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ARTICLE TYPE

Supplemental Information for

Modulation of Host Osseointegration during Bone Regeneration by Controlling Exogenous Stem Cells Differentiation Using a Material Approach

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Scaffold Characterization

To adjust the content of HA in the scaffold, the concentration of collagen in m-SBF was varied from 1.0 to 5.0 g/L. The resultant Col/HA scaffolds with different HA contents were then subjected to thermal gravimetric analysis (TGA) (TA Instruments TGA Q-500, New Castle, DE) to determine the HA content in the scaffolds. The lyophilized scaffold was hermetically sealed in an aluminum crucible. The scaffold sample was then scanned from room temperature to 800 °C at 1.0 °C/min.

The phase composition of HA incorporated into the scaffolds was verified by X-ray diffraction. Briefly, the scaffold was grounded into powder using and examined using an X-ray diffractometer (BRUKER AXS D5005, Bruker, Karlsruhe, Germany) with a copper target. The voltage and current setup were 40 kV and 40 mA, respectively. A step size of 0.02^{o} and a scan speed of 0.5^{o}/min were used.

BMSCs differentiation *in vitro*

The BMSCs were characterized with respect to transgene pOBCol3.6GFPCyan expression. To assess the potential of donor cells for osteogenic differentiation and associated reporter expression, primary BMSCs were trypsinized with 0.25% trypsin–EDTA and plated at a density of 2×10^{4} cells/cm^{2} in a 24-well plate in α-MEM ascorbic acid and 7mM β-glycerolphosphate at day seven. GFP expression in donor cells was visualized using a Zeiss Axio Imager Z1 equipped for fluorescence imaging.

IVIS fluorescence imaging of bone regeneration

In order to visualize the distribution of donor cells in the animal tissue after implantation, we introduced in vivo imaging system (IVIS, Caliper Life Science, Mountain View, CA) to track the pOBCol3.6GFP cells and xylene orange (XO) dye in the defect area. The dissected femurs with defect were imaged using IVIS with the fluorescence mode.

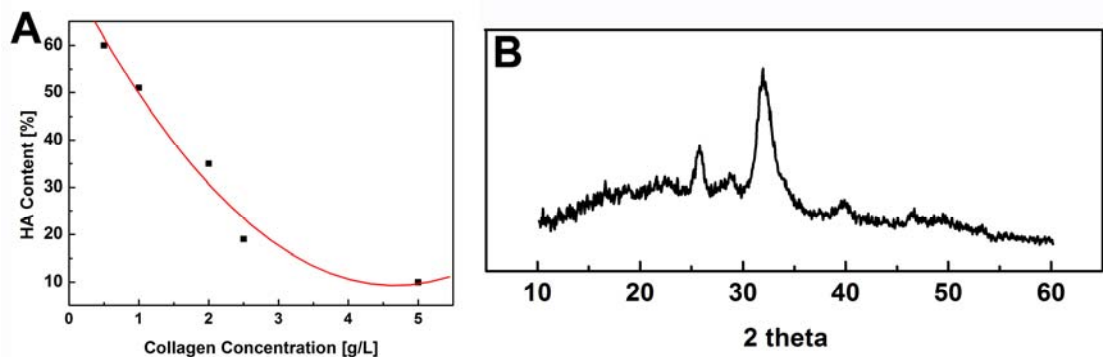

Fig S- 1 Col/HA scaffold characterization: (A) HA content in Collagen/HA scaffolds was characterized by TGA. The percentage of HA in the scaffolds were controlled by varying the concentration of collagen during processing; (B) XRD spectra of HA extracted from collagen/HA scaffolds.

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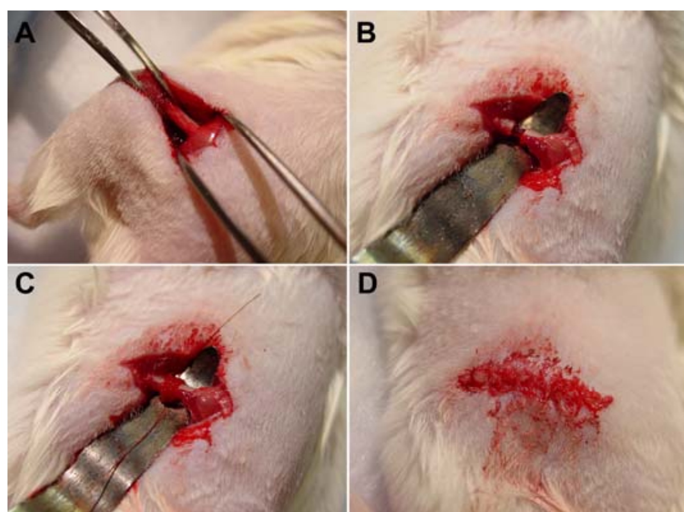

Fig. S-2 Photographs of the critically segmental bone defect model at different stage of surgical 5 procedure: (A) Expose the mouse femur by detaching muscle and connective tissue from the bone; (B) Creation of 3mm bone defect at the diaphysis of the femur, (C) Placement of MSC loaded collagen/HA scaffold into the segmental defect, (D) Close-up of the wound after fixation of the femur.

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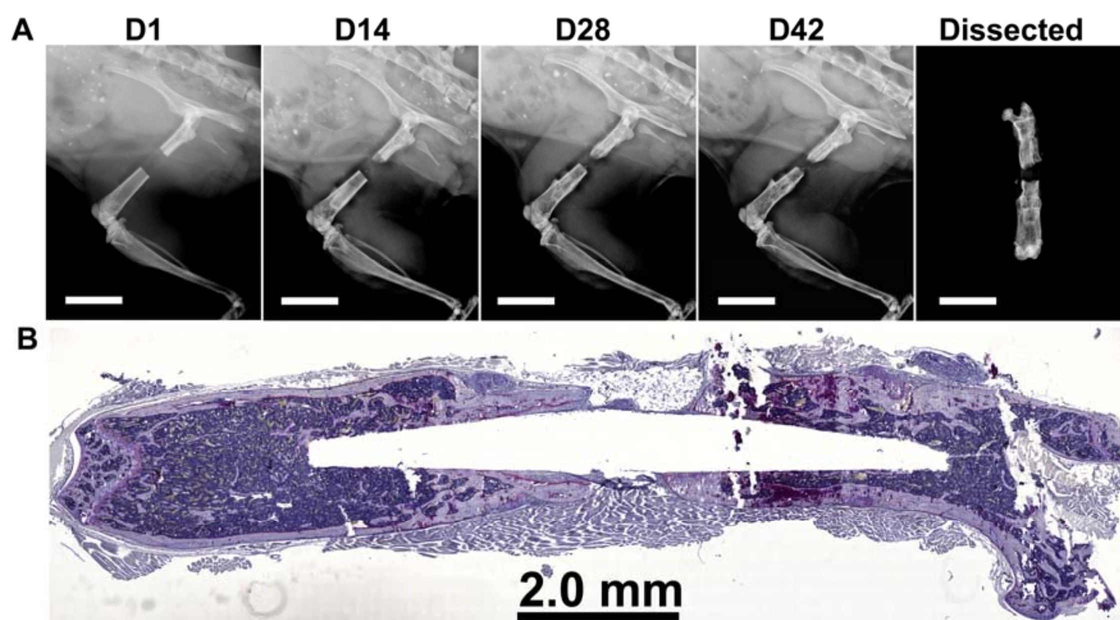

Fig S- 3 Bone defect healing without scaffold and donor cells: (A) Time lapse radiographs of segmental defects at different time points. The scale bars are 5.0 mm. (B) H&E staining of the bone defect on the femur at day 42.

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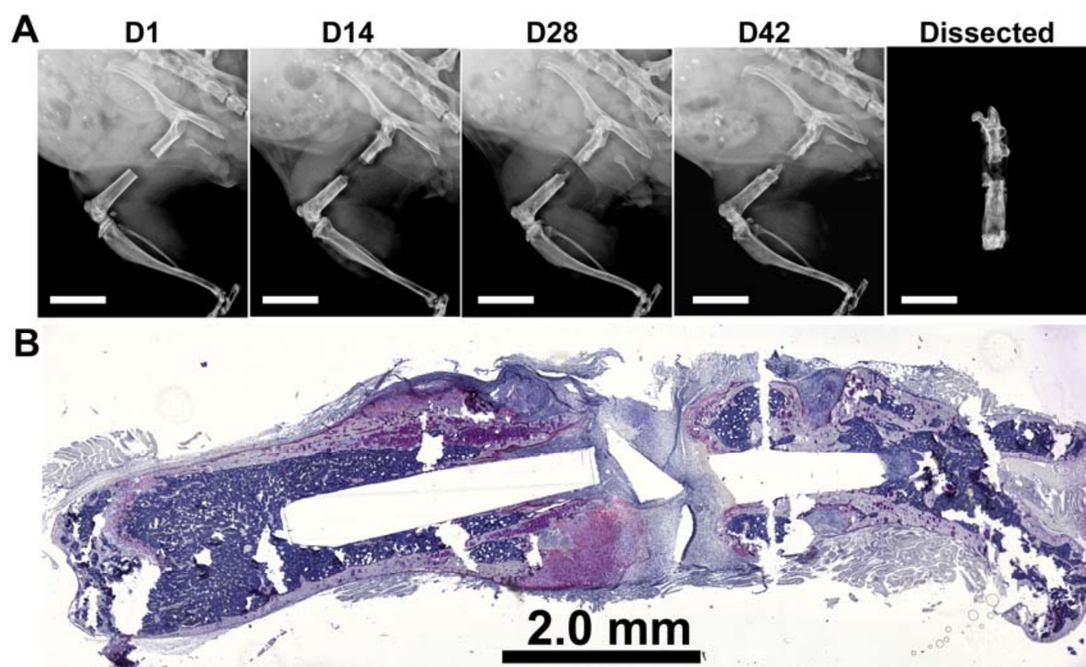

Fig S- 4 Bone defect healing with collagen scaffold only: (A) Time lapse radiographs of segmental defects at different time points. The scale bars are 5.0 mm. (B) H&E staining of the bone defect on the femur at day 42.

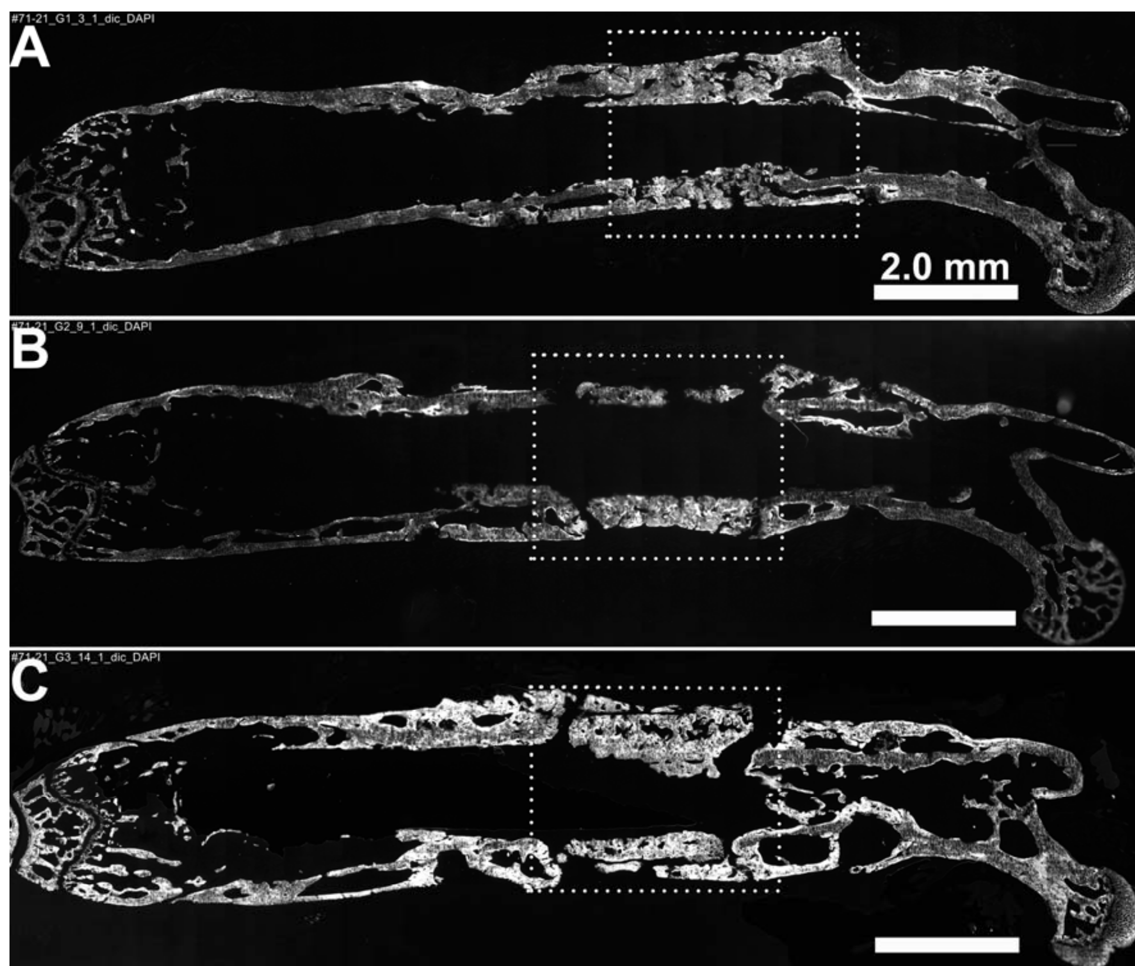

Fig S-5 Mineralization analysis of the whole femur loaded with different HA-content scaffolds: (A) No HA group, (B) Low HA group and (C) High HA group. New bone was bridged seamlessly with host bone tissue in No HA group while a gap was found between host and newly formed bone in HA-containing groups.

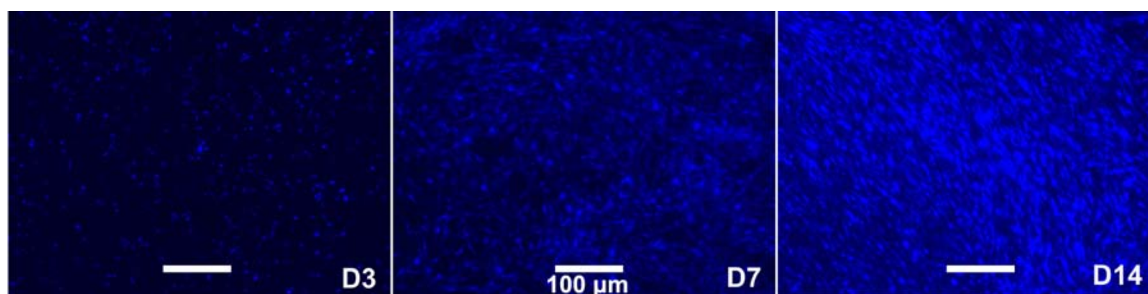

Fig S- 6 Cyan reporter expression of donor cell Col3.6GFPCyan during differentiation *in vitro*

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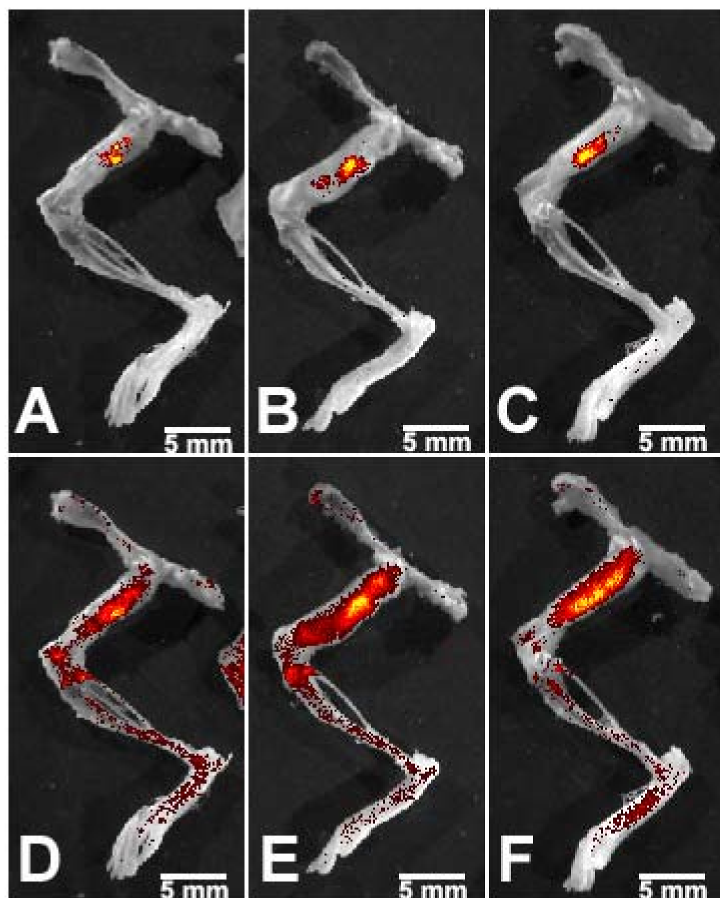

Fig S-7 Distribution of **donor BMSCs** in the recipient mice visualized by IVIS after 42 days of implantation: (A) Col3.6GFPCyan in No HA group, (B) (A) Col3.6GFPCyan in Low HA group, (C) (A) Col3.6GFPCyan in High HA group, 5 (D)XO in No HA group, (E)XO in Low HA group, **and (F) XO in High HA group**

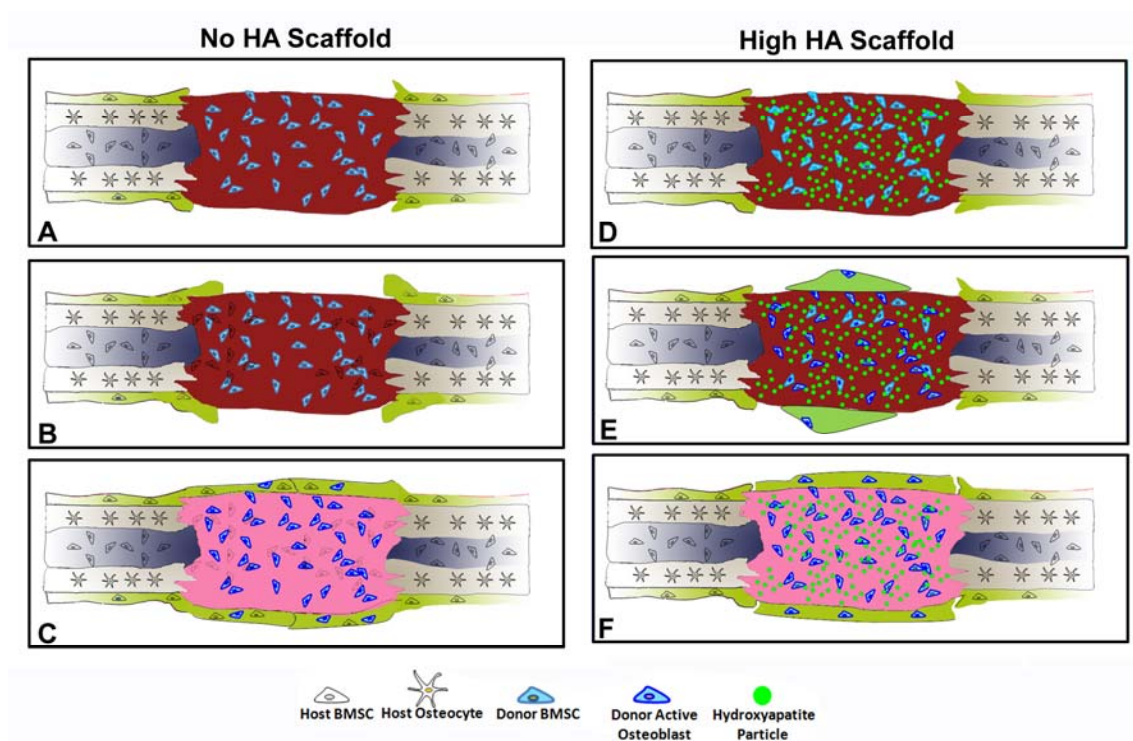

Fig S-8 The proposed donor cell-host-scaffold interactions associated with new bone regeneration. No HA Scaffold: (A) Early stage of healing (B) Intermediate stage of healing (C) Final stage of healing High HA Scaffold: (D) Early stage of healing (E) Intermediate stage of healing (F) Final stage of healing

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