SUPPLEMENTARY METHODS

Isolation, culture, and characterization of rat bone marrow-derived MSCs

MSCs were isolated from the bone marrow from the tibias and femurs of male Lewis rats (100-150g, Charles River) as described previously.[1,2] These cells were characterized as below.

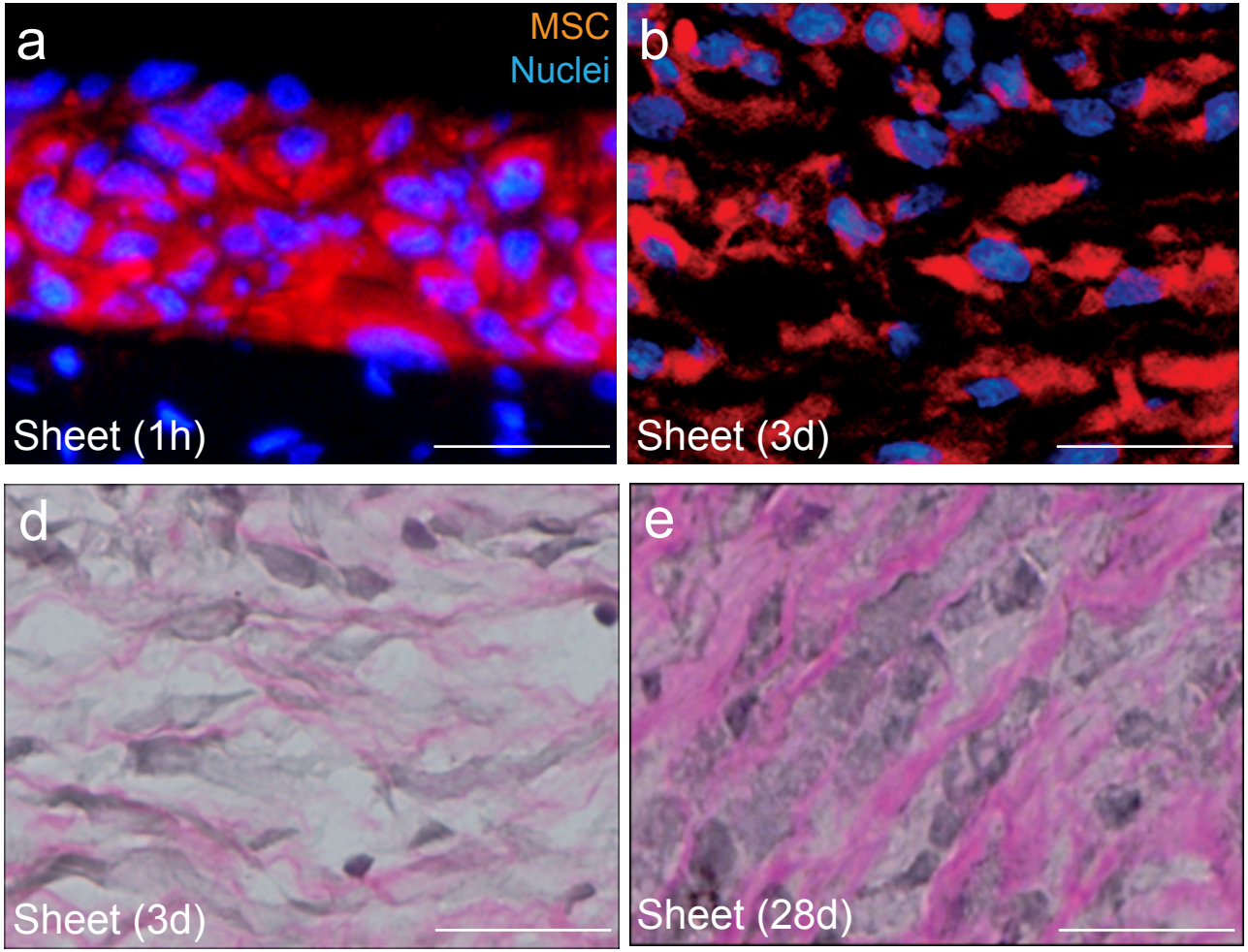
For cell surface marker characterization using flow-cytometry, 5×10^5 MSCs at passage 3-5 were resuspended in 100μL buffer (HBSS with 1% bovine serum albumin and 0.03% sodium azide) after washing. The cells were stained with 1:100 dilution of fluorescein isothiocyanate - or Alexa 647-conjugated anti-CD34 (Santa Cruz), CD45 (Chemicon), CD90 (Abcam), and CD29 (Biolegend) antibodies. Corresponding antibodies were used for negative controls. These cells were analyzed using the FACS Aria II and DIVA (Becton Dickinson).

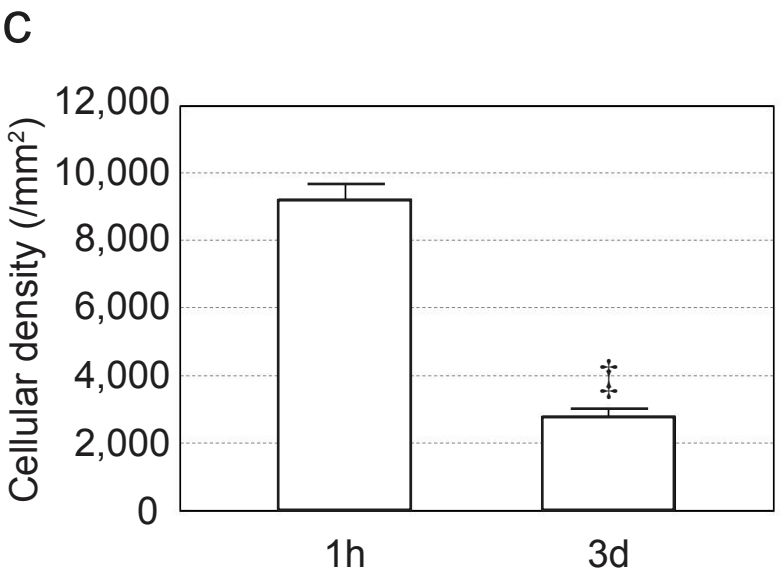
Also, cultured MSCs at passage 3-5 were transferred to 24-well plates and subjected to adipogenic or osteogenic differentiation medium, or proliferation medium as control, as previously described.[1-4] Adipogenic differentiation medium was α-minimal essential medium (α-MEM) supplemented with 100μM isobutyl methylxanthine (Sigma), 60 μM indomethacin (Fluka), 1 μg/ml insulin (Sigma), and 0.5 μM hydrocortisone (Sigma), while osteogenic differentiation medium was α-MEM supplemented with 0.1 μM dexamethasone (Sigma), 10mM β-glycerophosphate (Sigma), and.05 mM ascorbic acid (Sigma). Medium was changed every 2-3 days. After 3 weeks incubation, cells were fixed with 4% paraformaldehyde, and stained with Oil red O (Fluka) for detecting adipocytes containing lipid vacuoles or with Alizarin red (Fluka) to detect osteocytes containing calcium deposits.

SUPPLEMENTARY REFERENCES

1. Hayashi, Y, Tsuji, S, Tsujii, M, Nishida, T, Ishii, S, Iijima, H *et al.* (2008). Topical transplantation of mesenchymal stem cells accelerates gastric ulcer healing in rats. *Am J Physiol Gastrointest Liver Physiol* **294**: G778-786.
2. Nagaya, N, Kangawa, K, Itoh, T, Iwase, T, Murakami, S, Miyahara, Y *et al.* (2005). Transplantation of mesenchymal stem cells improves cardiac function in a rat model of dilated cardiomyopathy. *Circulation* **112**: 1128-1135.
3. Jaiswal, N, Haynesworth, SE, Caplan, AI and Bruder, SP (1997). Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *J Cell Biochem* **64**: 295-312.
4. Rochefort, GY, Delorme, B, Lopez, A, Hérault, O, Bonnet, P, Charbord, P *et al.* (2006). Multipotential mesenchymal stem cells are mobilized into peripheral blood by hypoxia. *Stem Cells* **24**: 2202-2208.

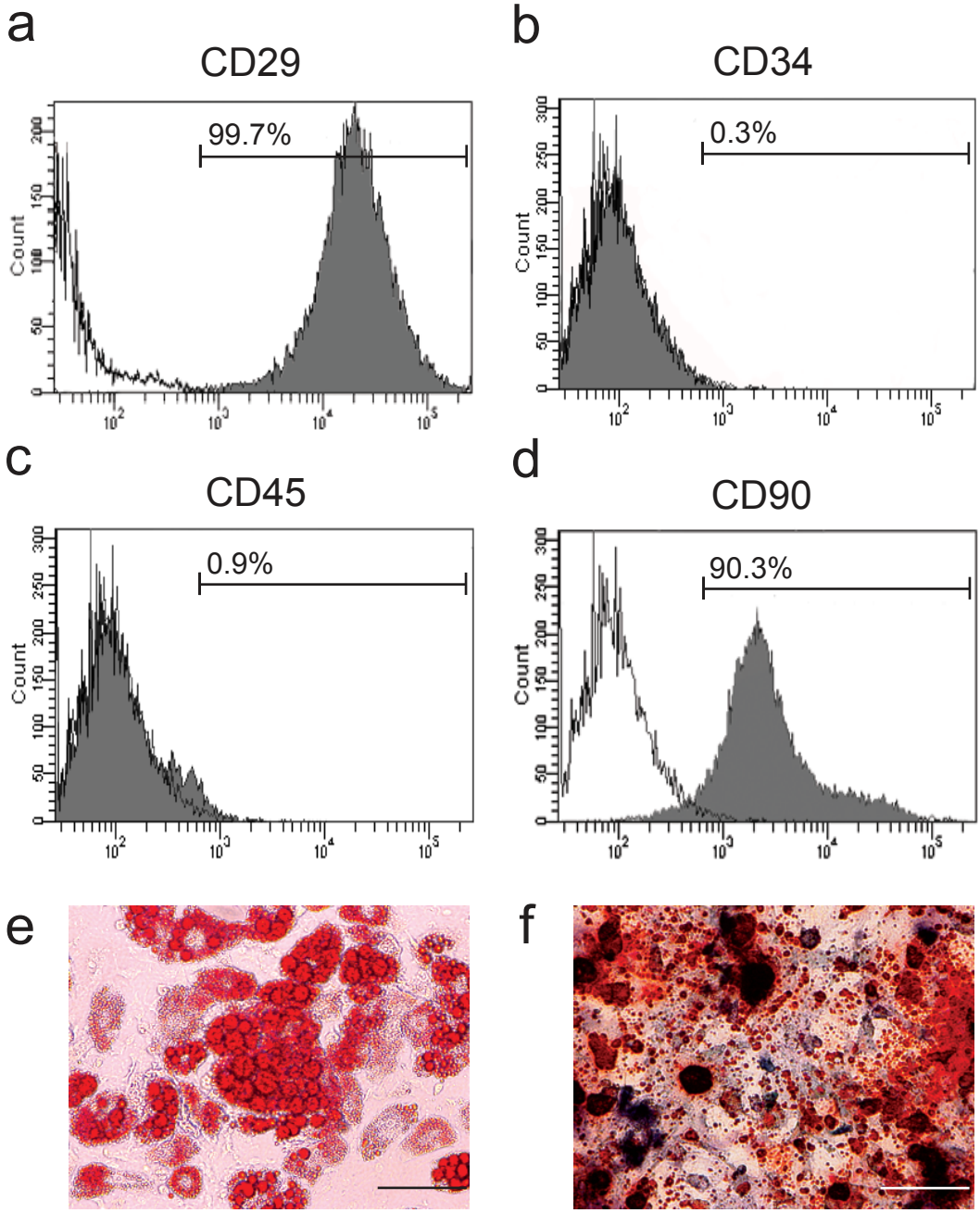
Supplementary Figure S1





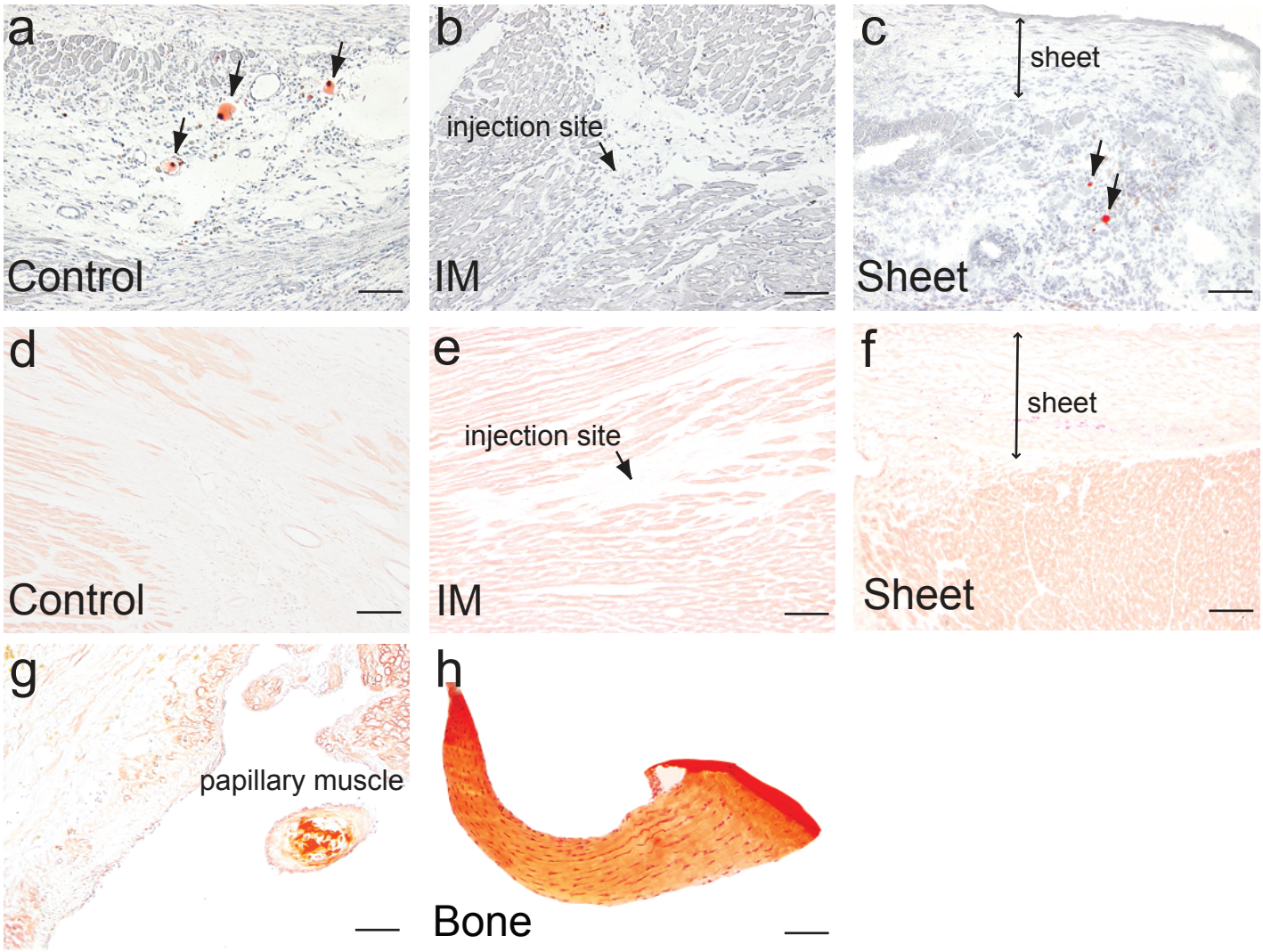
Supplementary Figure S1. Supplementary histological findings to Figure 1 (Less densely packed donor cells in the MSC-sheets after epicardial placement) Representative enlarged images of the MSC-sheets at 1 hour (**a**) and 3 days (**b**) after epicardial placement are shown. Orange signal for MSCs (DiI); blue for nuclei (DAPI). Note that donor MSCs (orange) were much more compacted with a significantly higher cell density at 1 hour compared to 3 days (**c**). ‡$p<0.05$ versus 1 hour, mean±SEM for n=5 in each group. Picrosirius red and hematoxylin staining detected extracellular collagen deposition in the MSC-sheets at day 3 (**d**), with an increasing tendency at day 28 (**e**). Scale bar = 30 mm in all images.

Supplementary Figure S2



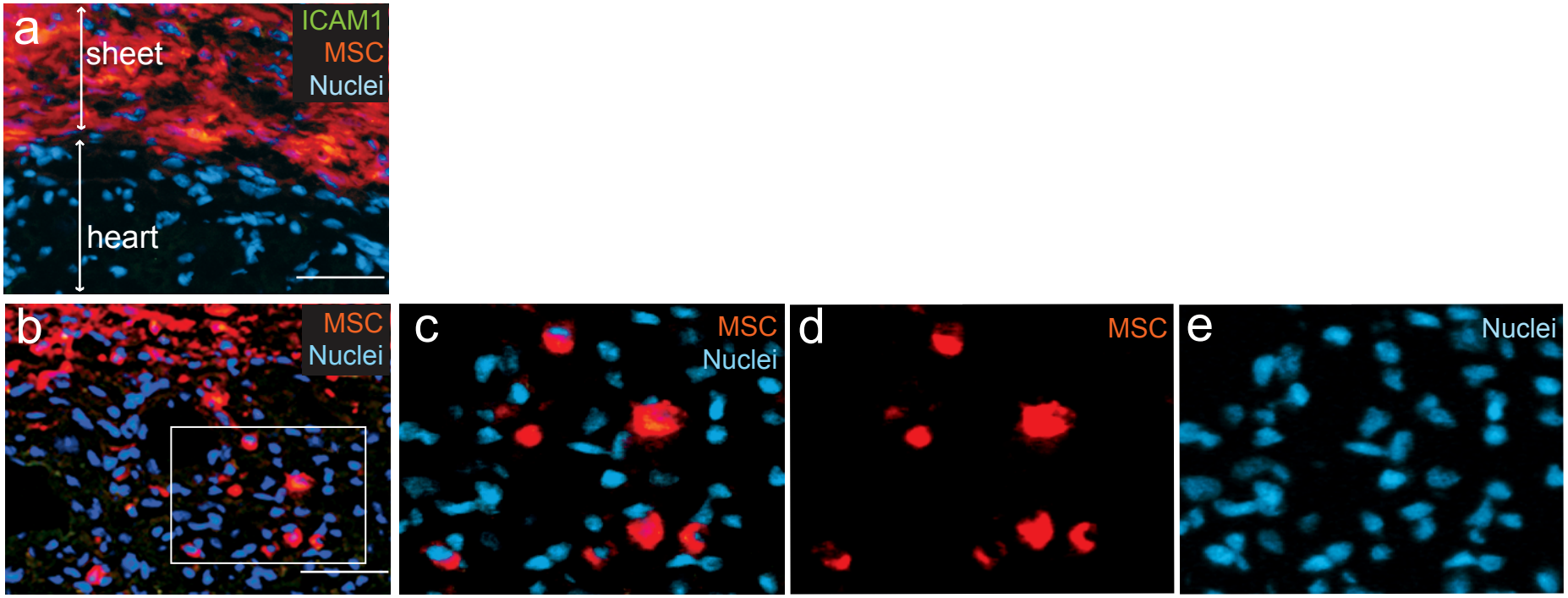
Supplementary Figure S2. In vitro characterization of rat bone marrow derived-MSCs
Flow-cytometric analyses showed that collected MSCs from rat bone marrow were mostly positive for CD29 (**a**) and CD90 (**d**) and negative for CD34 (**b**) and CD45 (**c**). A representative histogram is shown for each. After stimulation, MSCs were driven to Oil red O-positive adipocytes (**e**) or Alizarin red-positive osteocytes (**f**), demonstrating the mesenchymal multipotency of the MSCs. Scale bar = 100 μm in **e**, **f**.

Supplementary Figure S3



Supplementary Figure S3. Adipogenic and osteogenic differentiation of MSCs in vivo
Oil red O staining detected the existence of adipocytes (red signal; black arrows) in the infarcted myocardium (**a** for the Control group, **c** for the Sheet group). However, there were no such Oil red O positive adipocytes in the injection sites in the IM group (**b**) or within the MSC-sheets (**c**). Alizarin red staining showed the calcium deposition (dark orange) in the phalanx bone (positive control; **h**) and occasionally in the infarct areas (infracted papillary muscle; **g**). However, no such signal was observed in the border areas in the Control group (**d**), around injection sites of the IM group (**e**) or in the MSC-sheets in the Sheet group (**f**). Scale bar = 100 μm.

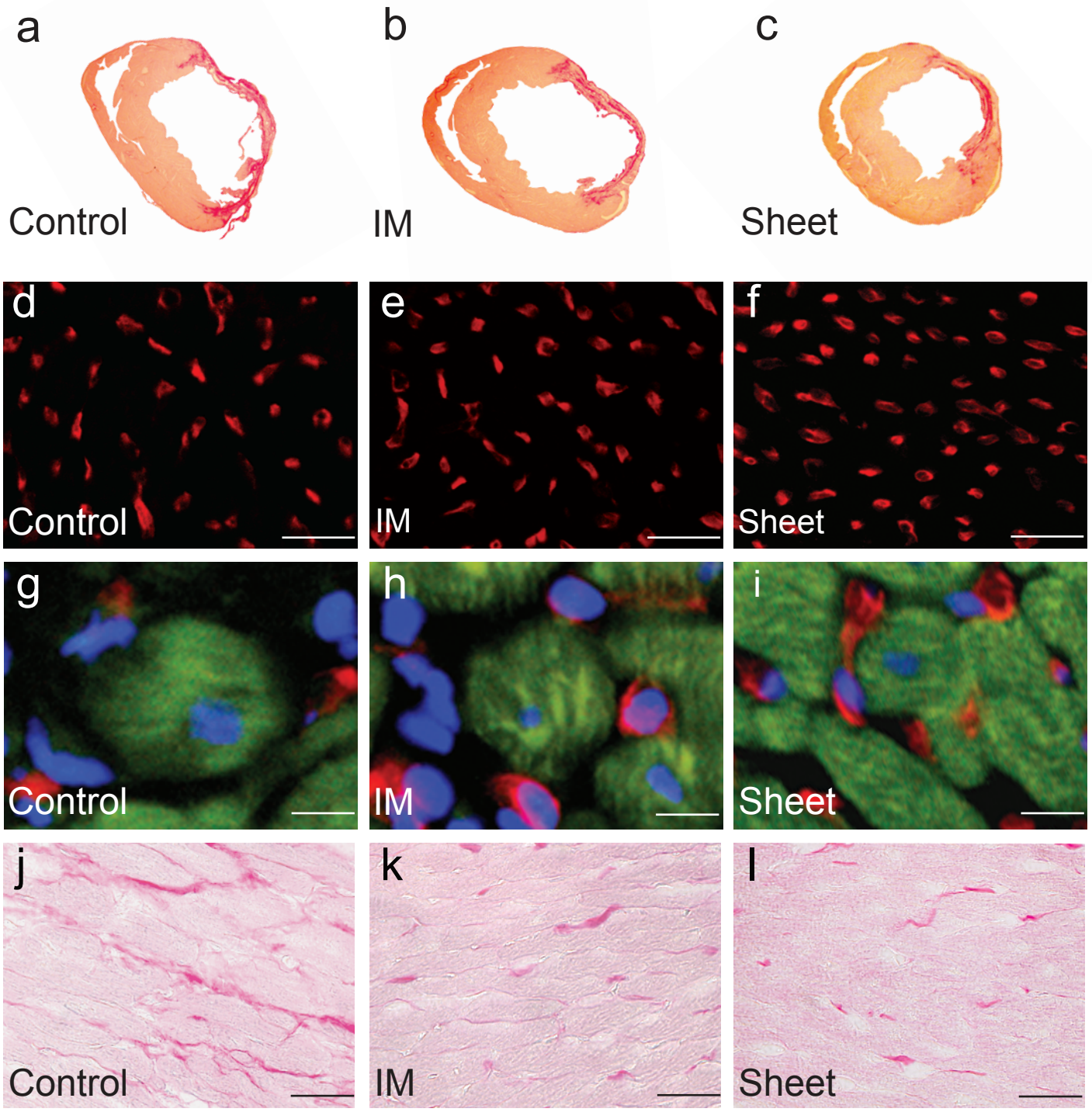
Supplementary Figure S4



Supplementary Figure S4. Supplementary histological findings to Figure 3 (Donor cell behaviors and changes in the epicardium after MSC-sheet therapy)

ICAM1 staining demonstrated that the epicardium continued to be absent until day 28 (**a**) after MSC-sheet therapy (refer to **Figure 3e-f**). There were occasionally detectable numbers of MSCs that had migrated into the host myocardium (**b** = **Figure 3h**). Enlarged images of white-gated area in **b** are presented here with **c-e** for each marker separate and merged, showing that DiI+ structures were nucleated cells and not debris. Green signal for ICAM-1 in **a** (; not observed); orange for MSCs (DiI); blue for nuclei (DAPI). Scale bar = 50 μm.

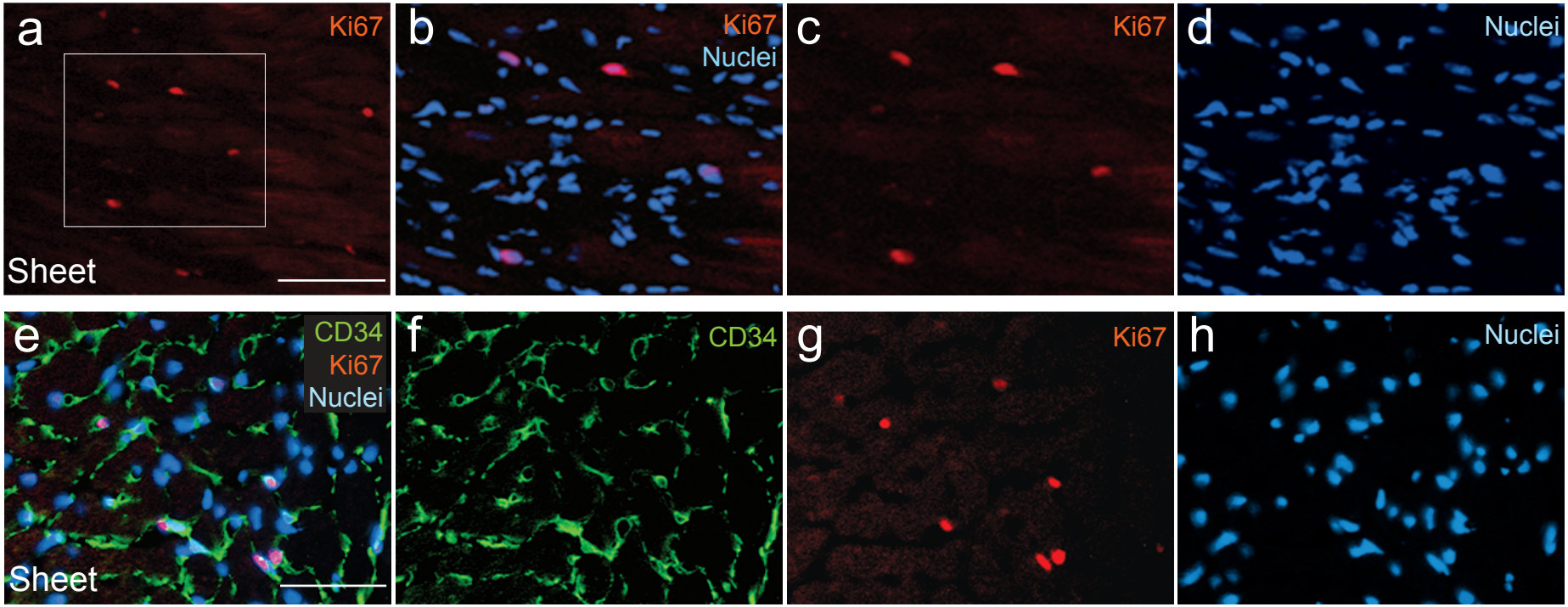
Supplementary Figure S5



Supplementary Figure S5. Supplementary histological findings to Figure 4 (Histological recovery of post-MI failing cardiac tissues by MSC-sheet therapy)

Representative microscopic images used for the calculated data in **Figure 4a-d** are presented here. Picrosirius red staining showed that the infarct size in the IM group (**b**) was reduced at day 28 compared to the Control group (**a**). Reduction of infarct size was further augmented in the Sheet group (**c**). Isolectin B4 staining (red) showed an increase in capillary density in the border area in the IM group (**e**) compared to the Control group (**d**) at day 28. Capillary density was further increased in the Sheet group (**f**). Cardiomyocyte size (cross sectional area) was smaller in the Sheet group (**i**) compared to the IM group (**h**) and Control group (**g**) at day 28. Extracellular collagen deposition (red) at the border area surrounding infarct assessed by picrosirius red staining at day 28 was reduced in the IM group (**k**) and the Sheet group (**l**) compared to the Control group (**j**). Red signal for isolectin B4; blue for nuclei (DAPI); green for cardiomyocytes. Scale bar = 30 μm in **d-f** and **j-l** and 10 μm in **g-i**.

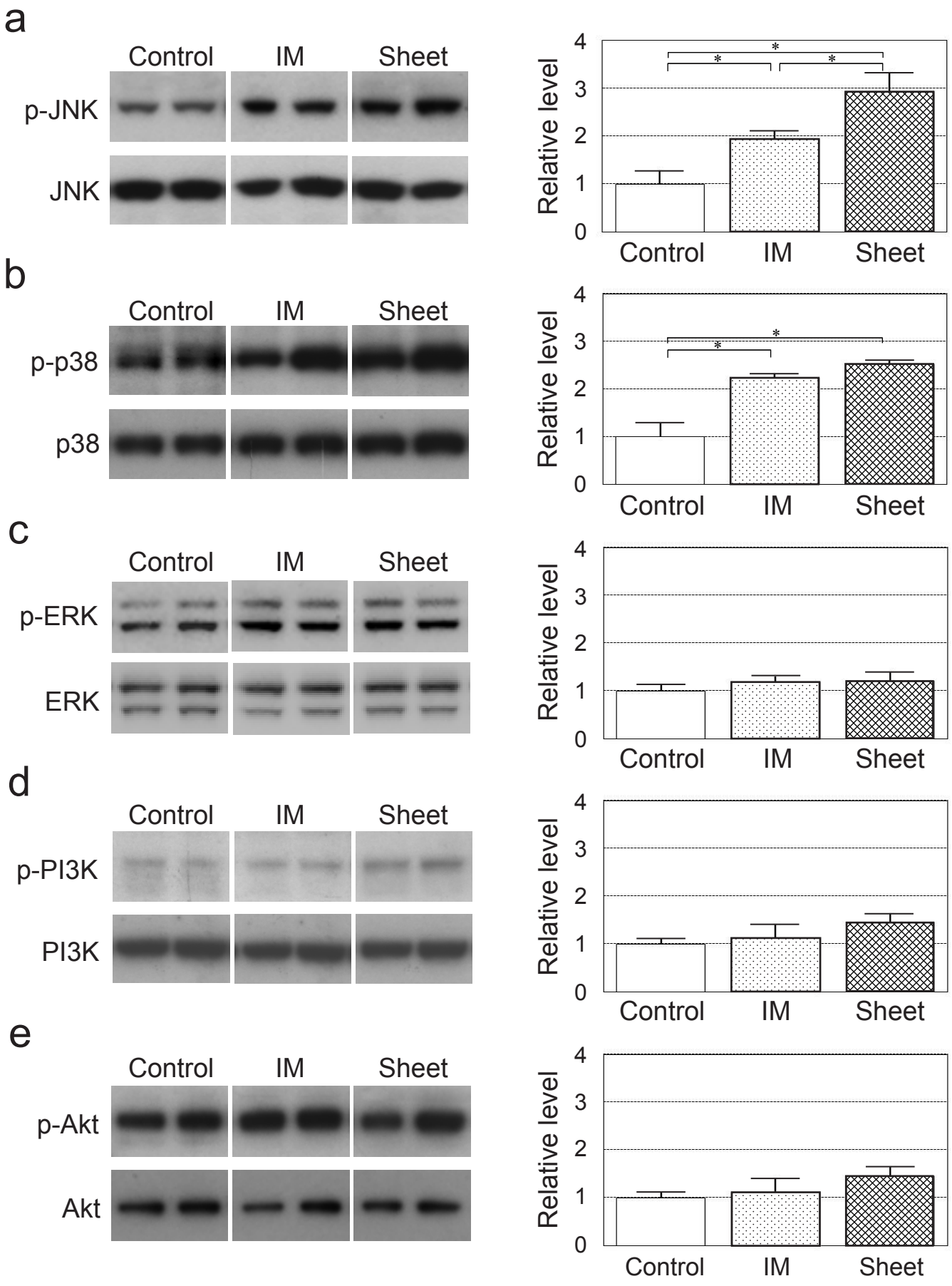
Supplementary Figure S6



Supplementary Figure S6. Supplementary histological findings to Figure 5 and Table 2 (Improved endogenous regeneration activity by MSC-sheet therapy)

The number of Ki67+ cells in the heart was increased in the Sheet group (refer to **Figure 5** and **Table 2**). Enlarged images of white-gated area in (**a** = **Figure 5c**) are presented here in **b-d** with each individual marker and merged image. Representative images of Ki67+/CD34+ cells in the border area at day 28 in the Sheet group are presented (**e-h**). Green signal for CD34 (**e-h**); orange for Ki67; blue for nuclei (DAPI). Scale bar = 30 μm (**a-d**), 50 μm (**e-h**).

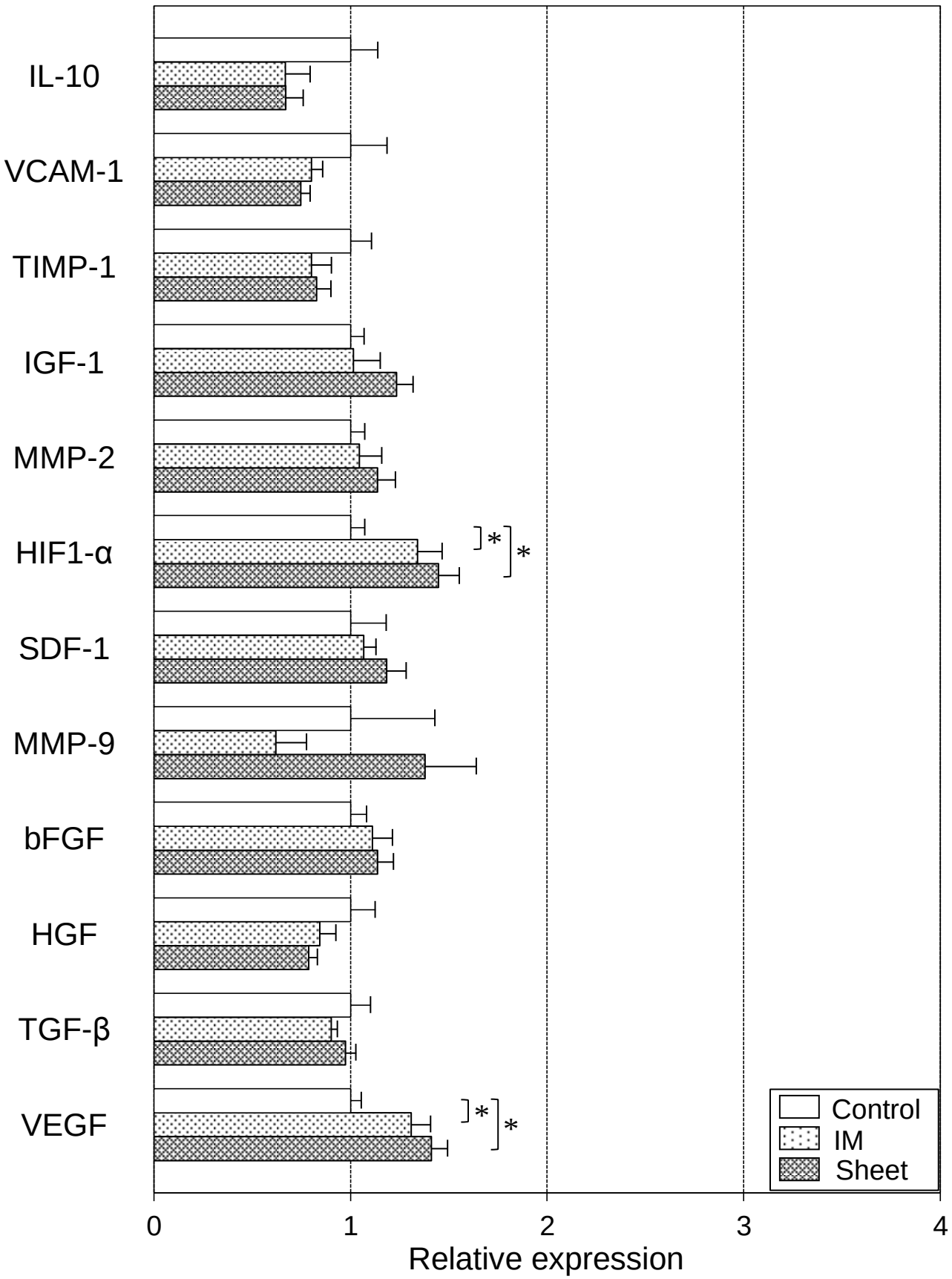
Supplementary Figure S7



Supplementary Figure S7. Elevated activation of paracrine effect-related signal pathways by MSC-sheet therapy

Western blotting demonstrated that phosphorylation of JNK and p38 MAPK, but not ERK1/2, PI3K, or Akt, was significantly increased in the Sheet group at day 3 compared to other groups. All expression levels were normalized to that in the Control group, which was assigned a value of 1.0. *$p<0.05$, mean±SEM for n=4-6 in each group.

Supplementary Figure S8



Supplementary Figure S8. Supplementary data to Figure 6 (Myocardial gene expression at day 28 after MSC-sheet therapy)

Quantitative RT-PCR showed that myocardial upregulation of most molecules observed at day 3 in the Sheet group (**Figure 6**) was diminished by day 28. All expression levels were normalized to that in the Control group, which was assigned a value of 1.0. *$p<0.05$, mean ± SEM for n=5-7 in each group.