Supplementary Data

In Vivo Bone Formation by iMPCs

Materials and methods

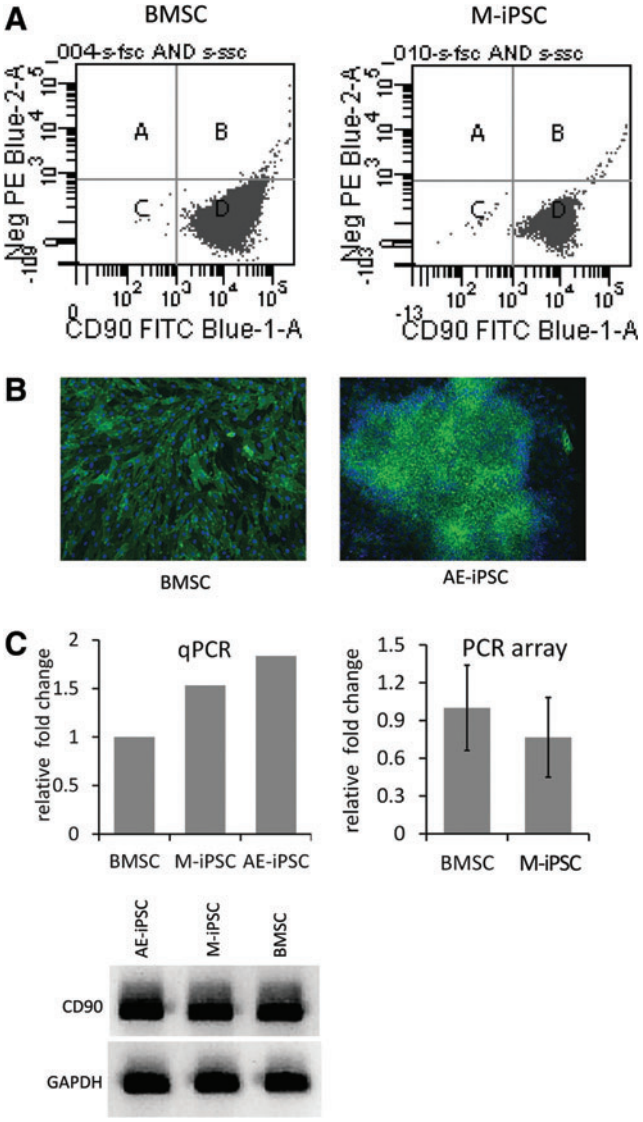
Induced pluripotent stem cell (iPSC)-derived mesenchymal stem cell (MSC)-like progenitor cells (iMPCs) (1×10^6 M-iMPC-GMs in 10 μL) were implanted with 90 μL collagen type I carrier with 1 μg recombinant human bone morphogenetic protein-2, BMP-2 into the left thigh muscle of 2- to 3-month-old NOD CB17-Prkdc^{scid}/J mice according to an IACUC approved protocol. As control, a similar implant without BMP-2 was made in the right thigh. The implant site was monitored by micro-computed tomography (micro-CT) for approximately 6 weeks postimplantation, after which the thigh muscle was harvested and examined histologically by H&E, Alizarin Red, and Masson's Trichrome staining.

Results

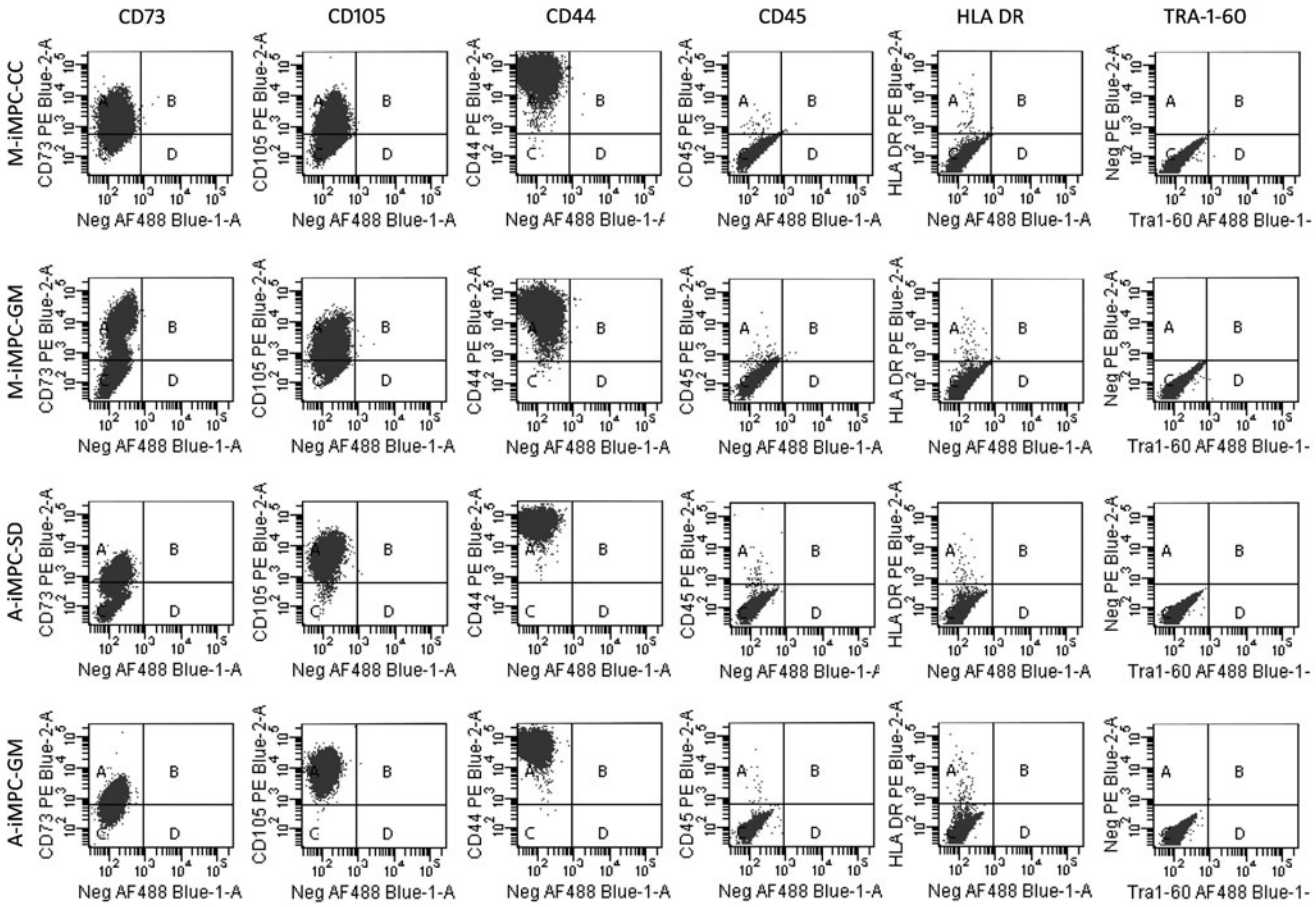
Bone formation was first observed only in the BMP-2-treated iMPC implant by micro-CT at week 2 postimplantation, and continued for approximately week 6 (Supplementary Fig. S7A). Mean bone density remained fairly constant from week 2 to 6 (318–339 mg hydroxyapatite/cm^3), although bone volume decreased from 0.28 to 0.09 mm^3 during the same period, suggesting tissue turnover and resorption. The presence of mineralized matrix was observed by histological staining (Supplementary Fig. S7B). In contrast, neither untreated iMPCs nor BMP-2 alone induced ectopic bone formation (Supplementary Fig. S7C).

Conclusions

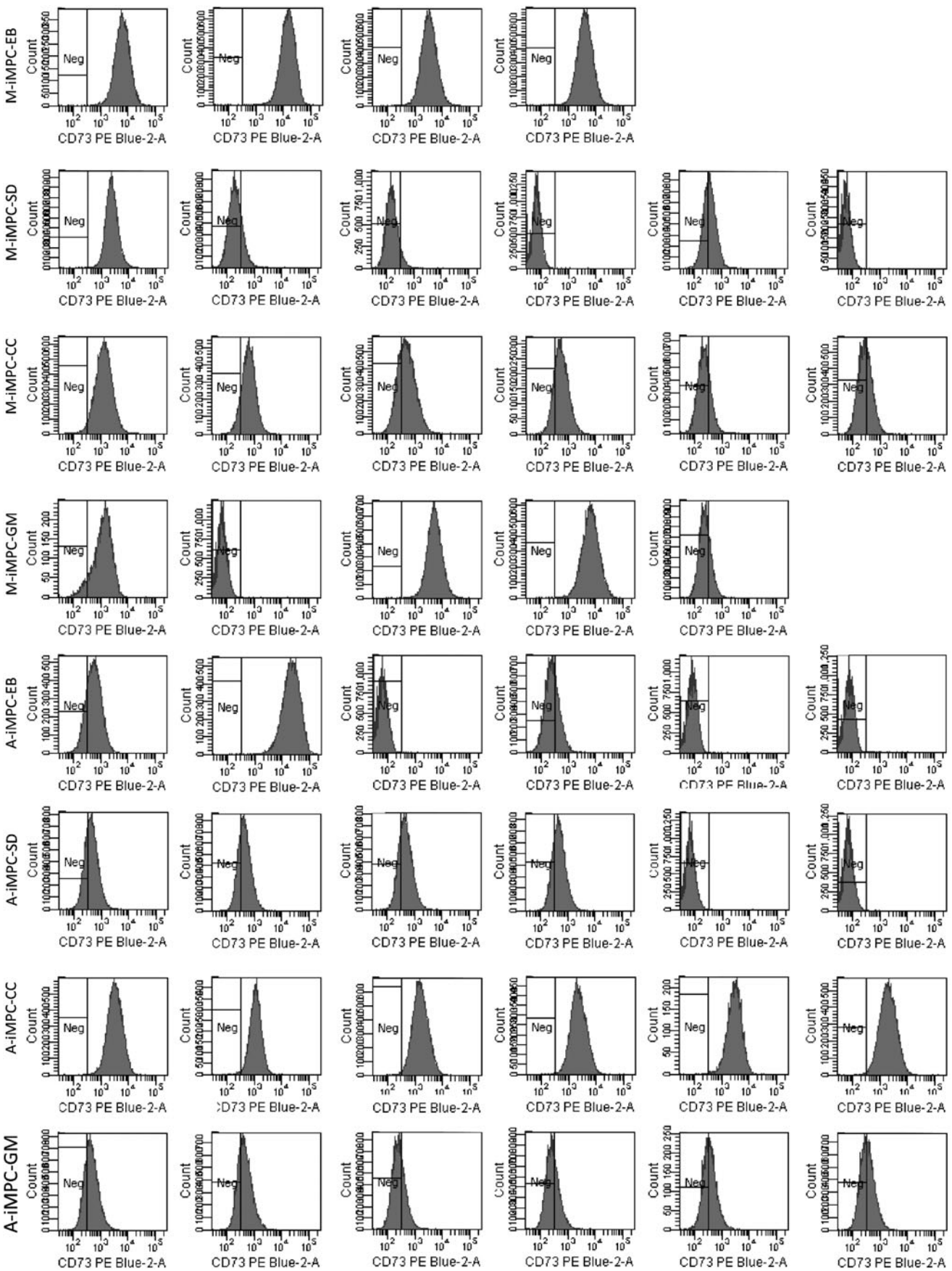
iMPCs were capable of BMP-2-induced osteogenesis on ectopic transplantation, and are, thus, MSC like. As anticipated, BMP-2 alone did not induce bone formation in this specific model, which is due to low retention at the implantation site.



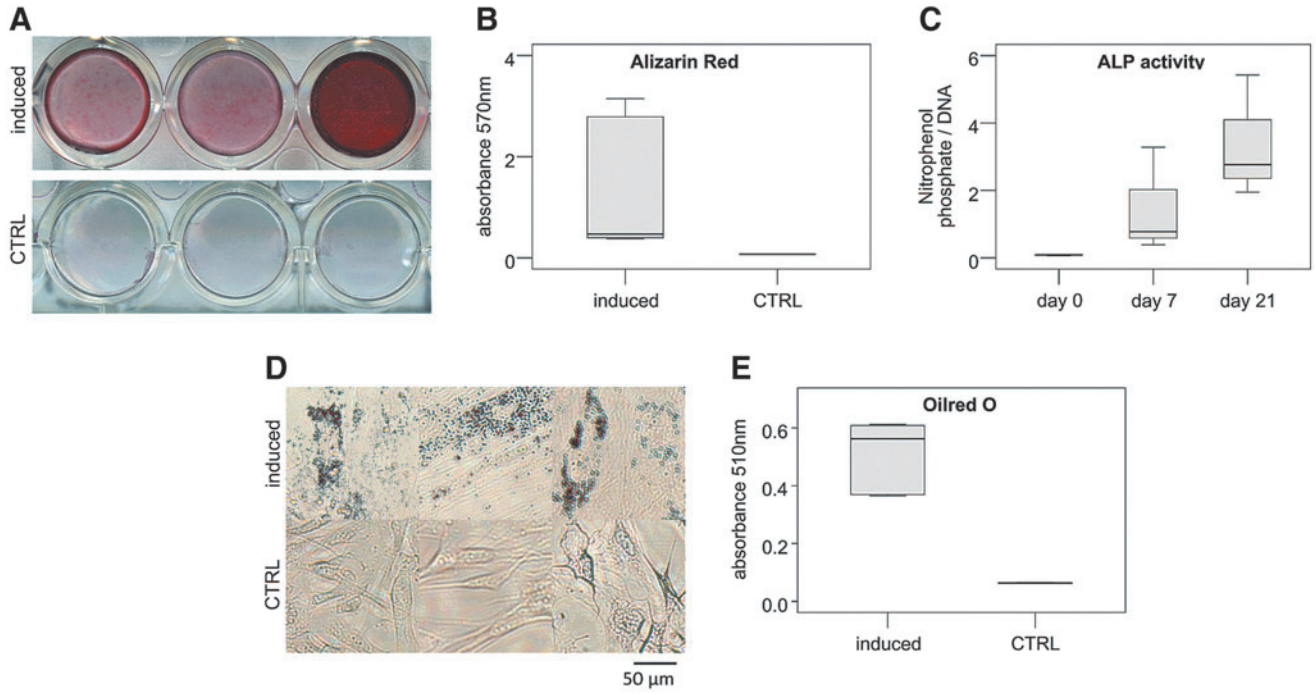
SUPPLEMENTARY FIG. S1. CD90 is expressed in BMSCs and iPSCs. **(A)** Flow cytometric analysis of CD90 in BMSCs (*left*) and M-iPSCs (*right*). **(B)** Immunofluorescence staining of CD90 (*green*) and DAPI counter staining (*blue*) in BMSCs (*left*) and AE-iPSCs (*right*). **(C)** qPCR analysis of CD90 expression in BMSCs, M-iPSCs, and AE-iPSCs (*upper left*) and agarose gel bands of qPCR products (*bottom*). CD90 expression in BMSCs and M-iPSCs according to qPCR array (*right*). qPCR, quantitative polymerase chain reaction.



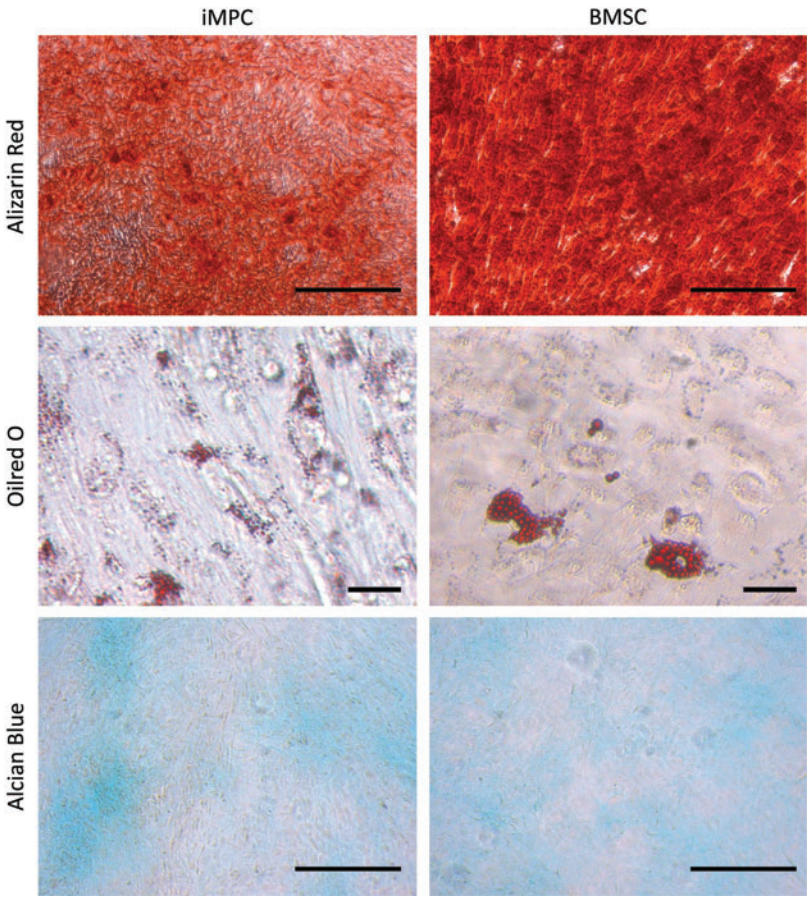
SUPPLEMENTARY FIG. S2. Flow cytometric analysis of surface marker expression in M-iMPC-CCs, M-iMPC-GMs, A-iMPC-SDs, and A-iMPC-GMs. Assessed surface markers included those typically present on BMSCs (CD73, CD105, and CD44), typically absent on BMSCs (CD45, HLA DR), and associated with pluripotency (TRA-1-60). Analysis of iMPC surface markers was performed with pools of four to six replicate cell lines. BMSCs, bone marrow stromal cells; CC, coculture; EB, embryoid body; GM, growth medium; iMPC, induced pluripotent stem cell (iPSC)-derived mesenchymal stem cell (MSC)-like progenitor cells; SD, spontaneous differentiation.



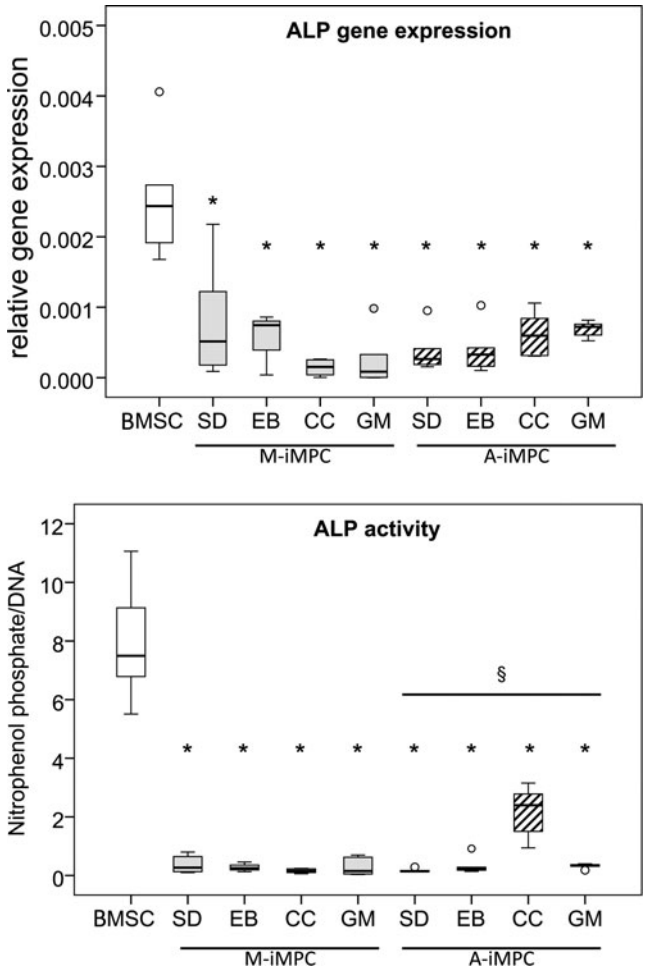
SUPPLEMENTARY FIG. S3. Flow cytometric analysis of CD73 expression in individual iMPC lines derived from M-iPSCs and AE-iPSCs as indicated. Four to six replicate cell lines were analyzed for each derivation method. CD73 expression was homogeneous within each cell line but deviated between replicate cell lines.



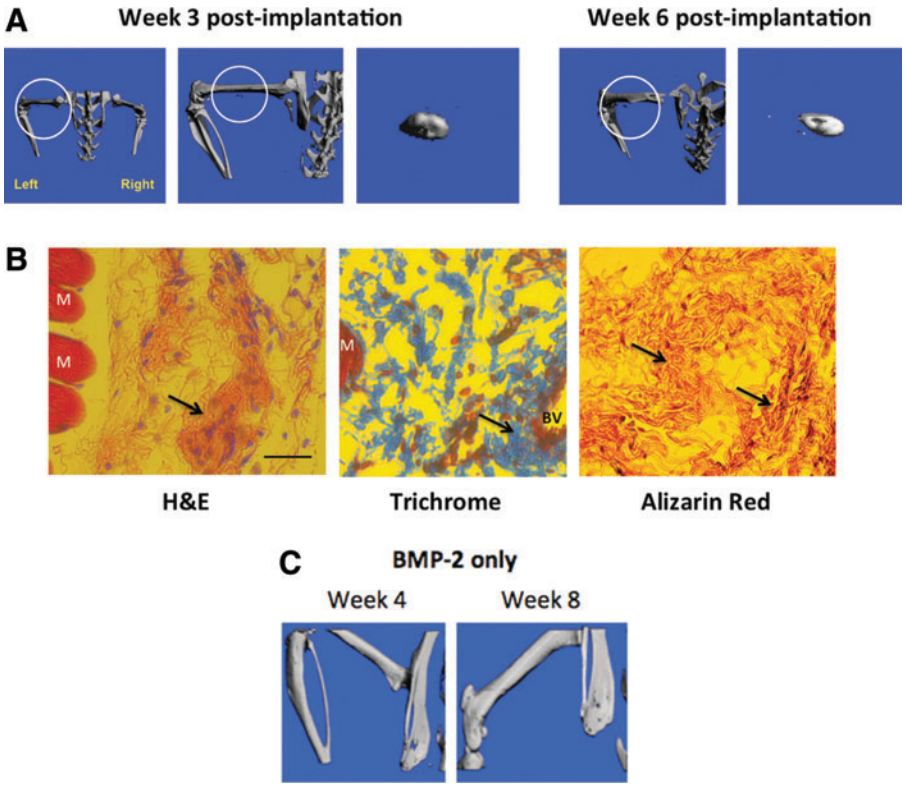
SUPPLEMENTARY FIG. S4. In vitro osteogenesis **(A–C)** and adipogenesis **(D, E)** of A-iMPCs in a preliminary experiment. In osteogenic medium, Alizarin Red staining **(A, B)** verified matrix mineralization in induced samples but not in non-induced controls. ALP activity **(C)** was negative on day 0 and strongly induced on days 7 and 21. In adipogenic medium **(D, E)**, Oil Red O staining verified production of lipid droplets in induced samples but not in non-induced controls. iMPCs, induced pluripotent stem cell-derived MSC-like progenitor cells. ALP, alkaline phosphatase.



SUPPLEMENTARY FIG. S5. Trilineage differentiation capacity of iMPCs. One M-iMPC-GM cell line (*left*) and BMSCs (*right*) were induced to undergo osteogenesis (*top*), adipogenesis (*middle*), and chondrogenesis (*bottom*) in monolayer culture. Alizarin Red staining indicates matrix mineralization, Oil Red O, formation of lipid vesicles, and Alcian blue, deposition of sulfated glycosaminoglycans. Scale bars represent 200 μm.



SUPPLEMENTARY FIG. S6. ALP gene expression on day 21 of osteogenic induction (*top*) and enzyme activity on day 7 (*bottom*) in BMSCs versus iMPCs. Values are median (*bars*), with the boxes representing first and third quartile and whiskers representing maximal and minimal values. Outliers are depicted as *circles*. Group sizes were $n=3$ for BMSCs (independent replicates from one donor) and $n=4$–6 for iMPCs (replicate cell lines). Significant differences ($p<0.05$) between iMPCs and BMSCs are designated by an *asterisk*. Significant differences ($p<0.05$) between M-iMPCs and A-iMPCs are designated by §.



SUPPLEMENTARY FIG. S7. In vivo BMP-2-induced bone by formation by iMPCs. **(A)** Micro-computed tomography imaging. Weeks 3 and 6 images shown (*circled*). **(B)** Histological staining of week 6 implants. (*Left*) H&E, (*middle*) Trichrome, and (*right*) Alizarin Red, showing evidence of matrix deposition and mineralization (*arrows*). BV, blood vessel; M, muscle. Scale bar = 20 μm. **(C)** BMP-2 alone did not induce ectopic bone formation.

Supplementary Table S1. Comparative Analysis of Gene Expression Profiles of iMPCs Versus MSCs

iMPC-EB vs. BMSC			*iMPC-SD vs. BMSC*			*iMPC-CC vs. BMSC*			*iMPC-GM vs. BMSC*		
	Fold regulation	P *value*		*Fold regulation*	P *value*		*Fold regulation*	P *value*		*Fold regulation*	P *value*
BDNF	10.814	0.001338	ITGA6	5.9571	0.038336	BDNF	6.498	0.037362	GDF15	8.849	0.034147
CSF3	4.813	0.030983	MCAM	4.8853	0.018162	CSF2	4.2759	0.000007	ICAM1	10.5502	0.021091
GDF15	9.7491	0.016135	PPARG	15.8182	0.021804	EGF	7.1965	0.000031	ITGA6	5.4728	0.003938
ICAM1	27.1627	0.007193	**SOX2**	29.627	0.007347	ITGA6	13.9898	0.000213	MCAM	4.2293	0.006948
MCAM	5.1584	0.040093	**ANPEP**	−5.595	0.001829	KDR	30.531	0.014242	NES	4.561	0.032144
NES	8.786	0.002814	**BGLAP**	−7.2534	0.005147	MCAM	6.3705	0.000002	**SOX2**	24.7001	0.002529
NOTCH1	7.0927	0.006026	BMP4	−22.6022	0.014924	NES	8.0444	0.026494	**ANPEP**	−9.4295	0.00125
POU5F1	17.8667	0.014796	HGF	−4.7863	0.011637	SMURF2	7.1157	0.003189	**BGLAP**	−8.2898	0.005226
PPARG	17.5012	0.046799	VCAM1	−40.5605	0.006716	**SOX2**	17.9056	0.000021	ENG	−5.4768	0.002128
PTK2	4.2113	0.025064				**ANPEP**	−16.604	0.000699			
SMURF2	4.7024	0.017529				**BGLAP**	−8.7761	0.005277			
SOX2	39.0144	0.006629				COL1A1	−4.6232	0.015443			
ANPEP	−4.1935	0.001796				ENG	−6.8749	0.000785			
BGLAP	−6.9754	0.005967				GDF5	−67.4121	0.023201			
BMP4	−15.3303	0.020994				HGF	−21.2355	0.001764			
GDF5	−15.167	0.032527				MMP2	−5.0184	0.001497			
THY1	−5.0549	0.035722				TGFB3	−6.5886	0.017613			
VCAM1	−5.1962	0.019326				VCAM1	−179.423	0.005701			

Recurring genes are printed in *bold*, up-regulation is marked *red*, and down-regulated genes are printed *green*.

CC, coculture; EB, embryoid body; GM, growth medium; iMPC, induced pluripotent stem cell (iPSC)-derived mesenchymal stem cell (MSC)-like progenitor cells; SD, spontaneous differentiation.