

Supplementary Figure 1

ECM gene expressions in MSCs under osteogenic or non-osteogenic conditions investigated by semi-quantitative RT-PCR analysis. *GAPDH* is an internal control.

Supplementary Figure 2

Negative controls of immunocytochemical analysis. Samples were stained with only anti-mouse secondary antibody or anti-rabbit secondary antibody as negative controls. Cell nuclei were counterstained with methyl green. Stem cell, Early stage, and Late stage indicate undifferentiated MSCs, the differentiating cells at the early and late stages of osteogenesis, respectively. Scale bar indicates 200 μm .

Supplementary Figure 3

Semi-quantitative analysis of ECM alteration during osteogenesis. Positive areas of (A) type I collagen, (B) fibronectin, (C) versican, (D) biglycan, and (E) decorin were semi-quantitatively measured from the results of immunocytochemical analysis. The results are expressed as a percentage normalized to the cell area.

Supplementary Table 1

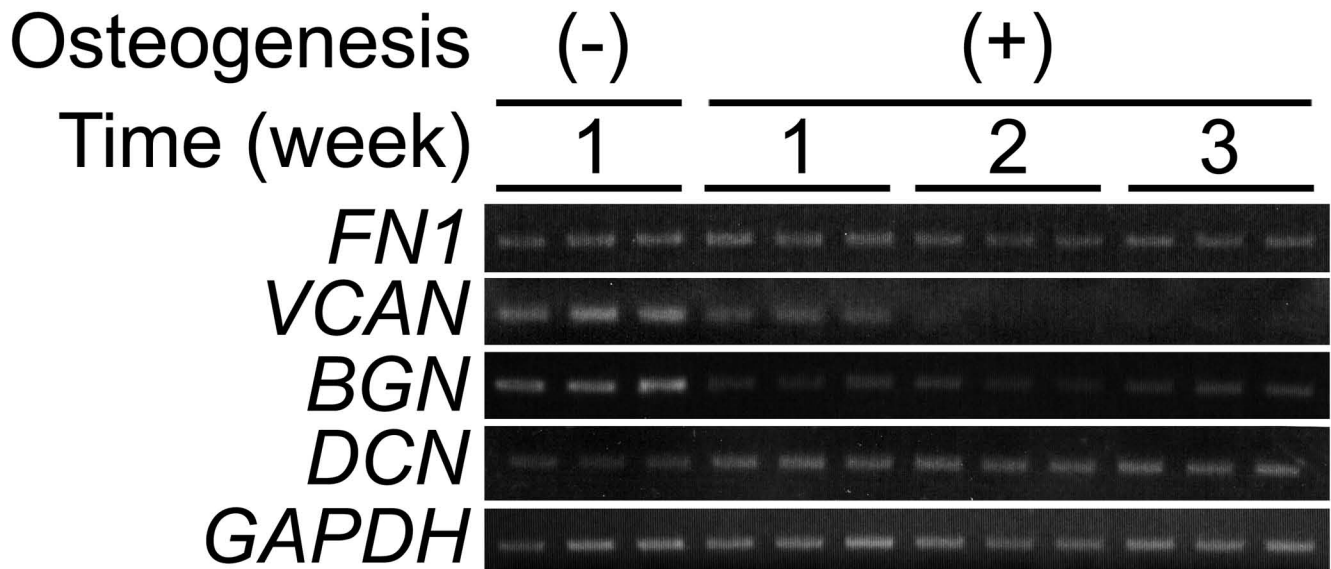
Supplementary Table 2: Primer sets for semi-quantitative RT-PCR analysis

mRNA		Oligonucleotide	Reference
<i>GAPDH</i>	Forward	5' - GGGCTGCTTTTAACTCTGGT-3'	[1]
	Reverse	5' - TGGCAGGTTTTTCTAGACGG-3'	
<i>FNI</i>	Forward	5' -ACCAATGCCAGGATTCAGAG-3'	
	Reverse	5' -ATACCACACCAGGCTTCAGG-3'	
<i>VCAN</i>	Forward	5' - GCACAAAATTTACCCCTGAC-3'	[2]
	Reverse	5' - GCACTGGATCTGTTTCTTCA-3'	
<i>DCN</i>	Forward	5' - GGCCACTATCATCCTCCTT-3'	[3]
	Reverse	5' - TTGTTGTTTTGCAGGTCTAGC-3'	
<i>BGN</i>	Forward	5' - CCCTCTCCAGGTCCATCCGC-3'	[4]
	Reverse	5' - GAGCTGGGTAGGTTGGGCGGG-3'	

FNI was designed by our laboratory.

Supplementary references

- [1] Tuli, R., Tuli, S., Nandi, S., Huang, X., Manner, P.A., Hozack, W.J., Danielson, K.G., Hall, D.J., and Tuan, R.S. (2003) *J. Biol. Chem.* **278**, 41227-41236
- [2] Read, J.T. Rahmani, M., Broomand, S., Allahverdian, S., McManus, B.M., and Rennie, P.S. (2007) *J. Biol. Chem.* **282**, 31954-31963
- [3] Bellucci, C. Lilli, C., Baroni, T., Parnetti, L., Sorbi, S., Emiliani, C., Lumare, E., Calabresi, P., Balloni, S., and Bodo, M. (2007) *Mol. Med.* **13**, 542-550
- [4] Chen, W.-B. Lenschow, W., Tiede, K., Fischer, J.W., Kalthoff, H., and Ungefroren, H. (2002) *J. Biol. Chem.* **277**, 36118-36128



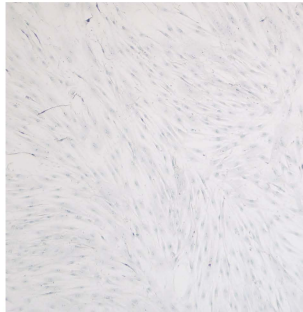
Supplementary figure 1 by Hoshiba et al.

Stem cell

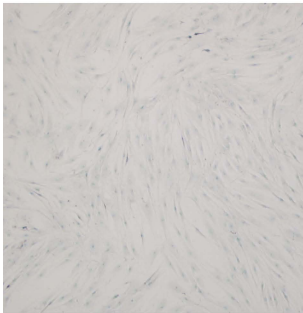
Early stage

Late stage

Anti mouse 2nd Ab
negative control



Anti rabbit 2nd Ab
negative control



— Bar: 200 μ m

Supplemental figure 2 by Hoshiba et al.

