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Supplemental Information

CD54-Mediated Interaction with Pro-inflammatory Macrophages Increases the Immunosuppressive Function of Human Mesenchymal Stromal Cells

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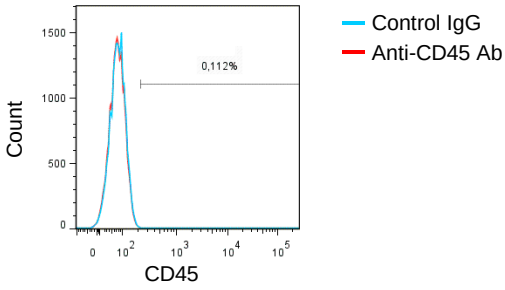
A



B

Markers	% of expression
CD90	99.7 ± 0.47
CD73	98.1 ± 1,9
CD146	23.4 ± 13.6
CD31	1.3 ± 1.2
CD45	0.3 ± 0.2

Figure S1: Phenotype of mesenchymal stromal cells.
The MSCs used for the experiment did not expressed the CD45 (histogram and table) and the CD31 but are positives for CD90 and CD73 (3 independent experiments).

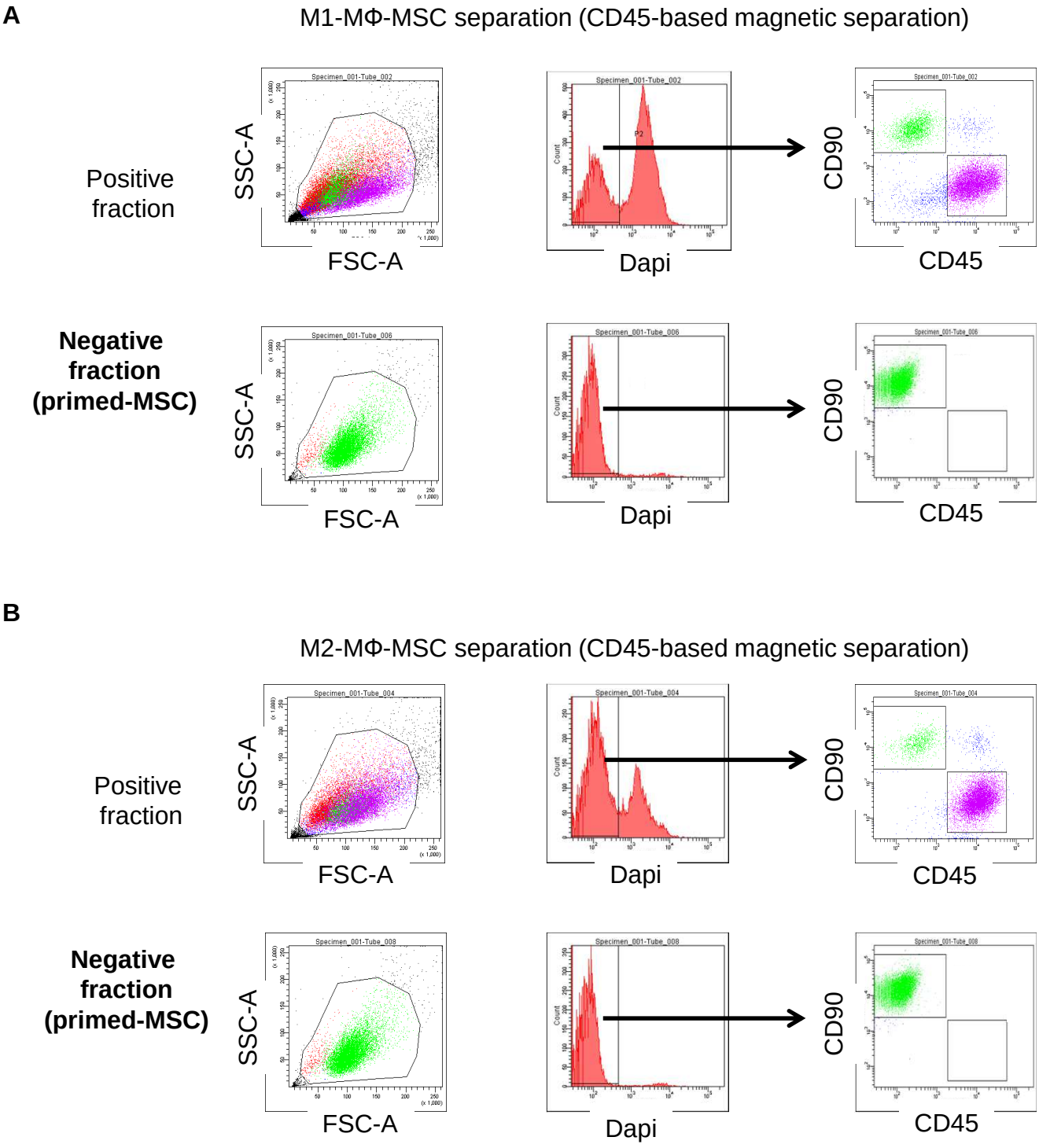


Figure S2: MSC sorting after co-culture with M1MΦ or M2MΦ macrophages
After 24 h of co-culture between M1MΦ (A) or M2MΦ (B) and MSCs, cells were harvested and MSCs were magnetically separated from macrophages. Viability and purity of positive and negative fraction was determined by DAPI labelling and staining with anti-CD90-FITC/anti-CD45 APC Vio770 antibodies.

A

Gene Symbol	RefSeq	Fold-Change (M1-MSC vs. MSC)	p-value (M1-MSC vs. MSC)	Fold-Change (M2-MSC vs. MSC)	p-value (M2-MSC vs. MSC)
CXCL9	NM_002416	91,36	2,2E-08	1,02	0,90
CXCL10	NM_001565	31,13	1,5E-06	-1,03	0,91
CD54	NM_000201	8,52	7,3E-04	1,47	0,31
PD-L1	NM_014143	6,76	1,1E-03	1,03	0,93
IDO1	NM_002164	5,93	1,4E-05	1,19	0,31
IL6	NM_000600	5,67	1,3E-03	1,73	0,13
VEGFA	NM_001025366	4,79	8,0E-03	1,03	0,94
TSG6	NM_007115	4,32	7,8E-03	-1,03	0,93
CD106	NM_001078	3,31	1,2E-03	2,37	0,00
HLA-G5	NM_002127	1,37	2,6E-02	-1,01	0,93
GALECTIN 9	NM_009587	1,95	8,6E-02	1,06	0,85
CD40	NM_001250	1,52	5,6E-02	-1,03	0,85
TGFB1	NM_000660	1,14	3,9E-01	-1,07	0,62
GALECTIN 1	NM_002305	-1,12	1,4E-01	1,07	0,34
HMOX1	NM_002133	-1,12	5,9E-01	-1,69	0,03
COX2	ENST00000361739	-1,32	8,7E-02	-1,06	0,68
CD58	NM_001779	-1,46	1,6E-01	1,15	0,57
GALECTIN 3	NR_003225	-1,49	1,1E-03	-1,12	0,14
CD86	NM_175862	1,23	5,9E-01	1,31	0,46
CD80	NM_005191	1,21	9,3E-02	1,06	0,56
CCL8	NM_005623	2,26	3,2E-03	1,13	0,51
IL1B	NM_000576	-1,01	8,9E-01	1,03	0,66
CD72	NM_001782	-1,04	7,9E-01	-1,15	0,29
CXCR7	NM_020311	1,03	9,2E-01	-1,50	0,12
ICAM2	NM_001099786	-1,13	4,0E-01	1,08	0,54
CCL1	NM_002981	-1,12	3,1E-01	-1,03	0,77
IL10	NM_000572	-1,13	3,3E-02	-1,09	0,10

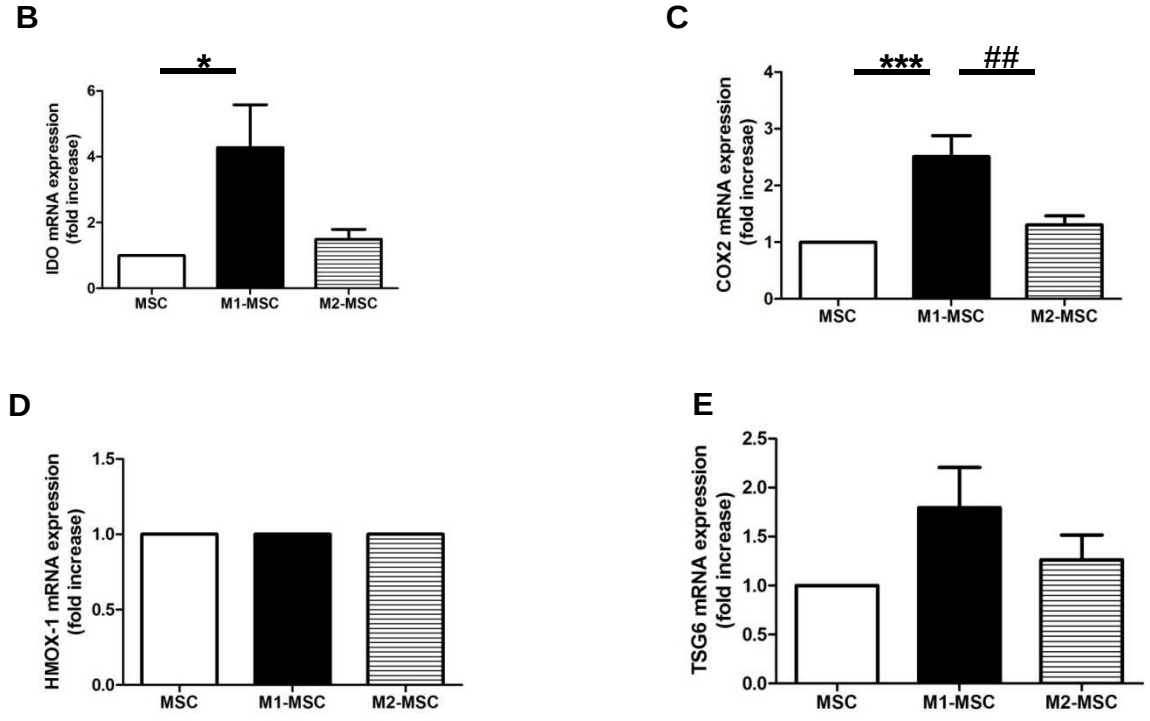


Figure S3: RNA expression of immunosuppressive molecules. MSCs were co-cultured with or without M1MΦ or M2MΦ for 24 h, then harvested, magnetically separated from macrophages and cultivated for 24 h. (A) Microarray analysis of Expression of genes coding for proteins involved in immunosuppression by M1MΦ- and M2MΦ–primed MSCs as compared with unprimed MSCs (Figure related to Figure 1). qPCR analysis of mRNA expression of *IDO* (B), *COX2* (C), *HMOX1* (D) and *TSG6* (E). Data are mean fold increase in expression ±SEM (5 independent experiments). **P<0.01, ***P<0.001, compared with MSCs. $^{\#\#}$P<0.01.

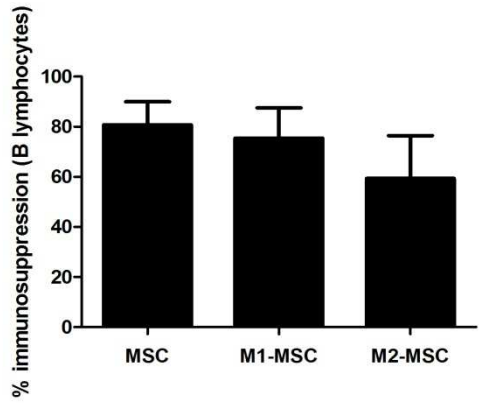


Figure S4 MΦ priming of MSC did not modify B-lymphocyte proliferation.
MSCs were unprimed or primed for 24 h with M1MΦ or M2MΦ. After separation, MSCs were added to CFSE-labelled B lymphocytes and stimulated with CpG for 7 days. Data are mean percentage immunosuppression ± SEM (3 independent experiments).

Supplemental experimental procedure

B lymphocyte immunosuppression: human B cells were isolated from PBMCs by using the B-cell isolation kit II (Miltenyi) and labeled with CFSE (2.5 µM, 10 min at 37 °C) (Life Technologies) according to the manufacturer's guidelines. 10^5-labeled B lymphocytes were plated alone as a positive control or with MSCs (ratio 1:1) and stimulated with 2 µg/mL CpG (InvivoGen) for 7 days. B lymphocytes were harvested and stained with anti-CD45-APC-Vio770 and anti-CD19-APC (BD) antibodies and fluorescence was analyzed on a FACS Canto II (BD) by using Diva software (BD). The proportion of immunosuppression induced by MSCs was calculated as [1 − (proliferation of B cells co-cultured with MSCs/proliferation of stimulated B cells alone)] × 100.

Supplemental movie legend (related to Figure 6)

(n=nomarsky; f=fluorescence)

Supplemental movie S1

MSC alone (big adherent cells) don't exhibit calcium mobilization and keep a stable blue color in a pseudocolor scale.

Supplemental movie S2

In contact with M1 macrophages (adherent rounded cells), MSC increase calcium mobilization and exhibit a yellow color intermittently from a pseudocolor scale.

Supplemental movie S3

In contact with M2 macrophages (adherent rounded cells), MSC don't increase calcium mobilization and keep a blue color from a pseudocolor scale.

Supplemental movie S4

In presence of T cells (small migrating cells), MSC don't increase calcium mobilization and keep a stable blue-green color from a pseudocolor scale.

Supplemental movie S5

In contact with M1 macrophages, anti-CD54 pretreated MSC (in center and in right of movie) don't increase calcium mobilization and exhibit a stable blue color from a pseudocolor scale.