

Suppl. Figure 1









Lane 1: Size marker, Lane2: Pre-diff, Lane 3: Induced diff, Lane 4: Positive control

h

ΗΝ**F3**β

 $\alpha FP$ 

Merge



m

 $HNF1\alpha$ 



Merge



Lane 1: Size marker, Lane2: Pre-diff, Lane 3: Induced diff, Lane 4: Positive control Suppl. Figure 2



## Suppl. Figure 3



## Supple. Figure 4

RAEC

RVSMC

2 .

0 -

NRCM









# Suppl. Figure 5

#### **Supplementary Information**

**Supplementary Figure 1. FACS analysis of hBMSC using multiple surface epitopes**. **a.** Various bone-marrow derived lineage specific markers, known HSC markers(CD34, CD133, Flk-1, Tie2) and major histocompatibility complex(MHC) class I(ABC) and II(DR) were not expressed. **b.** Positive control for FACS antibodies. We used peripheral blood for CD11b, CD13, CD14, CD45 and CD31 antibodies, magnetic column-selected (CliniMacs, Miltenyl Biotec, Germany) CD34 and CD133 cells from peripheral blood for CD34 and CD133 antibodies, HUVEC for CD146 antibody and fibroblast for CD71 and CD166 antibodies. **c.** Telomerase activity assessed by TRAP assay was detected in hBMSCs with both low (20, lane 2) and high (120, lane 3) PDs. Lane 1, positive control (T293 cell), Lane 4, negative control (human vascular smooth muscle cells).

Supplementary Figure 2. In vitro differentiation of hBMSC into neural and endodermal lineages. a. Morphologic characteristics of hBMSC in the neural differentiation culture showing typical appearance of neural cells. Scale bar = 100  $\mu$ m. b-e. IF staining of hBMSC after induction of neural differentiation demonstrates expression of multiple neural specific proteins: GFAP (astrocytes), GalC (oligodendrocytes), NF200 (neurons), and  $\beta$ -tubulin III (neurons). f. RT-PCR confirms de novo expression of neural lineage-specific genes, GFAP, MBP, oligodendrocyte, MAP2 (neurons), GAD (neurons), and Tau (neurons) after induced neural differentiation. g-l. Phenotypic characterization of hBMSC differentiation into endodermal (epithelioid) cells. IF localization of HNF 3 $\beta$ (FITC) and  $\alpha$ FP(Cy3) at day 10(g-i), and CK18 (FITC) and HNF 1 $\alpha$  (Cy3) at day 14 (j-l) in cultured hBMSC on Matrigel with media containing HGF, FGF-4 and DMSO. m. RT-PCR confirms de novo expression of endodermal lineage-specific genes, CK18, CK19,  $\alpha$ FP and albumin after induced endodermal differentiation. diff: differentiation. Heavy band in the lane of the size markers in RT-PCR (f and m) = 600bp. Original magnification x 200.

Supplementary Figure 3. Representative histologic illustration of capillary and CMC density, and myocardial fibrosis and representative gel photographs of Western blot and semiquantitative RT-PCR product. a. Representative immunohistochemical findings for CD31 in infarct myocardium 4 wks after cell transplantation showing significantly higher capillary density in hBMSC transplanted heart tissue. b. Representative findings of infarct area stained with H&E demonstrate notable salvage and regeneration of myocardium in an hBMSC-transplanted heart. c. Representative figures of Masson's trichrome-stained hearts showing significantly smaller area of

percent fibrosis (blue color) in hBMSC transplanted heart tissue. Scale bar, 100µm

**Supplementary Figure 4.** Fusion and Differentiation of hBMSC in co-culture with cardiomyocytes, smooth muscle cells and endothelial cells. **a-h.** Co-culture of hBMSC and NRCM for investigation of cell fusion and differentiation. We co-cultured hBMSC transfected with lentiviraleGFP (green fluorescence) (a, e), and NRCM transfected with adenoviral-LacZ (red fluorescence detected by  $\beta$ -galactosidase staining) (b, f). Seven days after co-culture, cells were stained with cTnI followed by AMCA-conjugated secondary antibody (blue fluorescence) (c, g). In the IF images, an hBMSC which has differentiated into CMC lineage is illustrated by red and blue fluorescence without green fluorescence (white arrows in a-d). Triple color positive cells are fusion cells expressing cTnI (yellow arrows in e-h). **i-l.** Co-culture of hBMSC and rat vascular smooth muscle cells (RVSMC). We co-cultured hBMSC transfected with lentiviral-eGFP (green fluorescence)(i) and RVSMC transfected with adenoviral-LacZ (red fluorescence detected by  $\beta$ -galactosidase staining)(j). Seven days after co-culture, cells were stained with calponin followed by AMCA-conjugated secondary antibody (blue fluorescence)(i) and RVSMC transfected with adenoviral-LacZ (red fluorescence detected by  $\beta$ -galactosidase staining)(j). Seven days after co-culture, cells were stained with calponin followed by AMCA-conjugated secondary antibody (blue fluorescence)(k). Arrows in i-l indicates fused hBMSCS (green) and RVSMC (red) which express calponin. **m.** Incidence of fusion and differentiation in co-culture of hBMSC with NRCM, RVSMC and RAEC, respectively. Original magnification x 400.

Supplementary Figure 5. Immunohistochemistry with caspase-3 and Ki-67. a. Representative immunohistochemical findings for activated caspase-3 and  $\alpha$ -sarcomeric actinin in periinfarct area at 1 wk after PBS injection revealing expression of caspase-3 (green fluorescence) within cardiomyocytes (red fluorescence) suggestive of apoptosis. **b.** Quantitative analysis of activated caspase-3 positive cells showed that hBMSC transplanted hearts have significantly lower number of apoptotic cells. **c.** Representative immunohistochemical findings for Ki-67 and  $\alpha$ sarcomeric actinin at 1 wk after hBMSC transplantation showing Ki-67 positive cells (green fluorescence) in periinfarct area. **d.** Ki-67 index was significantly higher in hBMSC transplanted hearts compared with TBMC or PBS treated hearts. \**P* < 0.05, \*\**P* < 0.01, vs. TBMC, PBS. Original magnification x 400.

		Product
Primers	Sequences	Size(bp)
Human		
Oct4-f	5'- GAG AAC AAT GAG AAC CTT CAG GAG A -3'	
Oct4-r	5'- TTC TGG CGC CGG TTA CAG AAC CA -3'	219
CD34-f	5'- ACC ACT TCC CTC ATC TCT CCT CCA A-3'	
CD34-r	5'- AGG GTG AGG GAG GCA GAG ACA GAA A-3'	421
KDR-f	5'- TGC AGG ACC AAG GAG ACT ATG T-3'	
KDR-r	5'- TAG GAT GAT GAC AAG AAG TAG CC-3'	458
Tie2-f	5'- ATC CCA TTT GCA AAG CTT CTG GCT GGC-3'	
Tie2-r	5'- TGT GAA GCG TCT CAC AGG TCC AGG ATG-3'	512
CD31-f	5'- AGG TCA GCA GCA TCG TGG TCA ACA T-3'	
CD31-r	5'- GTG GGG TTG TCT TTG AAT ACC GCA G-3'	387
VE-cadherin-f	5'- CTC TGC ATC CTC ACC ATC ACA G-3'	
VE-cadherin-r	5'- TAG CCG TAG ATG TGC AGC GTG T-3'	389
SM1-f	5'- TAA ACA CCT GCC CAT CTA CTC GG-3'	
SM1-r	5'- ATC TCA TCA TCC TGG GCT GCT GG-3'	732
SM22a-f	5'- CGG CTG GTG GAG TGG ATC ATA G-3'	
SM22a-r	5'- CCC TCT GTT GCT GCC CAT CTG A-3'	489
PDGFRb-f	5'- GCC TTA CCA CAT CCG CTC -3'	
PDGFRb-r	5'- TCA CAC TCT TCC GTC ACA TTG C -3'	443
GATA4-f	5'- AGA-CAT-CGC-ACT-GAC-TGA-GAA-C-3'	
GATA4-r	5'- GAC-GGG-TCA-CTA-TCT-GTG-CAA-C-3'	475
Nkx2.5-f	5'- CTT-CAA-GCC-AGA-GGC-CTA-CG-3'	
Nkx2.5-r	5'- CCG-CCT-CTG-TCT-TCT-TCA-GC-3'	233
A FP-f	5'- TGC AGC CAA AGT GAA GAG GGA AGA-3'	
AFP-r	5'- CAT AGC GAG CAG CCC AAA GAA GAA-3'	343
Alb-f	5'- TGC TTG AAT GTG CTG ATG ACA GGG-3'	
Alb-r	5'- AAG GCA AGT CAG CAG GCA TCT CAT C-3'	181
CK18-f	5'- GTA CTG GTC TCA GCA GAT TGA GGA G-3'	
CK18-r	5'- GCT TCT GCT GGC TTA ATG CCT CAG A-3'	499
CK19-f	5'- ATG GCC GAG CAG AAC CGG AA-3'	
CK19-r	5'- CCA TGA GCC GCT GGT ACT CC-3'	318
GFAP-f	5' - TCA TCG CTC AGG AGG TCC TT - 3'	
GFAP-r	5' - CTG TTG CCA GAG ATG GAG GTT- 3'	383
MAP2-f	5' - GAA GAC TCG CAT CCG AAT GG - 3'	
MAP2-r	5' - CGC AGG ATA GGA GGA AGA GAC T - 3'	527
MBP-f	5' - TTAGCT GAATTC GCG TGT GG - 3'	
MBP-r	5' - GAG GAAGTGAAT GAG CCG GTTA- 3'	374
GAD-f	5' - GCG CCA TAT CCA ACA GTG ACA G - 3'	
GAD-r	5' - GCC AGC AGT TGC ATT GAC ATA A- 3'	284
Tau-f	5 GTA AAA GCA AAG ACG GGA CTG G-3_,	
Tau-r	5 ATG ATG GAT GTT GCC TAA TGA G-3_	512/612
Rat*		
Nkx2.5-f	5'- CAG TGG AGC TGG ACA AAG CC-3'	
Nkx2.5-r	5'- TAG CGA CGG TTC TGG AAC CA-3' (32 cycles)	216
GATA4-f	5'- CTG TCA TCT CAC TAT GGG CA-3'	

### Supplementary Table 1. RT-PCR primer sequences and expected product size

GATA4-r	5'- CCA AGT CCG AGC AGG AAT TT-3' (32 cycles)	275	
MEF2c-f	5'- AGC AAG AAT ACG ATG CCA TC-3'		
MEF2c-r	5'- GAA GGG GTG GTG GTA CGG TC-3'(30 cycles)	407, 311	
Ang-1-f	5'- AGT CGG AGA TGG CCC AGA TAC AAC A -3'		
Ang-1-r	5'- TCC AGC AGT TGG ATT TCA AGA CGG G -3'(28 cycles)	169	
Ang-2-f	5'- TAC GTG CTG AAG ATC CAG CTG AAG G -3'		
Ang-2-r	5'- AGT TGG AAG GAC CAC ATG CGT CGA A -3'(30 cycles)	259	
PDGF-B-f	5'- CCG AGG AGC TTT ATG AGA TGC TGA G -3'		
PDGF-B-r	5'- AGC TGC CAC TGT CTC ACA CTT GCA T -3'(30 cycles)	479	
IGF1-f	5'- ACTTCAACAAGCCCACAGGCTA –3'		
IGF1-r	5'- TCCTTCTGAGTCTTGGGCATGT -3' (30 cycles)	189	
SDF1a-f	5'- ATGGGACGCCAAGGTCGTCG -3'		
SDF1ar	5'- TCGGGTCAATGCACA CTTGTCTGT-3`(32 cycles)	223	
GAPDH-f	5'- TCG GTG TGA ACG GAT TTG GCC GTA T-3'		
GAPDH-r	5'- AGC CCT TCC ACG ATG CCA AAG TTG T-3'(24 cycles)	505	
f. forward mining mining mining an			

-f: forward primer, -r: revers primer ( cycles) : RT-PCR cycles used for semiquantitative RT-PCR.