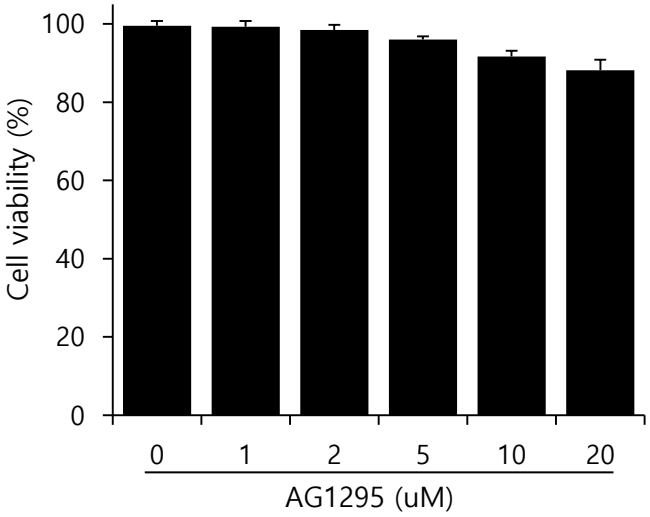
Supplementary materials

12 Figure, 1 table

Substance P enhances the therapeutic effect of MSCs by modulating their angiogenic potential

Hyun Sook Hong, Suna Kim, Yinji Jin, Yongsook Son

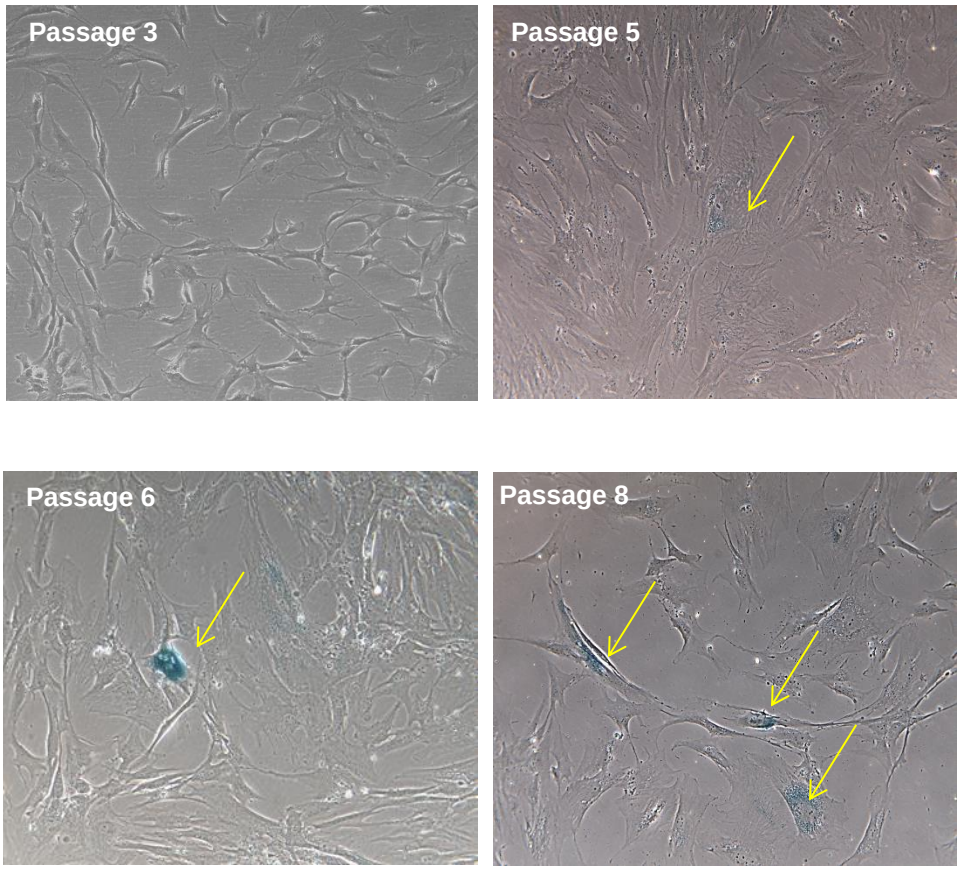
Supplementary Figure 1



Supplementary Figure 1. The evaluation of safety of AG1295 on MSCs

AG1295 was treated to MSC in several doses to check the effect of AG1295 on cell viability, The data are expressed as the mean ± S.D.

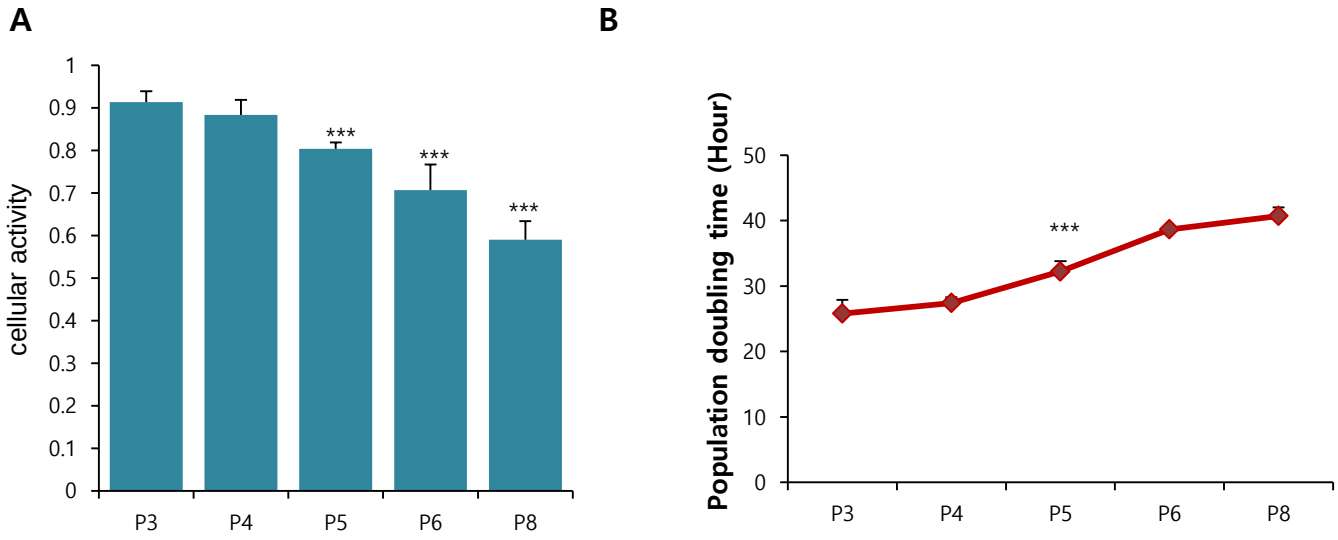
Supplementary Figure 2



Supplementary Figure 2. β-galactosidase staining of BM MSC in vitro

MSCs were cultured in vitro and passage 3, 5, 6 and 8 of MSC were stained with beta-galactosidase to assess cellular senescence. Yellow arrow: Beta galactosidase+ cells

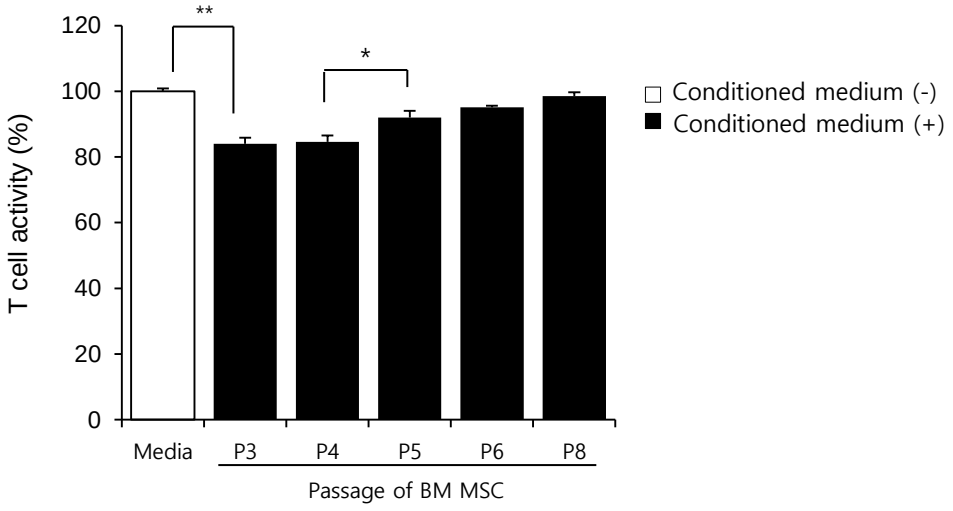
Supplementary Figure 3



Supplementary Figure 3. Population doubling time of BM MSC

MSCs were cultured in vitro and analyzed for cellular activity and population doubling time. (A) WST-1 assay was performed. (B) population doubling time was analyzed from p3 to p8 BM MSC. Values of $p < 0.05$ were interpreted as statistically significant (***$p < 0.001$, vs P3). The data are expressed as the mean ± S.D of triplicate experiments.

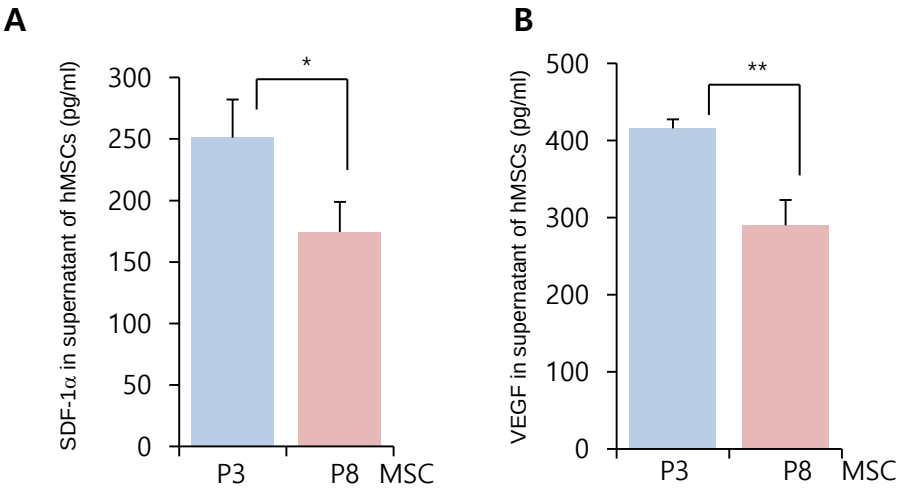
Supplementary Figure 4



Supplementary Figure 4. The immune suppressive effect of MSC on T cells

MSCs were cultured in vitro and conditioned medium of MSC (p3-8) was added to Jurkat T cells. 24h later, WST-1 assay was carried out. T cell activity was represented as a percentage relative to activity of T cells with cell-free media. Values of $p < 0.05$ were interpreted as statistically significant (*$p < 0.05$, **$p < 0.01$). The data are expressed as the mean ± S.D of triplicate experiments

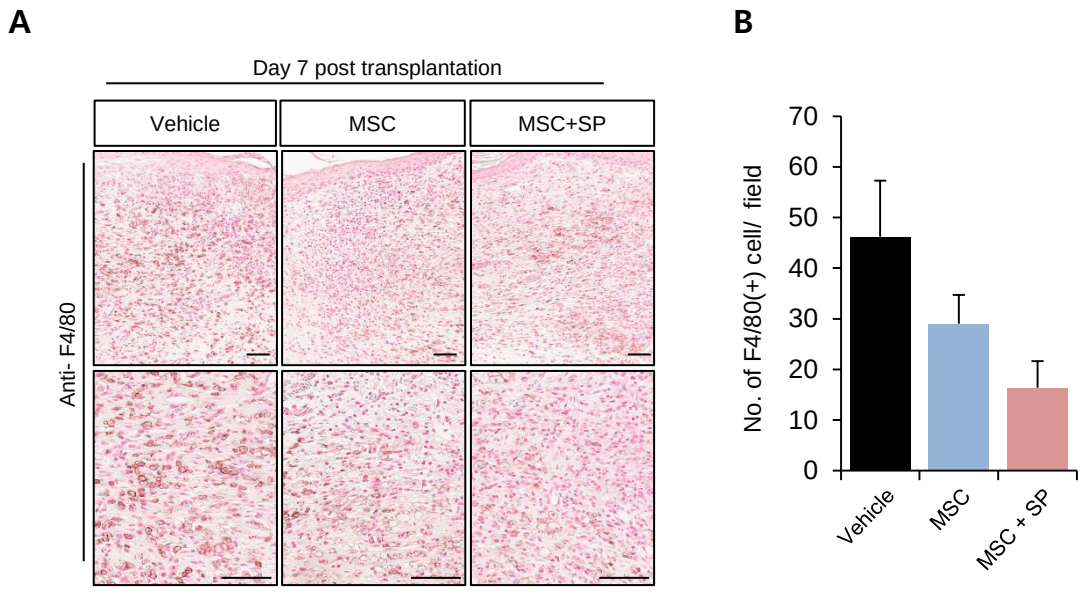
Supplementary Figure 5



Supplementary Figure 5. Ex vivo culture affects production of paracrine factor in MSCs

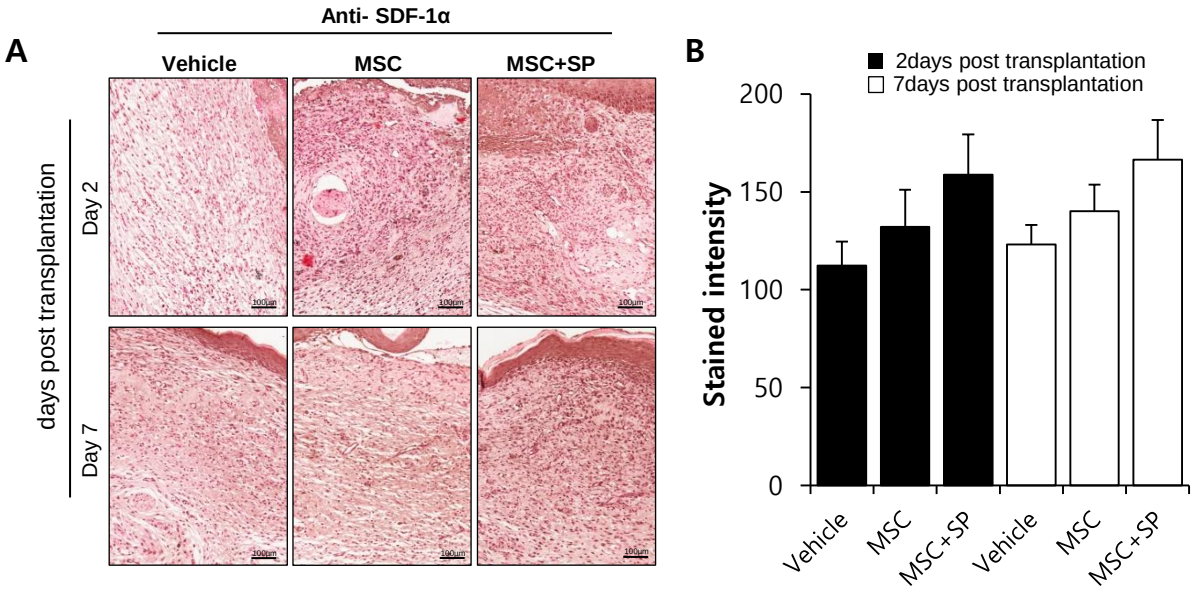
MSCs were cultured in vitro and passage 3 and 8 of MSC were evaluated for secretion of SDF-α (A) and VEGF (B). Values of $p < 0.05$ were interpreted as statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The data are expressed as the mean ± S.D of triplicate experiments

Supplementary Figure 6



Supplementary Figure 6. SP improves MSC-induced suppression of inflammation
Histological analysis of wounded tissue at day 7 post-wound was performed. (A) Samples of 4-μm thickness were stained for F4/80 (+) macrophages at the wound bed. (B) F4/80 + cells were counted.

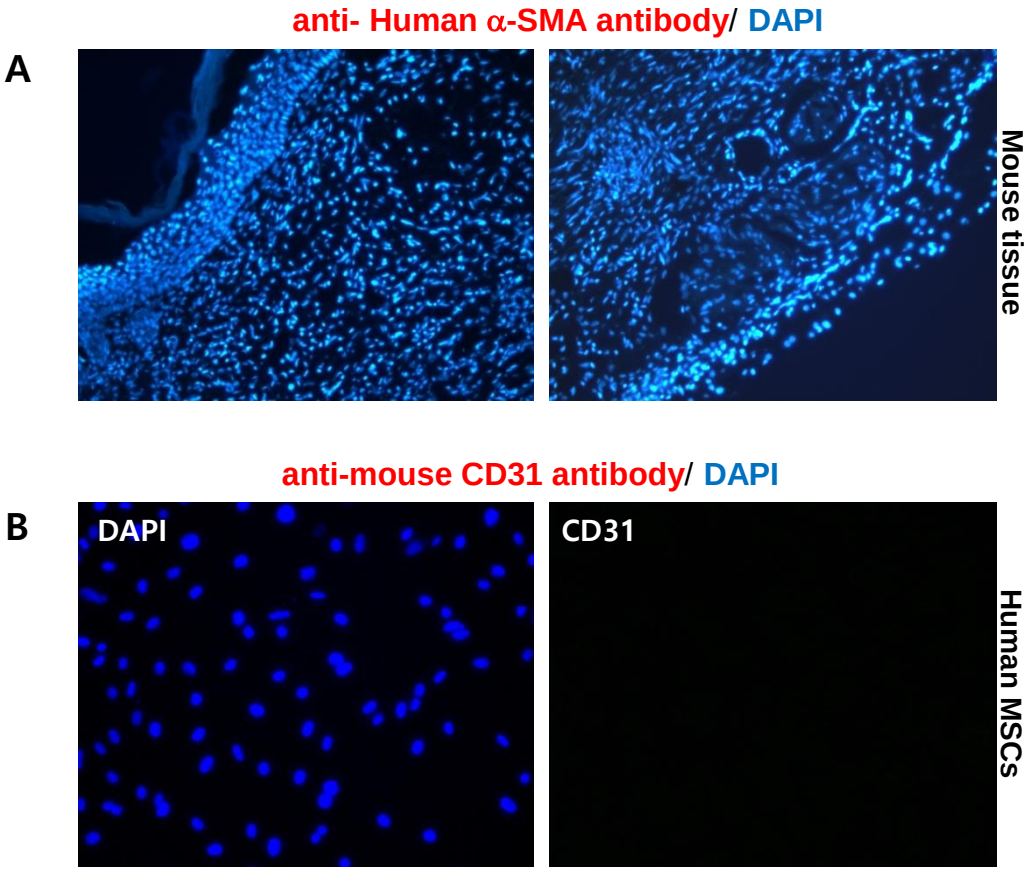
Supplementary Figure 7



Supplementary Figure 7. Immunohistochemical staining for SDF-1α at wound site.

Histological analysis for wounded tissue was performed. The secretion of SDF-1 was detected at wound site at day 2 and 7 post MSC transplantation. n=6 for each group. Scale bar: 100 μm

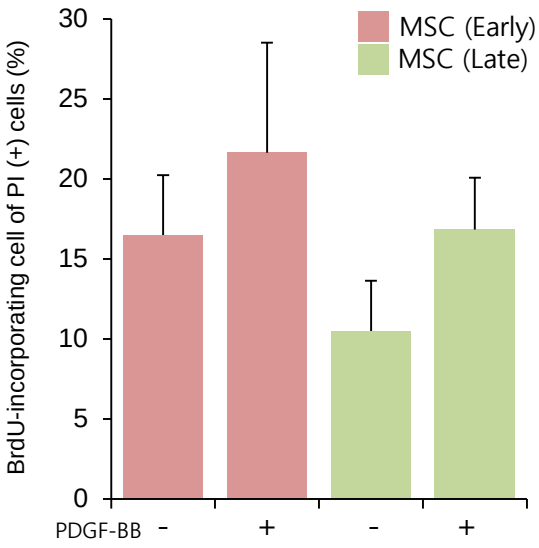
Supplementary Figure 8



Supplementary Figure 8. Examination for the reactivity of species-specific antibody.

(A) The reactivity of human α-SMA antibody on mouse tissue was examined. No activity of α-SMA antibody was detected. (B) Human MSCs was stained with anti-mouse CD31 antibody.

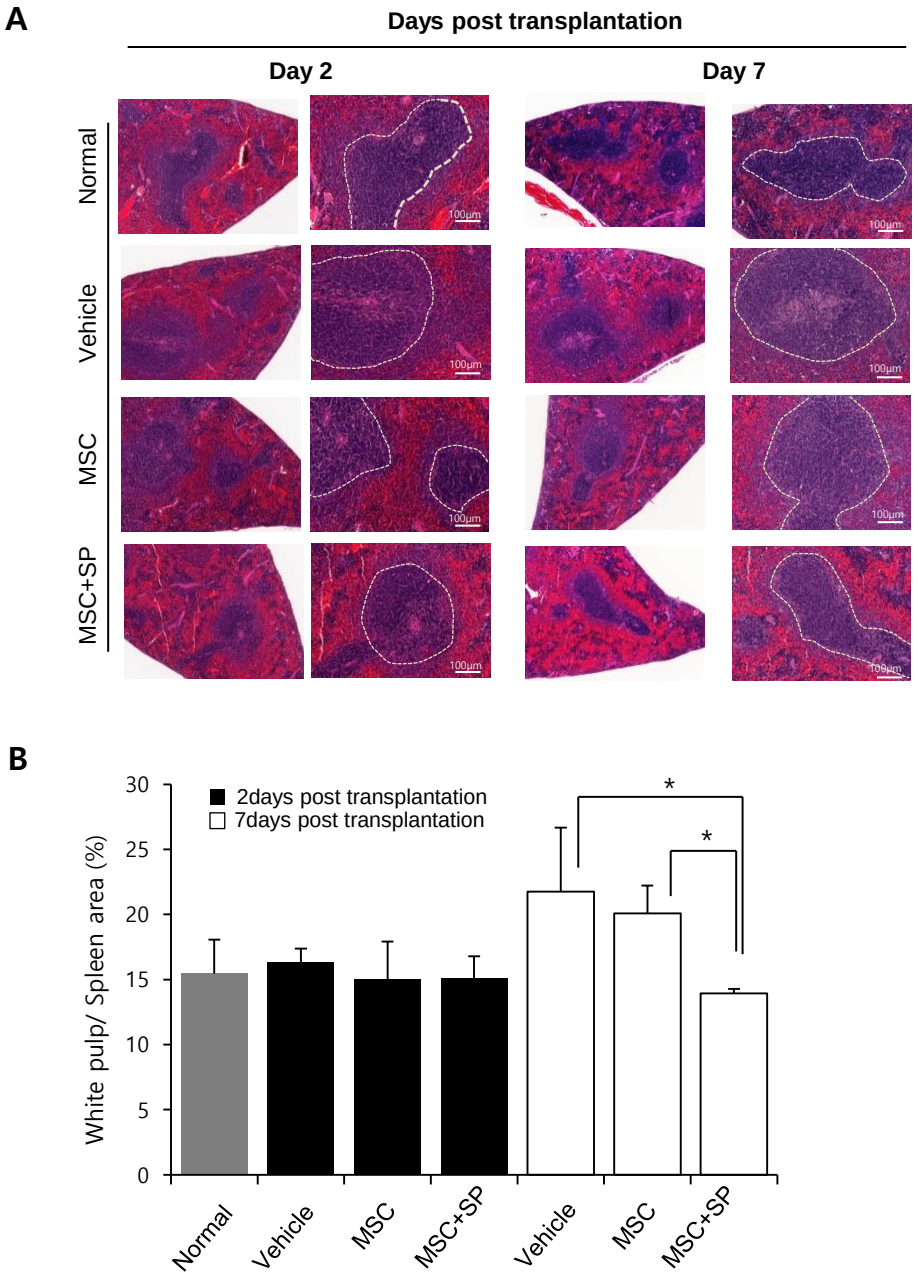
Supplementary Figure 9



Supplementary Figure 9. The effect of PDGF-BB on MSC proliferation in vitro.

PDGF-BB (20ng/ml) was added to MSC at early and late passage and then 24h later, BrdU incorporation assay was performed to determine the proliferating cell pool. BrdU was treated for 3 h before cell fixation.
BrdU (+) cells was quantified by counting BrdU (+) cell of total PI (+) cells and expressing the percentage.

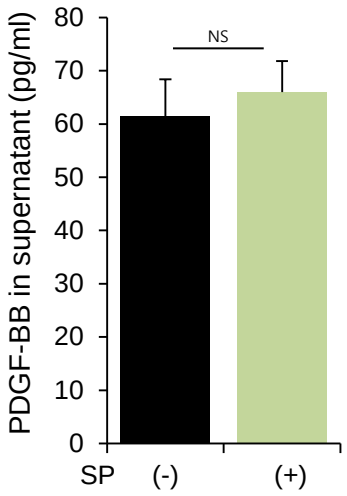
Supplementary Figure 10



Supplementary Figure 10. The histological analysis for spleen

Histological analysis for spleen was performed. (A) Hematoxylin and eosin staining was carried out at wound site at day 2 and 7 post MSC transplantation. White dotted line: white pulp area. Scale bar: 100 μm (B) Quantitative analysis for white pulp of total spleen area. Values of $p < 0.05$ were interpreted as statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The data are expressed as the mean ± S.D.

Supplementary Figure 11

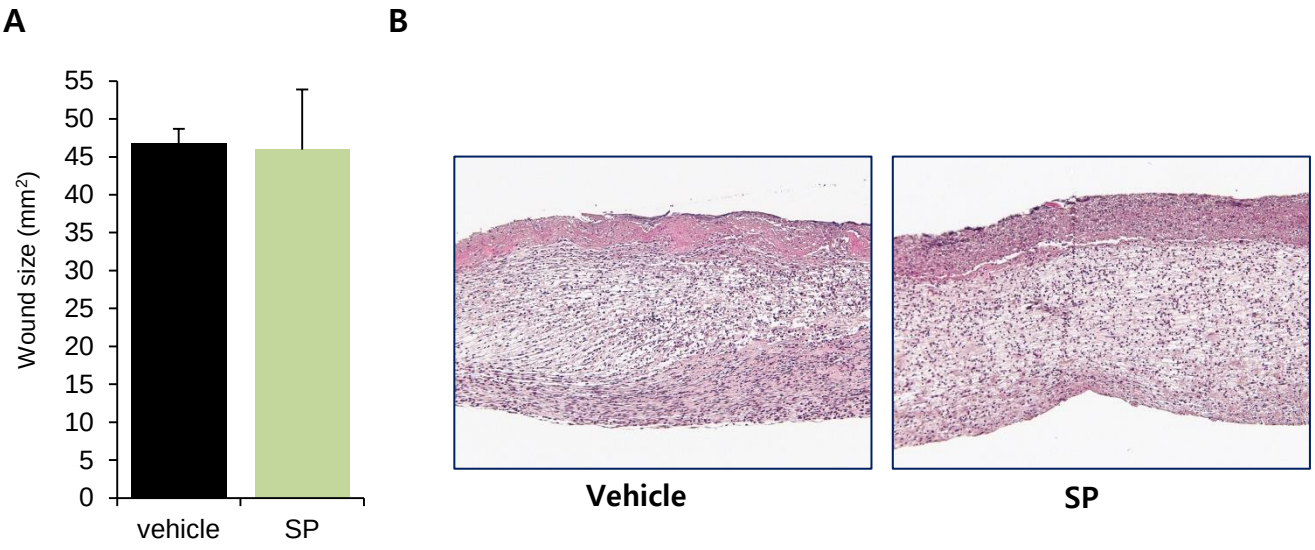


Supplementary Figure 11. The effect of SP on PDGF-BB secretion in mouse macrophage *in vitro*

Raw 264.7, monocyte macrophage cell line, was incubated in 24 well with LPS and then, SP was added in concentration of final 100nM. 24h later, supernatant was collected and PDGF-BB was quantified by ELISA.

NS: Non significant

Supplementary Figure 12



Supplementary Figure 12. The effect of SP on skin wound

To check the effect of SP on skin wound, SP was locally treated to wound site. 2 days later, wound size (A) and skin tissue was analyzed by H&E staining (B)

Supplementary table 1

Passage no.	3	8
Marker	Percentage of expression (%)	
CD29	98. 2 ± 0.01	98.3 ± 1.5
CD73	82.3 ± 0.09	79.4 ± 2.4
CD105	94.2 ± 3.4	98.8 ± 1.5
CD34	0.70 ± 0.01	0.8 ± 0.08
CD90	89.7 ± 0.09	88.7 ± 0.4

Supplementary table 1. Characterization of human MSC

Human MSC (hMSC, 2×106, Passage number 3 and 8) were analyzed by flow cytometry analysis. For the detection of MSC surface makers, cells were treated with FITC-conjugated antibodies against CD29, CD73, CD105, CD34, and CD90 and analyzed with FACs.