Table of content

| Number | Title | Page Number |
|--------------------|--|----------------|
| Appendix Figure S1 | MSCs depict reduced suppression of NET formation in the presence of LPS in co-cultures with neutrophils. | 2 |
| Appendix Figure S2 | Both non-primed MSCs and LPS primed MSCs suppress T cell proliferation in a mixed lymphocyte reaction. | 3 |
| Appendix Figure S3 | No significant difference in β2M+ MSC numbers in wounds injected with non-primed versus LPS-primed MSCs. | 4 |
| Appendix Figure S4 | LPS stimulation reveals repressed stimulation of CXCL6/IL-1β/IL-8 expression in TLR4 silenced MSCs. | 5 |
| Appendix Figure S5 | MSCs fulfill minimal criteria of stem cells. | 6-7 |
| Appendix Table S1 | RNAseq analysis: Top up and down regulated genes in MSCs following 6h and 24h of LPS stimulation. | 8 |
| Appendix Table S2 | Primers List. | 9 |



LPS Neu + PMA + LPS MSCs Neu + PMA + LPS MSCs



Β





Appendix Figure S1. MSCs depict reduced suppression of NET formation in the presence of LPS in co-cultures with neutrophils.

- А PMA activated neutrophils were co-cultured with MSCs in the presence of LPS at a concentration of 100 ng (lower row, right panel). No difference in the attenuated suppression of NET formation was observed compared to PMA activated neutrophils co-cultured with LPS primed MSCs (lower row, left panel). Non-primed MSCs, by contrast, led to a significantly stronger suppression of NET formation (upper row, outer right panel). Incubation of neutrophils with PMA alone served as positive control and DMSO treated neutrophils as negative control. Scale bars: 50µm. A representative experiment out of 3 independent experiments is depicted.
- Quantification of NET bound elastase indicative of NET formation. Statistical analysis was В performed using one way ANOVA, values are represented as mean ± SEM, n=3 biological replicates. 2



Appendix Figure S2. Both non-primed MSCs and LPS primed MSCs suppress T cell proliferation in a mixed lymphocyte reaction.

CFSE labeled PBMCs from buffy coat of healthy, unrelated, non-HLA-matched donor (effector cells A) were stimulated with irradiated PBMCs (stimulator cells B) in the absence or presence of untreated or LPS-treated MSCs. After 7 days, cells were stained for CD2 and 7-AAD. Live cells were identified by 7-AAD negativity and proliferation of CD2⁺ T cells was defined by CFSE dilution. Statistical analysis was performed using one way ANOVA, values are represented as mean \pm SEM, n=6 biological replicates.



Appendix Figure S3. No significant difference in β 2M+ MSC numbers in wounds injected with non-primed versus LPS-primed MSCs.

Quantification of human β 2M⁺ MSCs in the skin wounds that were treated either with PBS or LPS primed MSCs for 24h. Counting of β 2M⁺ MSCs in wound sections was performed 24h after the administration of MSCs. Statistical analysis was performed using unpaired t-test, values are represented as mean ± SEM, n=6 biological replicates. ns, non-significant.



Appendix Figure S4. LPS stimulation reveals repressed stimulation of CXCL6/IL-1 β /IL-8 expression in TLR4 silenced MSCs.

qPCR results demonstrating mRNA expression of CXCL6, IL-1 β and IL-8 upon silencing of TLR4 in LPS-primed silenced MSCs. Comparisons were made between 'Scr MSCs vs Scr MSCs + LPS', 'Scr MSCs vs TLR4 Si MSCs + LPS', and 'Scr MSCs + LPS vs TLR4 Si MSCs + LPS'. Statistical analysis was performed using one way ANOVA, values are represented as mean ± SEM, n=3 biological replicates.



Appendix Figure S5. MSCs fulfill minimal criteria of stem cells.

- A Flow cytometric analysis of cell surface marker expression on MSCs, including CD105, CD73, CD71, CD45, CD31, and HLA-ABC. Marker-specific antibodies labelled MSCs were shown in filled histograms, and the isotype control antibodies labelled cells were shown in empty histograms. They express typical MSC markers including CD73, CD90, CD105, and CD71 and do not express the leukocyte marker CD45, or endothelial marker CD31. They are MHC class I positive. MSCs in culture are plastic adherent.
- B-D MSCs were cultured in adipogenic, osteogenic and chondrogenic induction media and subjected to Oil-Red-O staining indicative of adipogenic differentiation, Alizarin Red-S staining indicative of osteogenic differentiation and aggrecan staining indicative of chondrogenic differentiation. Scale bar: 50µm.

Appendix Table S1

0 vs 6h LPS

0 vs 24h LPS

| Genes | 0 vs 6 hr Up | Genes | 0 vs 6 hr Down | Genes | 0 vs 24 hr Up | Gene | 0 vs 24 hr Down |
|---------------|---------------------------------------|---------------|----------------|---------------|---------------|---------------|-----------------|
| SOX6 | 5366.999 | FAM83H | -365,784 | STS | 67,3782 | FAM83H | -325,33 |
| CACNG8 | 2223,166 | MIR137,MIR137 | | TBC1D17 | 49.89488 | 0C554223 | -278 78 |
| CTC1 | 187 8022 | HG | -224,908 | | 44.04068 | EP300 | -181.54 |
| | 116 326 | EP300 | -212,861 | | 44,94068 | | -101,34 |
| | 111 9034 | LOC101927806 | | REXUI | 33,7176 | C100f118 | -138,31 |
| | 106 4952 | ,TRNAE1 | -107,972 | CELSR3 | 32,59232 | MIR137,MIR137 | 72.004 |
| | F8 24401 | C7orf43 | -67,9716 | IL8 | 19,40074 | | -73,904 |
| | 58,24491 | | -41,7484 | SULT1A1 | 17,19146 | POLM | -34,344 |
| | 48,25144 | | -31,1604 | DNAH12 | 15,61413 | LOC101928221 | -33,303 |
| TMCC3 | 42,46994 | | 21,7400 | CXCL6 | 15,24834 | MAGED4 | -20,716 |
| PISD | 30,51803 | RP11-887P2.5 | -13,8905 | LOC284454 | 14,569 | IRAK3 | -19,105 |
| GBP4 | 30,40338 | | -13,7568 | IL1B | 13.54567 | ILDR2 | -17,095 |
| CCL20 | 28,83222 | RGS7 | -12 6202 | C3 | 12.87294 | KSR2 | -13,994 |
| C15orf48 | 28,38211 | MAGED4 | -12,1152 | SYNGAP1 | 11 24296 | C5AR1 | -13.684 |
| STS | 28,19095 | PDCD4 | -11,8781 | GIMAP2 | 10 45927 | PRRG4 | -12 682 |
| MX1 | 27,33637 | PGR | -10,86 | | 0.012088 | | 12,002 |
| TNFSF13B | 25,11992 | ENPP5 | -10,5913 | | 9,912900 | RF3-322G13.3 | -12,429 |
| GNA15 | 24,45487 | LOC644662 | -10,5909 | | 9,030104 | RANBP3L | -11,293 |
| IL1B | 24,25987 | MIR4292 | -10,5909 | GNA15 | 9,595912 | GPR116 | -10,628 |
| CMPK2 | 23,96339 | RIMBP3 | -9,07791 | PCDHA1 | 9,466689 | PDCD4 | -10,549 |
| | , , , , , , , , , , , , , , , , , , , | | -9 07791 | 00101020071 | 0.265626 | LINC00982 | -10,472 |
| LOC101929580 | 22,19034 | TRNAC24 | -9,07791 | LOC 101930071 | 9,200000 | TRNAL16 | -8,7004 |
| CSF2 | 19,82831 | | , | RP11-290F20.3 | 8,889856 | RP3-400B16.1 | -8,7004 |
| SLC25A15 | 16.2507 | DLG3-AS1 | -9,07684 | CXCL1 | 8,864627 | RIMS4 | -8,496 |
| C3 | 15.86263 | | -0,32142 | TNFRSF9 | 8,832672 | I OC102723663 | -7 8602 |
| MIR433 | 15 4309 | KIAA0226 | -8,17031 | CCL5 | 8,448294 | TU 1 | 7,0002 |
| | 15 06397 | MAST1 | -7,56521 | SERPINB2 | 8,242717 | | -7,4045 |
| 0452 | 14 10926 | | -7,56516 | KCNJ5 | 8,179598 | | -7,4575 |
| | 13 50967 | | -7,30493 | HES7 | 8,051793 | HIGD2B | -7,4575 |
| | 13,30907 | AC016995.3 | -7,56495 | 0C101929609 | 8 0456 | LOC644189 | -7,4575 |
| | 13,19096 | BP3-322G13.5 | -7 56495 | | 0,0100 | TRNAC24 | -7,4575 |
| | 12,39108 | ARGFXP2 | -7,56489 | LOC100652930 | 8,0456 | FAM135B | -7,3347 |
| | 11,8392 | МҮВРН | -7,45951 | LOC100506974 | 8,0456 | GABRB3 | -6.7794 |
| IF112 | 11,51929 | | 7 07005 | CSF2 | 8,0456 | KIAA1456 | -6 5986 |
| CXCL6 | 11,4249 | LOC 102723729 | -7,37325 | LOC100499194 | 8.004881 | | 6 2180 |
| | | | 1,20022 | TMBIM1 | 7.75534 | | -0,2109 |
| LOC101926887 | 10,71493 | LOC102724423 | -6,80847 | CXCL5 | 7 6943 | | -6,2171 |
| CXCL1 | 10,41622 | LOC100507054 | -6,80847 | NME5 | 7 522541 | AC016738.3 | -6,2146 |
| GIMAP2 | 9,914156 | CYP21A2 | -6,78905 | CAMSAD3 | 7 244821 | TRNAR22 | -6,2146 |
| SERPINB2 | 9,896785 | | | | 7,244021 | MIR193A | -6,2146 |
| AQP6 | 9,890408 | LOC101929149 | | | 7,241055 | SNAR-G2 | -6,2146 |
| MX2 | 9,732969 | 8 | -6,65321 | LOC102723600 | 7,241055 | MIR6743 | -6,2146 |
| ICAM1 | 9,641783 | VWA8 | -6,50207 | DUSP8,LOC101 | | NAPSA | -6.2146 |
| C6orf58 | 9,253185 | NR1I3 | -6,49208 | 927562 | 7,241055 | TCP10I | -6,2146 |
| BATF2 | 9,152526 | KIAA1456 | -6,40336 | OR1J2 | 7,241055 | Ceorf165 | 6 21/6 |
| TNF-SF10 | 9,116247 | | 0.00000 | LOC101928549 | 7.241055 | 0001103 | -0,2140 |
| | | | -6,28089 | C6orf58 | 7 241055 | RP11-423C15.3 | -6,2146 |
| LOC102723726. | | ATP1A3 | -6,22498 | NTE4 | 7 237693 | MIR7845 | -6,2146 |
| TNF-AIP2 | 8,245517 | ACP5 | -6,05279 | | 6.075081 | НССАТЗ | -6.2146 |
| MEOX1 | 8,146441 | ADH4 | -6,05208 | | 6.010070 | WNT10B | -6 2146 |
| RNF170 | 8,095892 | | 6.05105 | | 6,919079 | | 6.2140 |
| IFIT3 | 8,058102 | 100101920074 | -0,05195 | LHFPL4 | 6,436471 | LOC 101928153 | -0,2140 |
| ARNT2 | 8,00183 | HNRNPA1P10 | -6,05195 | LOC102724715 | 6,436471 | LOC10192/919 | -6,2146 |
| | , | RP11-480112 7 | -6 05105 | DGCR8 | 6,332785 | C2CD4A | -6,2146 |
| C20orf195 | 7,945181 | | -0,00190 | LOC101928841 | 6.305234 | DNAH9 | -6,2146 |
| FLJ45079 | 7,931315 | RP5-858B6.3 | -6,05195 | RAPGEF5 | 6.235132 | AC016995.3 | -6.2146 |
| ADH1C | 7,931315 | 00:101930434 | -6 05195 | FTCD | 6 155773 | SNORD105B | -6 205 |
| | ., | | 0,00100 | | 0,100110 | 5.15.10.000 | 0,200 |

Appendix Table S2

Primers List

| Genes | Forward primer | Reverse primer |
|---------|---------------------------|----------------------------|
| (human) | • | |
| (numan) | | |
| | | |
| IL-1β | AATCTGTACCTGTCCTGCGTGTT | TGGGTAATTTTTGGGATCTACACTCT |
| | | |
| | | |
| 11.8 | ATGACTTCCAAGCTGGCCGTGGCT | TCTCAGCCCTCTTCAAAAACTTCTC |
| | | |
| | | |
| | | |
| CXCL6 | AGAGCTGCGTTGCACTTGTT | GCAGTTTACCAATCGTTTTGGGG |
| | | |
| | | |
| MvD88 | GACATGGTTAGGCTCCCTCA | GCACATGGGCACATACAGAC |
| , = = = | | |
| | | |
| | | |
| I ILR4 | CGGAGGCCATTAIGCIAIGI | |
| | | |
| | | |
| IL6 | AGACAGCCACTCACCTCTTCAG | TTCTGCCAGTGCCTCTTTGCTG |
| | | |
| | | |
| β-actin | TCACCCACACTGTGCCCATCTACGA | CAGCGGAACCGCTCATTGCCAATGG |
| | | |
| | | |
| | | |