

A Novel S100A8/A9 Induced Fingerprint of Mesenchymal Stem Cells associated with Enhanced Wound Healing

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Short Title: The S100A8/9 Fingerprint of Mesenchymal Stem Cells

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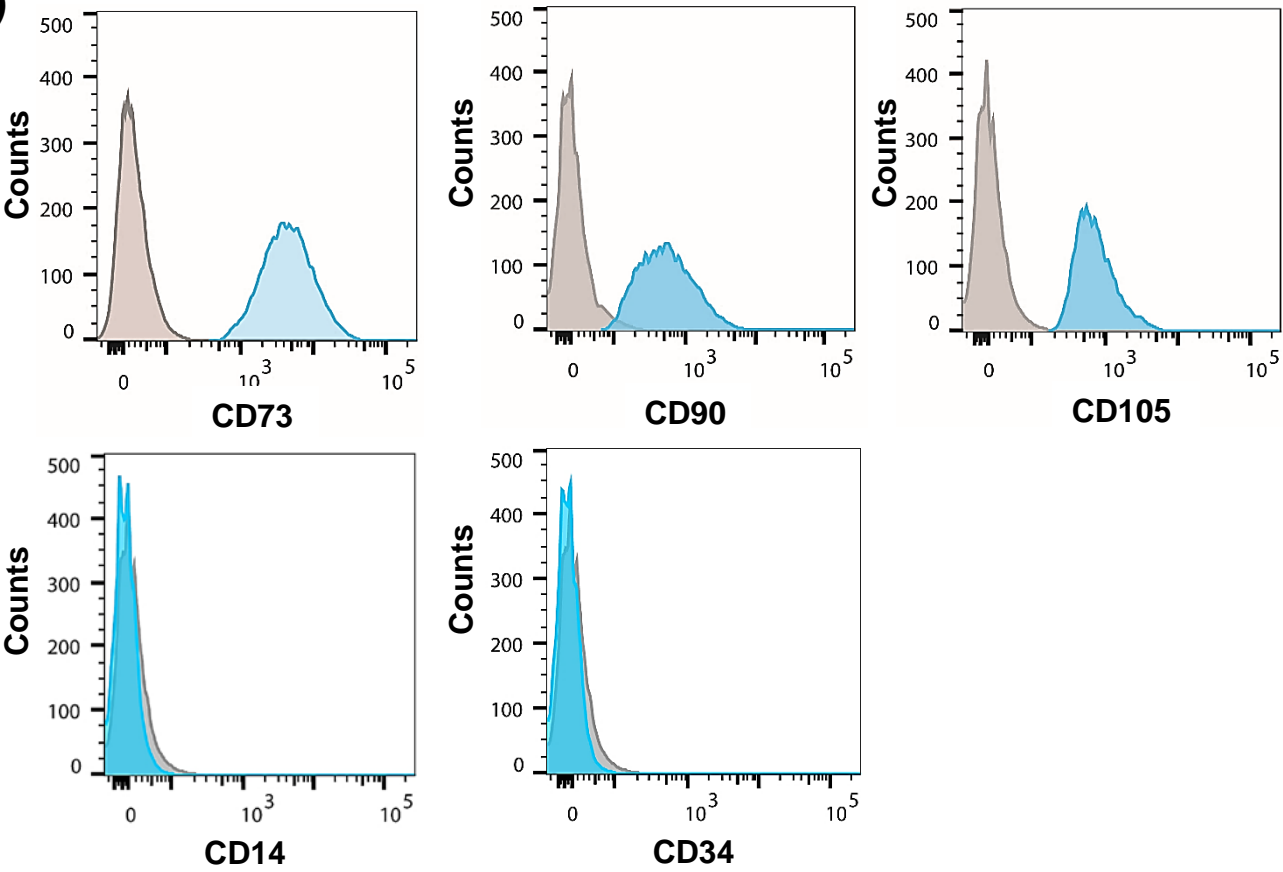
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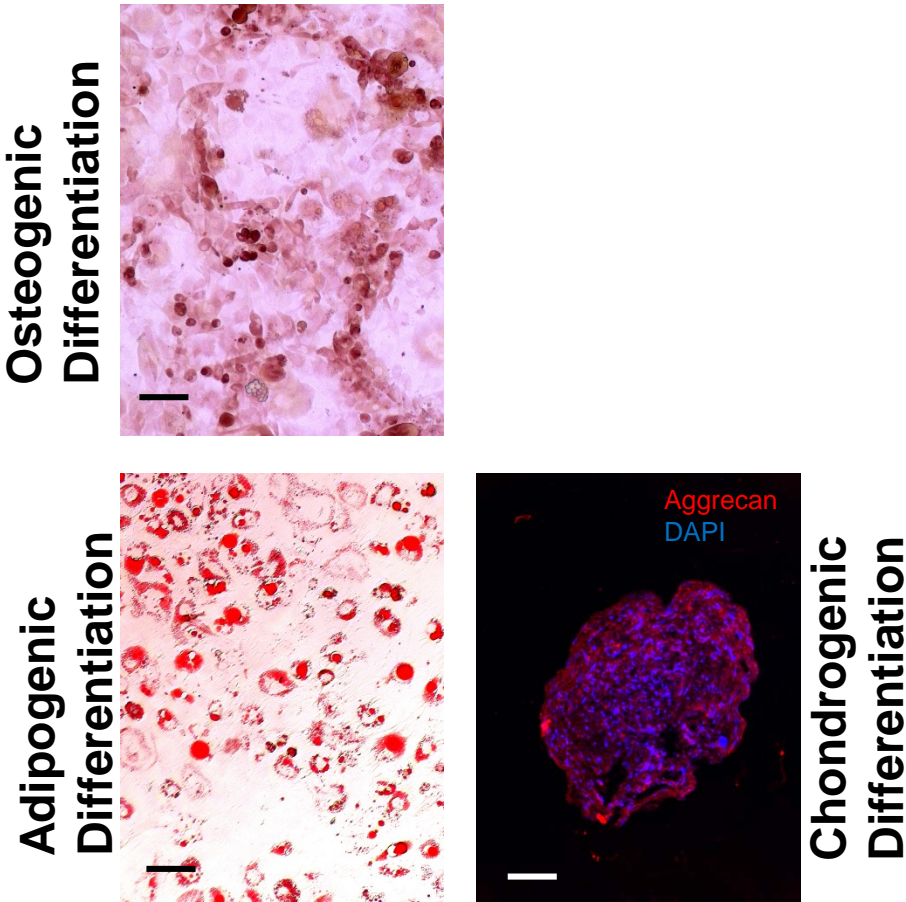
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Supplementary Figure. 1

(a)

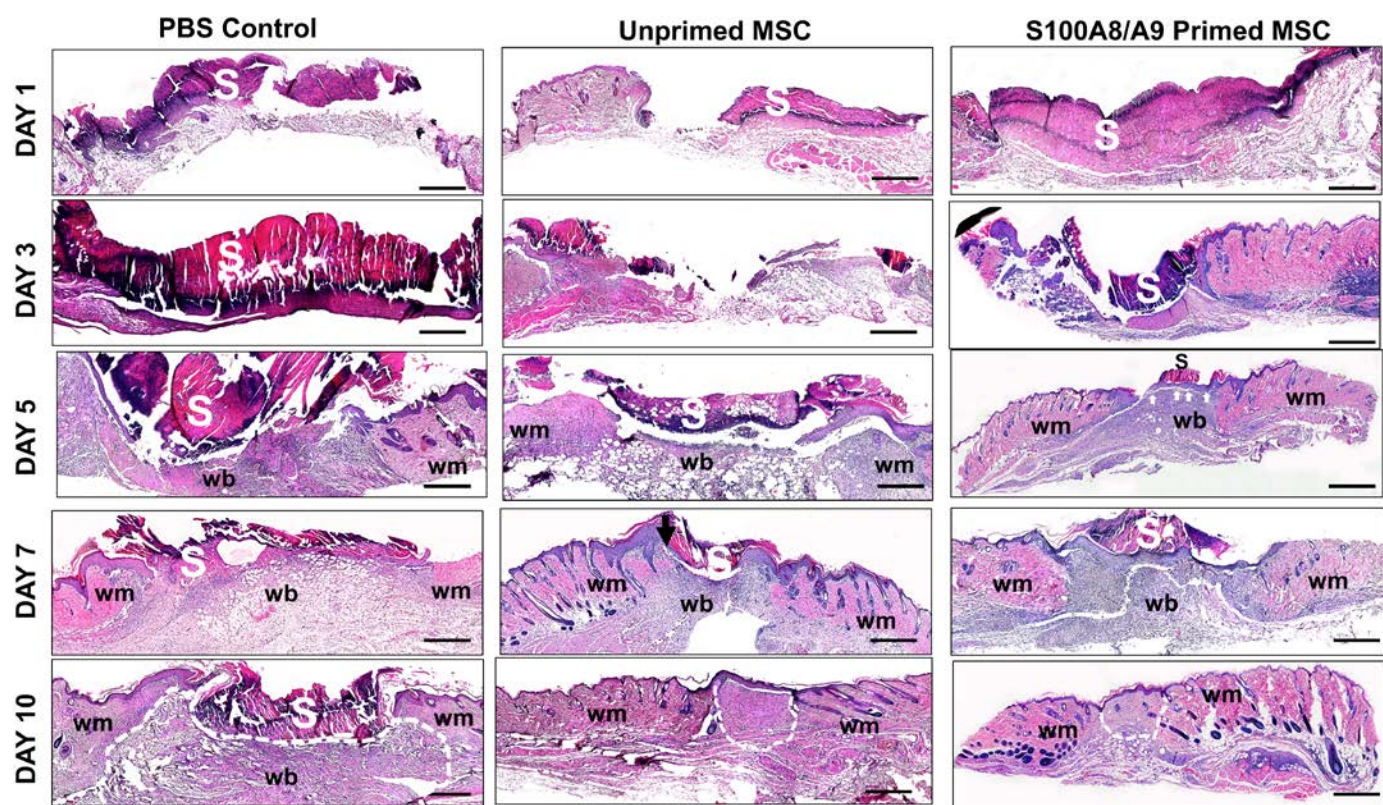


(b)



Supplementary Figure. 1 (a) Expression of surface markers of AT-MSC was analysed by flow cytometry, including CD73, CD90, CD105, CD14 and CD34. Stem cell specific surface marker expression on MSCs were highlighted by blue histograms and the isotype control stained MSCs were highlighted by grey histograms. (b) 10^5 MSCs were seeded and were cultured in adipogenic induction, osteogenic induction and chondrogenic induction medium for 21 days. The cells were fixed with PFA and stained with Oil-Red-O staining indicative for adipogenic differentiation and with Alizarin-Red-S for osteogenic differentiation. An antibody against human aggrecan was used to stain for MSCs with chondrogenic differentiation. Scale bar 200 μ M

Supplementary Figure 2.

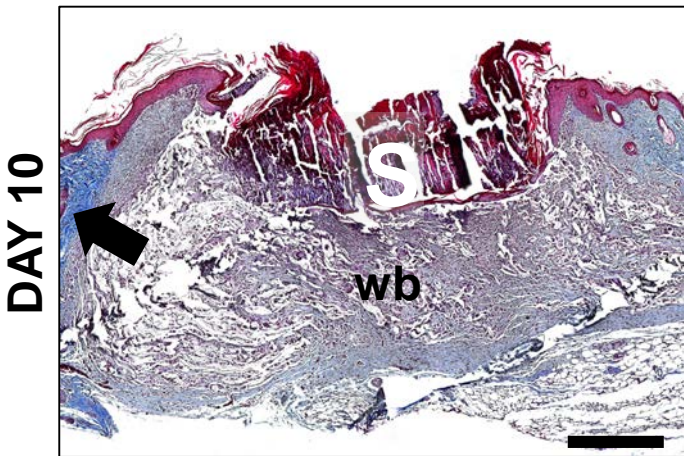


Supplementary Figure 2. Injection S00A8/A9 of primed MSCs into wounds result in acceleration of wound healing.

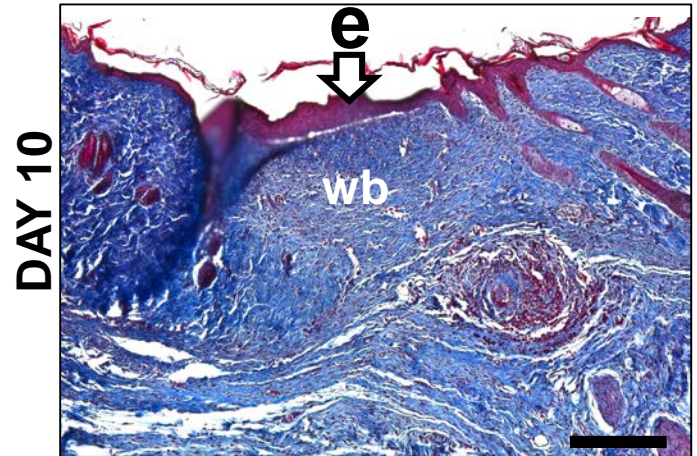
Representative microphotographs depict H&E staining of PBS injected wounds, MSC injected wounds and wounds injected with S100A8/A9 primed MSCs at different time points after wounding. Of note, there is reduced scab formation (S) in MSC injected wounds, and even less in wounds injected with S100A8/A9 primed wounds. Re-epithelialisation occurs already at day 5 in wounds injected with S100A8/A9 primed MSCs (white arrows), while MSC injected wounds show re-epithelialisation only at day 10. No re-epithelialisation was observed in PBS injected control wounds at day 10 after wounding. Wound area at day 10 is very large in PBS injected wounds (stippled line) as compared to MSC injected wounds and S100A8/A9 primed MSCs injected into wounds. S Scab, wb wound bed, wm wound margin. Scale bars: 200 μ m.

Supplementary Figure 3.

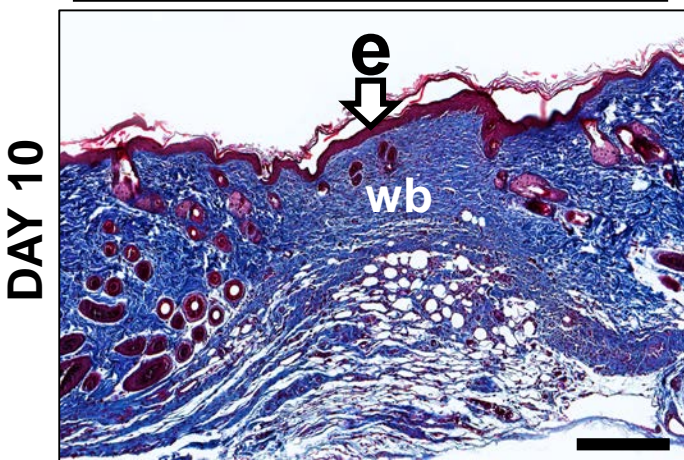
PBS injected control



MSC injected control



S100 primed MSCs



Supplementary Figure 3. Injection of S100 primed MSCs into wounds reveals a faster deposition of collagen mimicking unwounded skin.

Representative photomicrographs depict Trichrome stained sections of full thickness wounds at day 10 after wounding. Note that the PBS injected control wound (left top panel) do not depict blue staining in the wound bed (wb) as compared to the blue staining (collagen deposition) of the wound margin (↑). This indicates that there is almost no collagen deposition in the wound bed. By contrast, at day 10 after wounding collagen deposition already occurred in MSC injected wounds (right top panel) and in wounds injected with S100A8/A9 primed MSCs (lower left panel). Of note, wounds injected with S100A8/A9 primed MSCs depict a collagen deposition which is in a wavy basket weaver structure similar to unwounded skin of the wound margin. By contrast, unprimed MSC injected wounds show a cell rich dense collagen which in its structure is still distinct from that of unwounded skin at 10 days after wounding. Please note that there is still severe scab formation (S) without any reepithelisation in PBS injected control wounds as opposed to no scab formation in MSC and S100 A8/A9 primed MSCs which were injected into wounds. While MSCs injected wounds still depict a thickening of the epidermis, the reepithelised epidermis in S100A8/A9 primed MSCs which had been injected into wounds have an almost normal thickness of the epidermis. S scab, e epidermis, wb wound bed. Scale bars: 20µm.

Supplementary Tab. 1

Gene	5' End	3' End
<i>MMP27</i>	GCCAATTCATGCCACGTCTC	AAGCCGGTTCTGTTAGCACA
<i>DSCC2</i>	CATCTGCGGAGATTGTTGCG	AAAGTCAAAGGGTGGGCCAT
<i>SPOCK2</i>	GTGAGAGGTTTGGGGAGGTG	TCCATGGAGTTTCACGGTCG
<i>PRSS36</i>	TCCGCTGCTCACTGTTTCAT	CAGGACCAGGGCGAATACTC
<i>ICOSLG</i>	CATTGGCTGCTGCATAGA	GGATTCTCTGTGATCTTGTCTC
<i>CCR7</i>	TGTGGGCATCTGGATACT	CCACCTGGATGGTGATAAAG
<i>OIT3</i>	CCTAGATCCTTGTTCTGCTTAC	CATGGTTGTCACATAGAGGAG
<i>IL-32</i>	CTTTCTGAGTGTCACCGTTATT	AGAAGTAGGGAGGAGCATTAC
<i>SERPINA9</i>	CCACCACCAAGTTCATAGTC	ATACCGTCTGTGGCTTTATTT
<i>PI15</i>	GACCATGGACCTTCTTACTTAC	CTTGACCAACTGGAGAATAGAG
<i>β-Actin</i>	TGAGAGGGAAATCGTGCGTG	TGCTTGCTGATCCACATCTGC
<i>Mir-582-5p</i>	UAACUGGUUGAACAAACUGAACC	
<i>RNAUB</i>	GTTGGCTCTGGTGCAGGGTCCGA GGTATTTCGCACCAGAGCCAACAA AAATAT	

Supplementary Table. 1 Primer sequences used to quantify the mRNA through real time qPCR