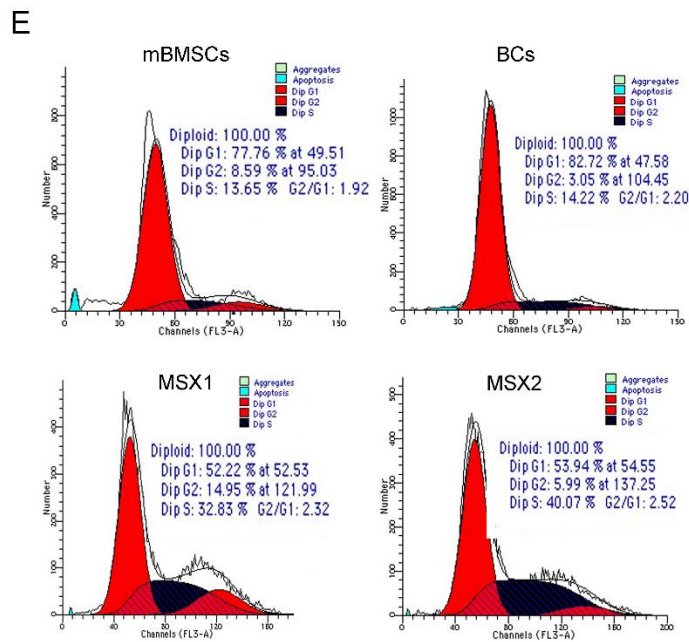
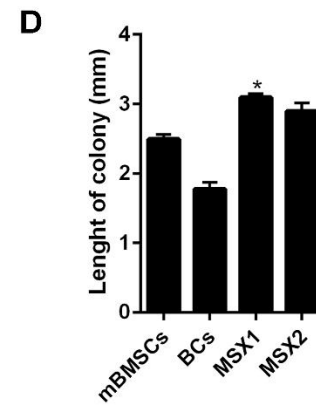
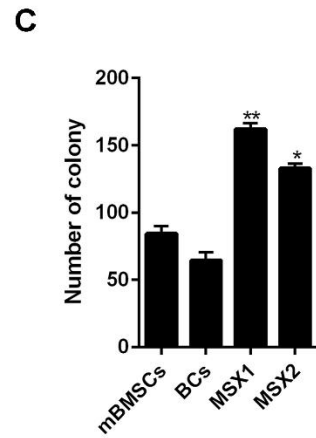
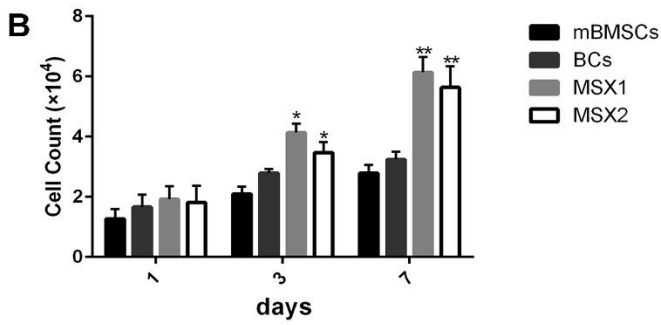
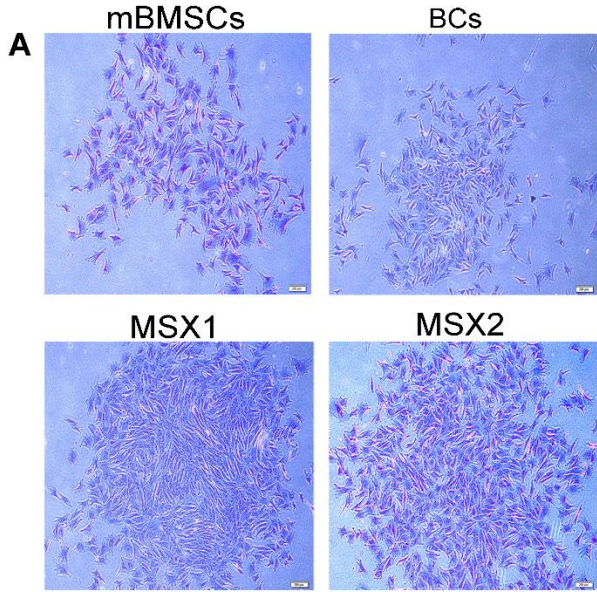


Figure 1S. Analysis of cell proliferation, colony forming and cell cycle assay. A, Trypan blue staining of colonies formed in the cultured mBMSCs, BCs and MSX groups. B, Histogram shows proliferated cells in the MSX groups compared to BCs and mBMSCs, as assessed by MTT. C, Histogram shows the number of colony formed in the BCs, mBMSCs, MSX1 and MSX2 groups. D, Histogram represents the length of colony in the BCs, mBMSCs, MSX1 and MSX2 groups. E, Flow cytometry analysis shows the number of cells in G0/G1, S and G2 phases. F, Histogram shows the percentage of cells at various phases of cell cycle. Data are presented as means \pm SD. ** $p < 0.01$ and * $p < 0.05$.

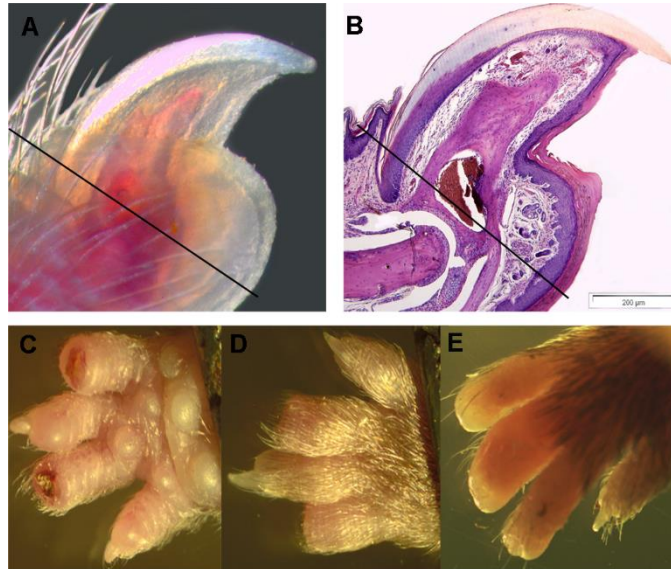


Figure 2S: Proximal digit tip amputation model. Non-regenerative proximally amputated mouse digit tip. A. Whole mount alizarin red and B. Hematoxylin and eosin (H&E) staining showed the exact amputation site of the digit tip. C. Whole mount forelimbs Showed scar formation in the digit tips immediately after amputation. D, E. Whole mount analysis in Nrm. Reg and Sham groups confirmed that the amputated models are non-regenerative.

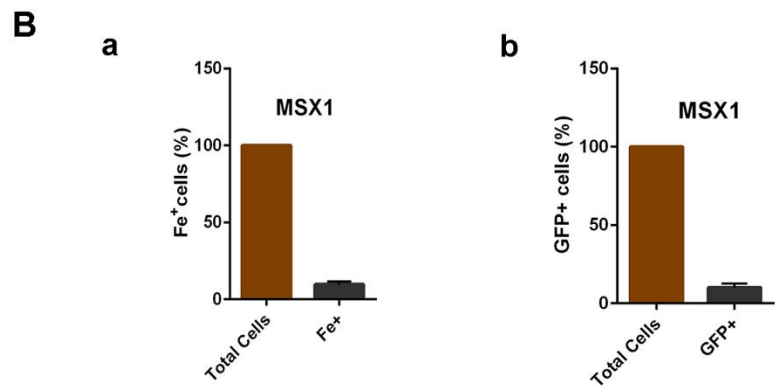
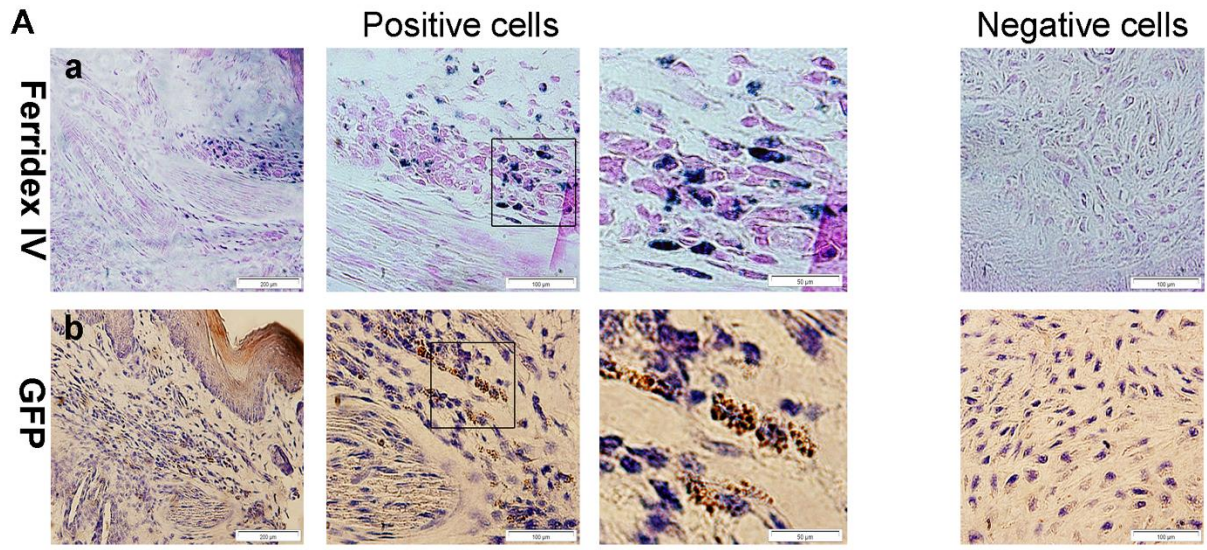


Figure 3S: Cell tracking of Ferridex and GFP labeled cells after 6 WPI. Cells labeled with Ferridex and GFP were tracked at the injury site 6WPI in the MSX1 group. A. Prussian blue staining results showed the presence of Ferridex labeled cells (blue) at the injury site after 6 WPI (a). Immunohistochemistry staining against GFP confirmed expression of the *Msx1* gene in BICs (b). B. The percentage of Ferridex and GFP labeled cells as measured by Image-J software on immunostained slides in the MSX1 group (a, b).