

Human Mesenchymal stem cells program macrophage plasticity by altering their metabolic status *via* a PGE₂-dependent mechanism

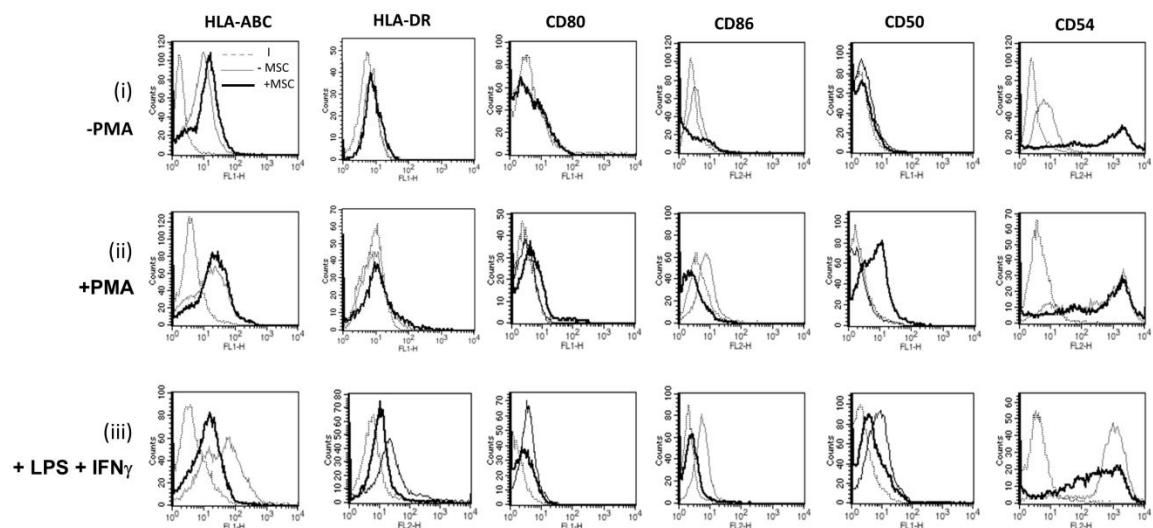
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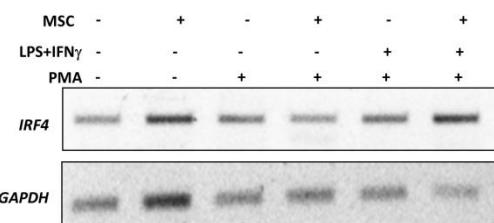
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S1



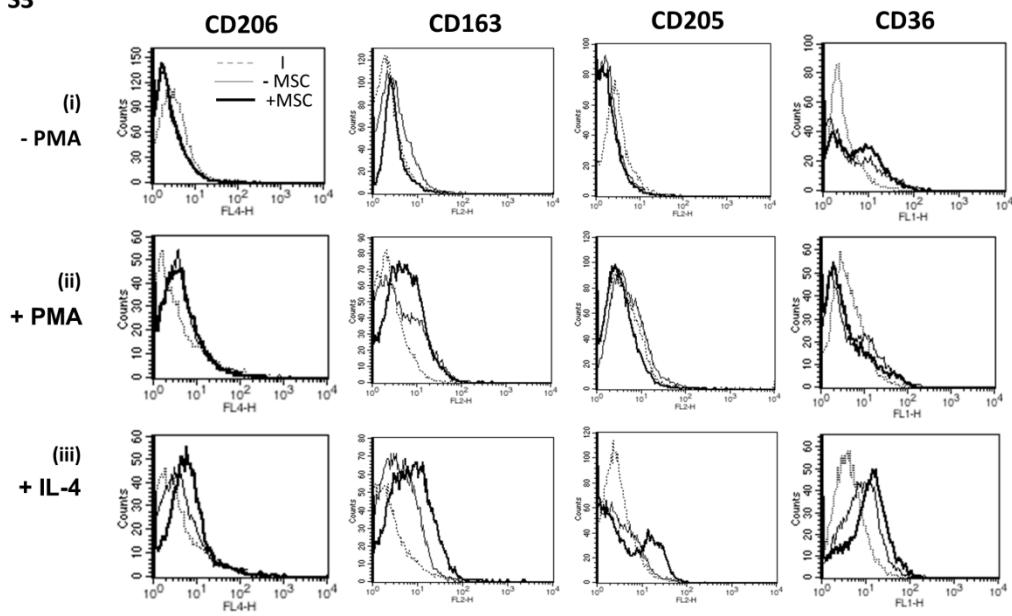
Supplementary figure 1: MSCs influence surface expression of key co-stimulatory and immune-adhesive ligands on PMA differentiated and M1 polarized human THP-1 Cells. Flow cytometry of surface antigens associated with APC-capacity of THP1 (i) PMA differentiated THP1 Mφ (ii) M1 polarized THP1 cells (iii). Histogram overlays depict relevant isotype control (I; black dotted line), Thick black line and thin black line indicate surface expression of the specific ligand with and without MSCs respectively. Histograms are representative of three independent experiments.

S2

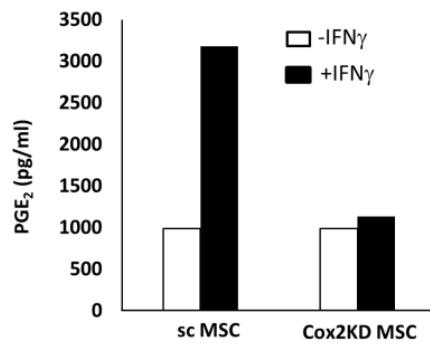


Supplementary figure 2: *IRF4* transcript levels in MSC-macrophage co-cultures evaluated on a 1% agarose gel. PCR was run for 20 cycles for *GAPDH* and 25 cycles for *IRF4*.

S3



Supplementary figure 3: MSCs influence surface expression of key scavenger receptors on PMA differentiated and M2 polarized human THP-1 Cells. Flow cytometry of surface antigens associated with scavenger receptors on THP1 (i) PMA differentiated THP1 Mφ (ii) M2 polarized THP1 cells (iii). Histogram overlays depict relevant isotype control (I; black dotted line), Thick black line and thin black line indicate surface expression of the specific ligand with and without MSCs respectively. Histograms are representative of three independent experiments.



Supplementary figure 4: *Cox2* KDN resulted in decrease in PGE₂ secretion in IFN γ primed MSC. MSCs were primed with 50 ng/ml of IFN γ for 48 hours and PGE₂ was analyzed in the culture supernatants as described in material and methods.

Supplementary Table 1**Primers used for real time PCR analysis**

GENE	FORWARD PRIMER (5'-3')	REVERSE PRIMER (5'-3')
<i>ALOX15</i>	CTTAAGGACGACGCCCTGGTT	AGTTTCCCCACCGGTACAAC
<i>BAX</i>	GCCGTGGACACAGACTCC	AAGTAGAAAAGGGCGACAACC
<i>BCL2</i>	CACCTGTGGTCCACCTGAC	ACGCTCTCCACACACATGAC
<i>CCL13</i>	CAGATGCACTCAACGTCCTA	TGGTTCTGAAGATGACAGCCTT
<i>CCR7</i>	GGCTGGTGGTGTGACCTAT	ACGTAGCGGTCAATGCTGAT
<i>CD11b</i>	CCATAGCCAGCGGATAGCAG	CTTGCCACTTCCCTGGGATT
<i>CD14</i>	CACAGTGGGAGCCGCACAGG	ACGTTGCGTAGGCGCAAGCT
<i>CD206/MRC1</i>	CCAGGGCGAAAGCCAGGGTG	GTCGTGGCTTCGGTGGGTG
<i>CD36</i>	ACTGAGGACTGCAGTGTAGGA	ACAAGCTCTGGTTCTTATTACA
<i>CD68</i>	TGGGTGGGATCATCTCCAGT	CCCCTGGCTGCCAGTA
<i>CD86</i>	TGGAGAGGGAAGAGAGGTGAACA	GCCCATAAGTGTGCTCTGAA
<i>CPT1α</i>	CCAGACGAAGAACGTGGTCA	ATCTTGCCGTGCTCAGTGAA
<i>DC-SIGN</i>	GAACCTGGCACGACTCCATCA	GTTGGGCTCTCCTCTGTTCC
<i>GAPDH</i>	CGACCACTTGTCAAGCTCA	AGGGGTCTACATGGCAACTG
<i>HK2</i>	GATTCACCAAGCGTGGACT	CCACACCCACTGTCACTTG
<i>IDO</i>	CCTGAGGAGCTACCATCTGC	TCAGTGCCTCCAGTTCTTT
<i>IL-10</i>	AAGCCTGACCACGCTTTCTA	ATGAAGTGGTTGGGAATGA
<i>IL-12β</i>	CCAAGGGGTGACGTGGGAG	GGTGGGTCAAGTTGATGATGTCCC
<i>IL-6/IFNβ2</i>	GAACTCCTCTCCACAAGCG	TTTTCTGCCAGTGCCTCTTT

GENE	FORWARD PRIMER (5'-3')	REVERSE PRIMER (5'-3')
<i>IRF4</i>	CTCTTCAAGGCTTGGCACT	AGGGTAAGGCCTTGTCA
<i>IRF5</i>	TAGAGGCTACCCAGGAGCAA	GCCCACCTCCAGAACACCTTA
<i>MSR1</i>	TTCAACGCAGGAATGTGTCA	AGCTGTCATTGAGCGAGCAT
<i>p53</i>	GAAGACCCAGGTCCAGATGA	TTCTGGAAAGGGACAGAAGA
<i>PCNA</i>	CCTGGCCATGGCGTGAACC	AGCGCCAAGGTATCCCGT
<i>PHOX p22</i>	GGGCCATGTGGGCCAACGAA	CACGCCCGCCACAATGGAGT
<i>PHOX p47</i>	CGCAGGTCGTCCATCCGCAA	CGGACGCTGTTGCGGCGATA
<i>PHOX p91</i>	TGTGTGAATGCCGAGTCAA	ATGGATGGCAAGGCCAATGA
<i>SIRT1</i>	CACACTTTAGACCAAGCAGCTAA	TTTCTCCCCAGTACTAGAACCAAC
<i>SOD2</i>	GGAACAAACAGGCCTTATTCCAC	GAGCTTAACATACTCAGCATAACGA
<i>TGFβ1</i>	CCTACATTGGAGCCTGGAC	TGTCCTTAAATACAGCCCC
<i>TGM2</i>	GCCCACATCACCAACACAC	GTAGAGGTCCCTCTCAGCCA
<i>TNFα</i>	TGGCCAATGGCGTGGAGCTG	GTAGGAGACGGCGATGCGGC

Cycling conditions (a) Real time PCRs: Initial denaturation at 50°C at 2 min and 95 °C for 10 min. cycling at 40 cycles: Denaturation at 95 °C for 15 sec and Annealing/extension at 60 °C for 1 min.
(b) RT-PCR: Initial denaturation at 95 °C for 5 min; Denaturation at 95 °C for 30 sec, 61 °C for 45 sec and 72 °C for 30sec; final extension 72 °C for 10min.