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Supplemental Information

Single-cell spatiotemporal analysis reveals cell fates and functions of transplanted mesenchymal stromal cells during bone repair

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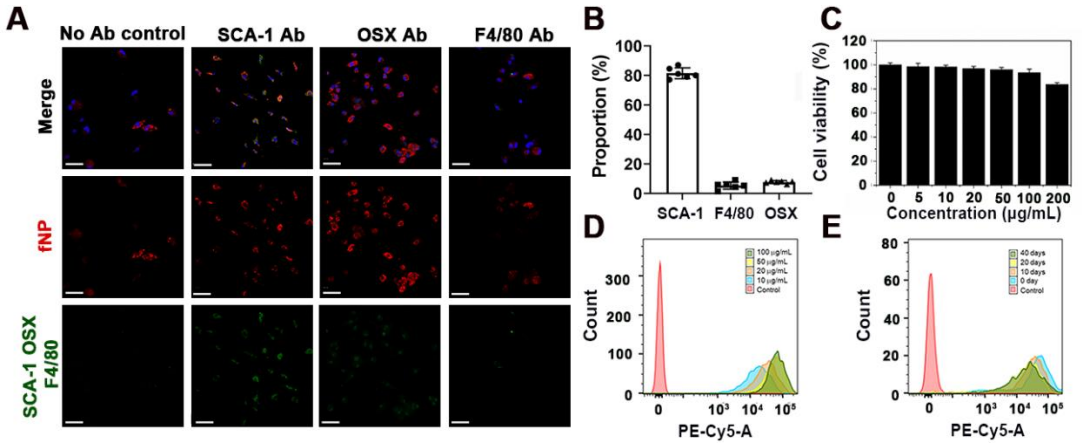

Figure S1. Mouse BMSCs labeling by fNP, related to Figure 1. (A) Representative fluorescence images for fNP, SCA-1, OSX and F4/80 in BMSCs after 50μg/ml fNP incubation for 12h; scale bare=20μm. (B) Quantitative assessment for the proportion of SCA-1+, F4/80+ and OSX+ cells versus BMSCs. (C) Metabolic viability of BMSCs after incubation with 0, 5, 10, 20, 50, 100 and 200μg/ml fNPs for 10 days. (D) Flow cytometry histograms of BMSCs labeling efficiency after incubation with 0, 10, 20, 50 and 100μg/ml fNPs for 12h. (E) Flow cytometry histograms and fluorescent images of BMSCs after incubation with 50μg/ml fNPs for 12h and subculture for 40 days; n=6 for each group.

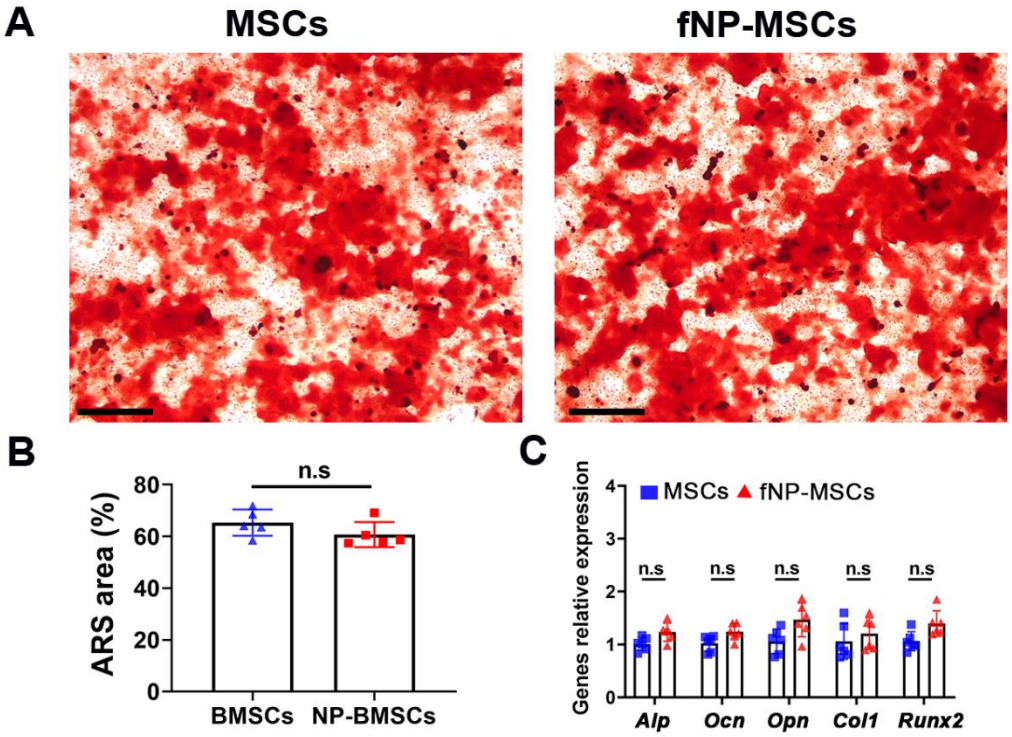

Figure S2. Effect of nanoparticles on osteogenic differentiation of BMSCs in vitro, related to Figure 1. (A) osteogenic differentiation capacities of MSCs with and without fNP labeling demonstrated by alizarin red staining (ARS); scale bare=50μm. (B) Quantitative assessment of area proportion of ARS. (C) Representative gene expression analysis for osteogenic genes from labeling/normal MSCs; n=6 for each group.

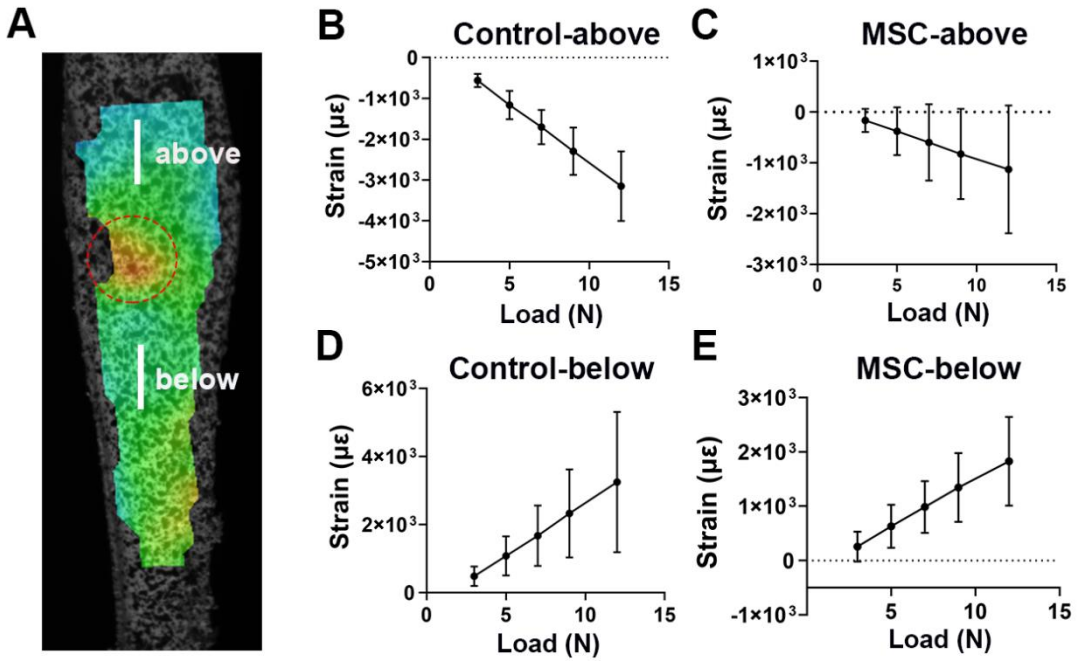

Figure S3. DIC analysis of surface strain of tibial defect and surrounding bone, related to Figure 2. (A) Representative strain map on the medial side of the tibia isolated from 11 weeks old female C57BL/6 mice, 10 days after the creation of a circular defect (red dotted circle). (B and C) Quantitative analysis of tensile strain 1mm above/below defect region.

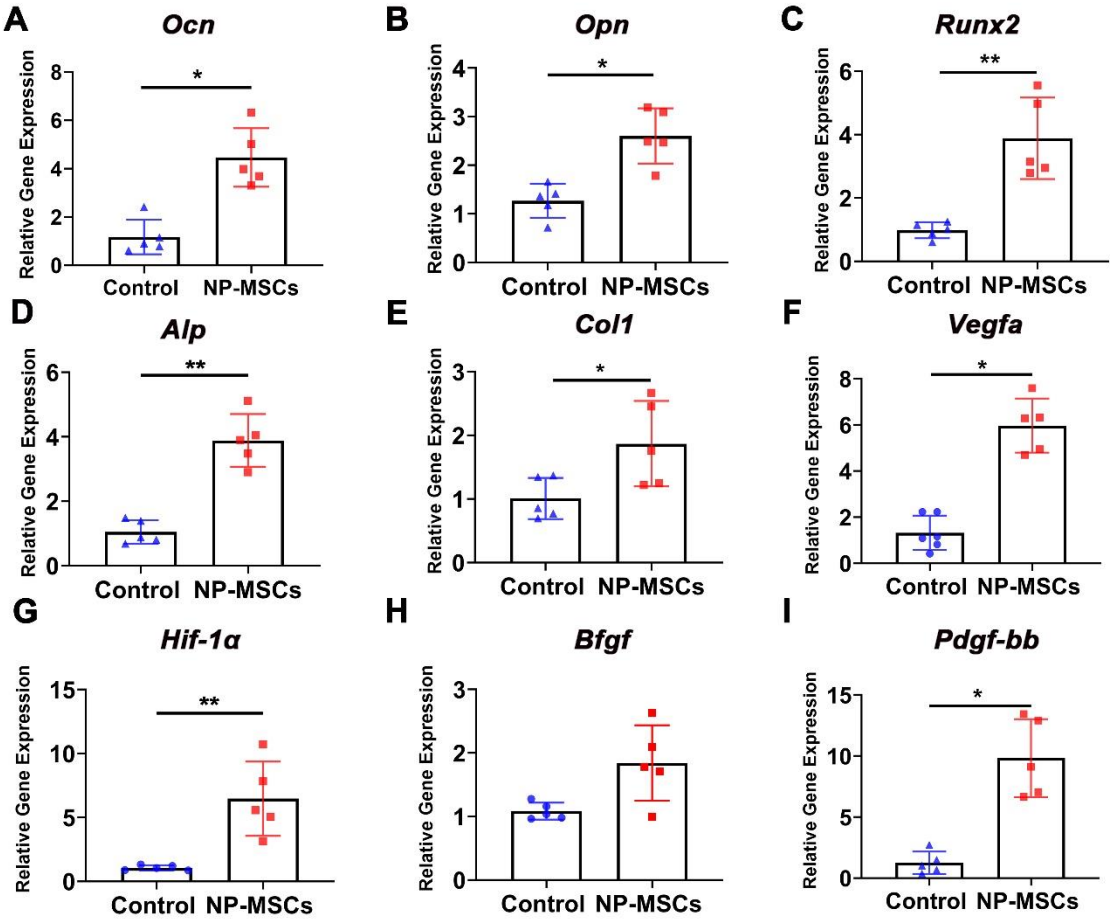

Figure S4. Relative gene expression analysis for defect region from PSD 10 samples, related to Figure 4. Relative gene expression of (A) *Runx2*, (B) *Alp*, (C) *Ocn*, (D) *Opn*, (E) *Col1*, (F) *Vegfa*, (G) *Hif-1α*, (H) *Bfgf* and (I) *Pdgf-bb* in defect area on PSD 10. *: P<0.05; **: P<0.01. n=6 mice for each group.

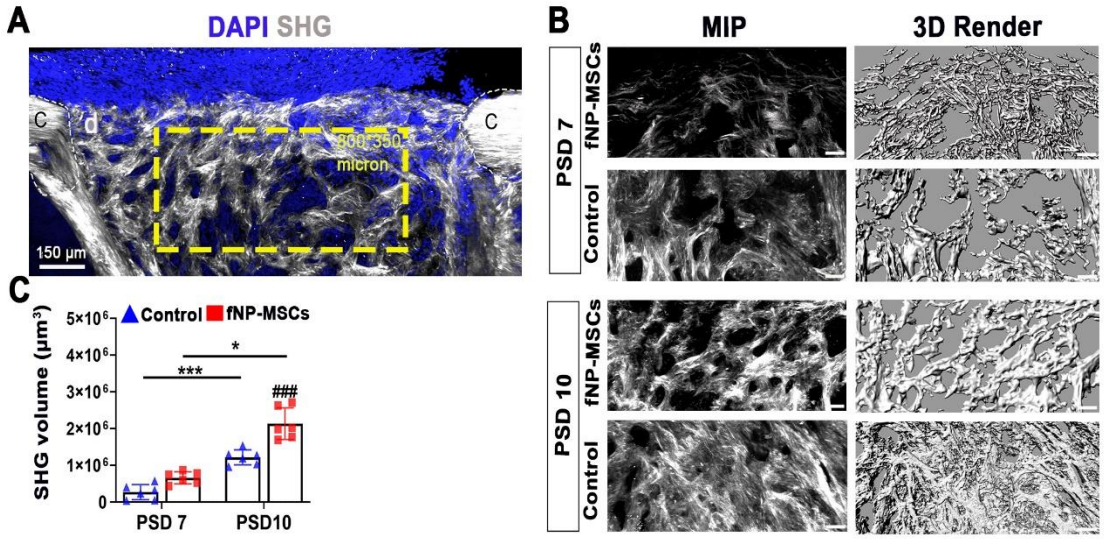

Figure S5. Local-delivered fNP-MSCs increased collagen fibers formation during bone defect repair, related to Figure 4. (A) Representative fluorescence images for collagen fibers (SHG, gray) and DAPI in three-dimensional space within tibial defect; scale bar= 50µm; c: cortical bone; d: defect area; yellow dotted line: volume of interest (VOI); (B) MIP and 3D rendered surface of collagen fibers from (a) on PSD 7 and 10; scale bar= 50µm; (C) Quantitative assessment for collagen fibers volume in defect area at each time point; MIP: maximum intensity projection; SHG: second harmonic generation. *: P<0.05; ***: P<0.001; ###: P<0.001; n=6 mice for each group.

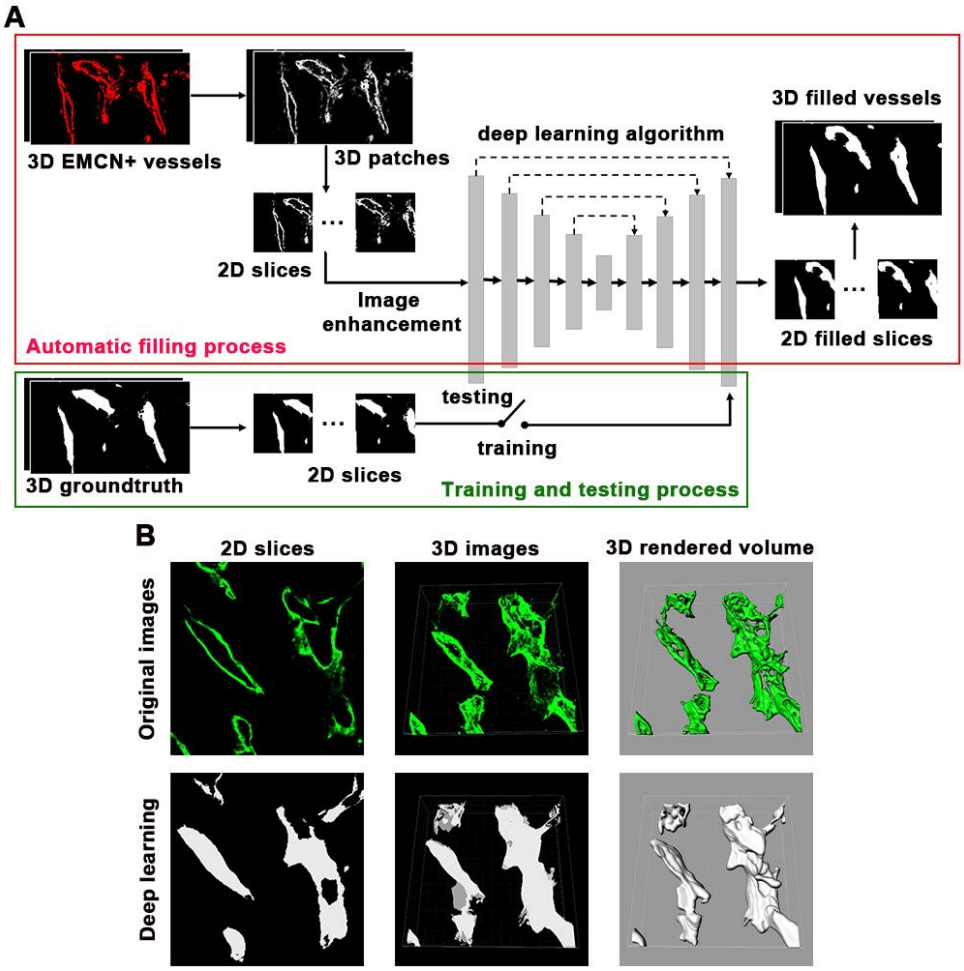

Figure S6. 3D digital rendering of the blood volume by utilizing deep learning algorithm, related to Figure 6. (A) The framework of filling the blood volume from EMCN+ vessels in defect by utilizing deep learning algorithm: the 3D groundtruth, which was manually filled according to 3D vascular images, was separated to create 2D section, and applied to train deep learning algorithm; this well-trained algorithm was utilized to automatically fill blood volume from 3D EMCN+ vessels; green box: training and testing process; red box: automatic filling process. (B) Qualitative evaluation of the filled vessels.

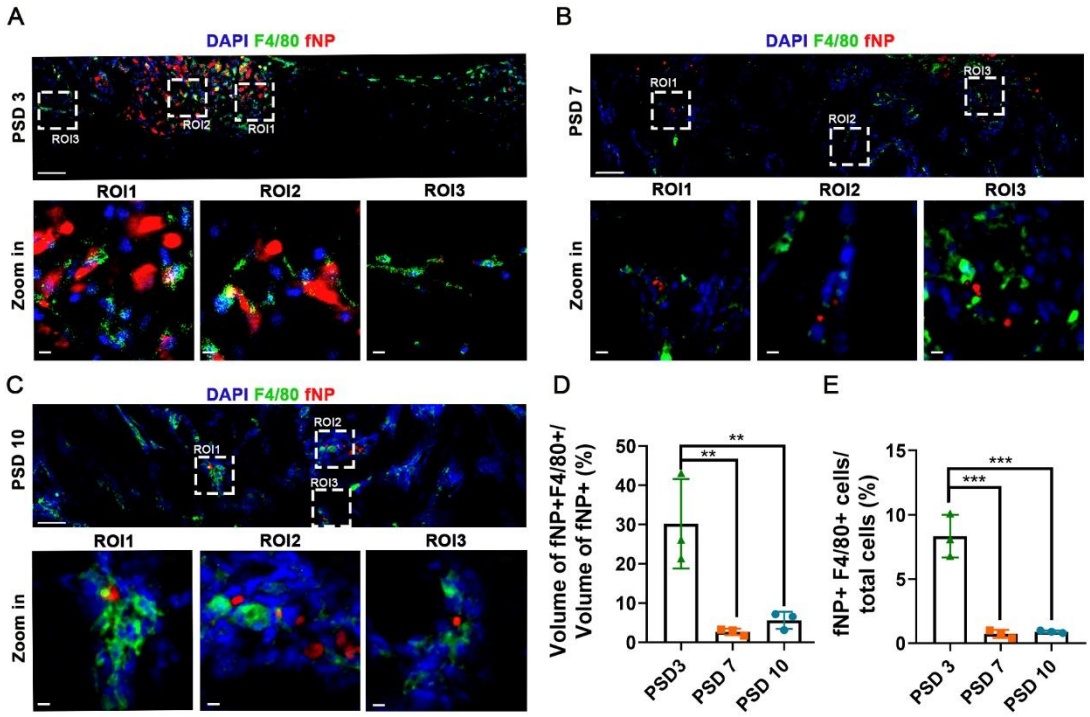

Figure S7. Co-localization of fNP with macrophages in bone defect area, related to Figure 7. Representative confocal microscopy images of macrophage marker F4/80 and nanoparticle signals (fNP) in defect area on (A) PSD 3, (B) PSD 7 and (C) PSD 10; with zoomed-in images below. Scale bar = 50μm for large images; Scale bar= 5μm for zoomed-in images. (D) Quantification of proportion of fNP+ F4/80+ volume over total fNP+ volume. (E) Quantification of proportion of fNP+F4/80+ cells over total cells. n=3; **: P<0.01; ***: P<0.001

Table S1. The primer sequences for tissue q-PCR, related to Figure 4.

Primer name	Sequence (5'-3')
Alp F	GGACAGGACACACACACACA
Alp R	CAAACAGGAGAGCCACTTCA
Runx2 F	TCCCTGAACTCTGCACCAAG
Runx2 R	ATCTGGCTCAGGTAGGAGGG
Ocn F	CTGACAAAGCCTTCATGTCCAA
Ocn R	GCGCCGGAGTCTGTTCACTA
Opn F	AGAGCGGTGAGTCTAAGGAGT
Opn R	TGCCCTTTCCGTTGTTGTCC
Osx F	ATGGCGTCCTCTCTGCTTG
Osx R	AAGGTCAGCGTATGGCTTCT
Vgfa-F	GCACCCATGGCAGAAGGAGG
Vgfa-R	CCTTGGTGAGGTTTGATCCGCATA
Hif-1α-F	TCAAGTCAGCAACGTGGAAG
Hif-1α-R	TATCGAGGCTGTGTCGACTG
Ppar-γ-F	AGCTGAATCACCCAGAGTCC
Ppar-γ-R	TGCAATCAATAGAAGGAACACG
Pdgf-bb-F	TTTCTCACCTGGACAGGTCG
Pdgf-bb-R	AAAGAGTGGACCTGTCCAGC
Bfgf-F	GGAGAAGAGCGACCCTCACATCAAG
Bfgf-R	CCAGTTCGTTTCAGTGCCACATACCAA
18S_1 F	GAGAAACGGCTACCACATCC
18S_1 R	CCTCCAATGGATCCTCGTTA