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# Inflammatory modulation of hematopoietic stem cells by Magnetic Resonance Imaging (MRI)-detectable nanoparticles

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**Experimental Section**

***Preparation and characterization of NP210-PFCE.*** PLGA (Resomers 502 H; 50:50 lactic acid: glycolic acid) (Boehringer Ingelheim) was covalently conjugated to fluoresceinamine (Sigma-Aldrich) according to a protocol reported elsewhere[1]. NPs were prepared by dissolving PLGA (100 mg) in a solution of propylene carbonate (5 mL, Sigma). PLGA solution was mixed with perfluoro-15-crown-5-ether (PFCE) (178 mg) (Fluorochem, UK) dissolved in trifluoroethanol (1 mL, Sigma). This solution was then added to a PVA solution (10 mL, 1% w/v in water) dropwise and stirred for 3 h. The NPs were then transferred to a dialysis membrane and dialysed (MWCO of 50 kDa, Spectrum Labs) against distilled water before freeze-drying. Then, NPs were coated with protamine sulfate (PS). For this purpose, NPs (1 mg/mL) and PS (1 mg/mL) were incubated for 15 min under agitation, at room temperature. After the incubation period, the NPs were dialyzed (MWCO of 10-12 kDa) against distilled water and freeze-dried. Under these conditions, approximately 13 µg of protamine sulfate existed per mg of NP. Particle size and zeta potential of the NPs were determined using light scattering via Zeta PALS Zeta Potential Analyzer and ZetaPlus Particle Sizing Software, v. 2.27 (Brookhaven Instruments Corporation), according to protocols described by us[2].

***Quantification of internalized NPs.*** HUVECs and $CD34^+$ cells were transfected with fluorescent-labelled NPs (500 µg NPs for $4\times10^5$ HUVECs or for $1\times10^6$ $CD34^+$ cells) in M200 medium (Invitrogen, Carlsbad, USA) or QBSF-60 (Quality Biological Inc., Gaithersburg, USA) for 4 h at 37ºC. After incubation, the cells washed 3 times with medium and incubated either with CD31 microbead kit (for HUVECs) or CD34 microbead kit (for $CD34^+$ cells) from Miltenyi for 30 min at +4ºC. After incubation period, cells were centrifuged and supernatant was removed. Cells were resuspended in cell culture medium and passed through MS columns from Miltenyi to remove particles that were not internalized. Finally, magnetic beads were removed and cells were lysed in 1N sodium hydroxide (NaOH) solution. Fluorescence was measured using Gemini XPS

fluorescence microplate reader at excitation and emission wavelengths 485 nm and 520 nm, respectively.

For inductively coupled plasma mass spectrometry (ICP-MS) analyses, $CD34^+$ cells were transfected with SPIONs (30 nm in size, coated with poly-L-lysine, obtained from BioPAL Inc., Worcester, MA, USA) as described above, followed by the addition of nitric acid (69%, v/v) on top of cell pellet. ICP-MS analyses were performed for the quantification of Fe.

***Electroporation and MRI studies.*** $CD34^+$ cells were suspended in QBSF medium supplemented with 50 ng/mL Flt-3, 50 ng/mL SCF and 10% BSA at a concentration of $1\times10^6$ cells/mL. The cells were electroporated in a volume of 500 µL in 0.4 cm Bio-Rad Gene Pulser cuvettes with or without 2 mg/mL NP210-PFCE particles using Bio-Rad Gene Pulser Xcell electroporation system. The voltage was set to 220 Volt and pulse length was 38 ms. Immediately after electroporation, the cells were seeded into V-shaped 96-well plates at a concentration of 200,000 cells/mL in 200 µL medium and incubated at 37°C. After 24 hours, labeled cells were washed several times and incubated with CD34 microbead kit for 15 min at +4ºC. After incubation, cells were centrifuged and supernatant was removed. Cells were resuspended in cell culture medium and passed through MS columns from Miltenyi to remove particles that were not internalized. Then, magnetic beads were removed from the cells, cells were fixated with 4% PFA for 15 min at room temperature, washed several times and resuspended in PBS for MRI measurements.

Gradient Echo MRI was performed on a 7T horizontal bore (60 G/cm, inner bore diameter 120 mm) system (Varian), using Direct Drive console (VJ NMR) with a linear transmit and receiver. For both $^{19}F$ and $^{1}H$ imaging, coronal SEMS images were taken at matrix size of 128, 120 averages were taken, field of view was 40 × 40 cm, 9 imaging planes each of 2 mm depth no spacing between imaging planes, TE/TR = 5/80 ms with a flip angle of 90 degrees.

***$^{19}F/\ ^{1}H$ in vivo MRI.*** 10 million $CD34^+$ cells were isolated via indirect selection using EasySep system. The beads were detached from the cells, cells were left to be stabilized for 24 hours and then labeled with NPs (500 µg NPs for $1\times10^6$ $CD34^+$ cells) in M199 medium (Sigma, USA) for 4 h at 37ºC. Cells were injected into the myocardium of SD rats after LAD. Within 30 minutes of injection, rats were euthanized. The heart was removed and set into gelatin and MRI was done on these.

MRI analysis was performed using a 9.4T horizontal bore (60 G/cm, inner bore diameter 120 mm) system (Varian), using Direct Drive console (VJ NMR) with a linear transmit and receiver. $^{19}F$ MRI was performed to detect NP-labeled cells while $^{1}H$ MRI was done for anatomy of the heart. $^{19}F$ MR images were acquired using a purpose built circular surface coil tunable to $^{19}F$. For $^{1}H$ imaging, coronal GEMS images of the hearts were taken at matrix size of 128 (later zero filled to 256), 30 averages, field of view 35 × 35 cm, 10 imaging planes of 1mm depth, TE/TR = 4/1.2 ms with a flip angle of 70 degrees. The $^{1}H$ images were later isotropically zero-filled by a factor of two and filtered (modified third-order Butterworth filter) before Fourier transformation. For $^{19}F$ imaging, coronal GEMS images of the hearts cloned from the $^{1}H$ images were taken at matrix size of 256, 60 averages, field of view 35 × 35 cm, 1 imaging plane of 10mm depth, TE/TR = 4/1.2 ms with a flip angle of 90 degrees. All images were Fourier transformed. At all times a phantom was present to allow shimming and pulse calibration, and to aid orientation within the surface coil.

***Isolation of $CD34^+$ cells from umbilical cord blood (UCB).*** All human UCB samples were collected from donors, who signed an informed consent form, in compliance with Portuguese legislation. The collection was approved by the ethical committees of Dr. Daniel de Matos Maternity Hospital in Coimbra and Hospital Infante D. Pedro in Aveiro. The samples were stored in sterile bags containing 35 mL of citrate-phosphate-dextrose anticoagulant solution. $CD34^+$ cells were isolated from mononuclear cells obtained from UCB samples after Ficoll (Histopaque-1077

Hybri Max; Sigma-Aldrich, St. Louis, USA) density gradient separation. $CD34^+$ cells were positively selected (2 times) using the mini-MACS immunomagnetic separation system (Miltenyi Biotec, Bergisch Gladbach, Germany), according to the manufacturer's instructions. These cells express high levels of CD31 (approximately 85%), incorporate moderate levels of Ac-LDL (approximately 13%) and showed no expression of definitive endothelial marker vWF [3]. Therefore these cells are $CD34^+CD45^+CD31^+KDR^-vWF^-CD14^-$ [3]. $CD34^+$ cells were immediately used for ROS, Bioplex, viability assays and genetic analysis without further treatment.

***Differentiation of $CD34^+$ cells into ECs.*** Isolated $CD34^+$ cells were incubated with fluorescent-labeled NPs (500 µg NP for $10^6$ $CD34^+$ cells) in serum-free M199 medium (Invitrogen, Carlsbad, USA) for 4 h at 37ºC. After incubation, the cells washed 3 times with PBS and the cells labeled with NPs were selected using a FACSAria II cell sorter (BD Biosciences, USA). The sorted cells were transferred onto 1% (w/v) gelatin-coated 96-well plates ($2\times10^4$ cells/well) and incubated in endothelial growth medium (EGM-2; Lonza, Gaithersburg, MD, USA) with 20% (v/v) fetal bovine serum (FBS; Invitrogen, Carlsbad, USA) and 50 ng/mL vascular endothelial growth factor ($VEGF_{165}$; PrepoTech Inc., Rocky Hill, USA), at 5% $CO_2$, 37°C. After 5 days and then every other day, half of the volume of the medium was replaced with fresh one. At the end of the differentiation assay, expression of EC markers was evaluated by fluorescence-activated cell sorting (FACS) and immunofluorescence staining. The functionality of the cells was evaluated by their ability to metabolize acetylated low-density lipoprotein (DiI-Ac-LDL; Biomedical Technologies, Stroughton, USA) and form microvessels on top of Matrigel™ (BD Biosciences, San Jose, CA, USA), according to protocols previously described by us[4].

***FACS analyses.*** Cells were trypsinized, centrifuged and finally resuspended in PBS supplemented with 5% (v/v) FBS. The single cell suspensions were aliquoted ($1.5 \times 10^5$ cells per condition) and stained with either isotype controls or antigen-specific antibodies: anti-human PECAM1-FITC (BD

Biosciences Pharmingen), KDR/Flk1-PE (R&D Systems, Minneapolis, USA), CD34-PE (Miltenyi Biotec). Cells were analyzed without fixation. FACS Calibur (BD Biosciences, San Diego, CA) and BD Cell Quest Software (BD Biosciences, San Diego, CA) were used for the acquisition and analysis of the data.

***Immunostaining analyses.*** Cells were fixed with 4% (v/v) paraformaldehyde (EMS, Hatfield, USA) for 15-20 min at room temperature. After permeabilizing the cells with 0.1% (v/v) Triton X-100 (Sigma-Aldrich) for 10 min, whenever required, and blocking for 30 min with 1% (w/v) bovine serum albumin (BSA) solution (Sigma-Aldrich), the cells were stained for 1 h with the following primary mouse anti-human monoclonal antibodies: PECAM1, CD34, von Willebrand factor (vWF), α-smooth muscle actin (α-SMA) (all from Dako, Glostrup, Denmark) and VE-cadherin (VE-CAD) (Santa Cruz Biotechnology, Santa Cruz, USA). In each immunofluorescence experiment, an isotype-matched IgG control was used. The binding of primary antibodies to specific cells was detected with anti-mouse IgG Cy3 conjugate (Sigma-Aldrich). The nuclei of the cells were stained with 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). After the indirect labeling, the cells were examined with a fluorescence microscope (Carl Zeiss International, Germany).

***Cell proliferation and viability analyses.*** HUVECs and $CD34^+$ cells were transfected with fluorescent-labeled NPs (500 μg NP for $4\times10^5$ HUVECs or for $1\times10^6$ $CD34^+$ cells) in M200 medium (Invitrogen, Carlsbad, USA) or QBSF-60 (Quality Biological Inc., Gaithersburg, USA) for 4 h at 37ºC. After incubation, the cells washed 3 times with medium and labeled cells were collected using FACSAria II cell sorter. After cell sorting, HUVECs and $CD34^+$ cells were counted and seeded on 1% gelatin-coated 24-well ($0.25\times10^5$ cells/well) or 96-well plates ($1\times10^4$ cells/well). At different time points, the cells were counted and ATP production was measured using Cell Titer Glo® Luminescent Cell Viability Assay kit according to manufacturer's instructions. Briefly, the equal volumes of CellTiter-Glo® Reagent and cell culture medium present in each well were mixed

and incubated at room temperature for 2 min on an orbital shaker to induce cell lysis. Then, the cells were transferred into opaque-walled multiwell plates and luminescence measurements were done using Power Wave XS (Bio-TEK, VT, USA) luminometer. The medium without cells was used as a blank control.

Cell membrane integrity was assessed by a lactate dehydrogenase (LDH) assay kit (Sigma-Aldrich). Half of the culture volume of the culture medium was removed into a flat-bottom 96-well plate. The LDH assay mixture was added to each sample in a volume equal to twice of the medium removed for testing. The plate was incubated at room temperature for 30 min in the dark. Absorbance measurements were done at the wavelength of 490 nm with the background measurement at the wavelength of 690 nm. LDH release from each sample was normalized to LDH release in untreated cells.

***ROS analyses.*** The production of intracellular reactive oxygen species (ROS) was measured by flow cytometry using the oxidation-sensitive probe, 5-(and-6)-carboxy-2",7"-dichlorodihydrofluorescein diacetate (carboxy-$H_2$DCFDA; Invitrogen, Carlsbad, CA, USA). Briefly, carboxy-$H_2$DCFDA working solution (10 µM) was prepared in cell culture medium without serum or other supplements. HUVECs in 24-well plates ($2\times10^5$ cells/well, in 500 µL EGM-2) were incubated with different concentrations of NPs (500 and 1000 µg/mL) for 4 h at 37ºC. After incubation time, the cell media were collected and used immediately for Bioplex assay. The cells were washed with serum-free M200 for 3 times and the medium was replaced with EGM-2 for 24 h samples. These cell samples were incubated at 37ºC for additional 20 h. 4 h samples were immediately incubated in 500 µL carboxy-$H_2$DCFDA working solution at 37ºC in dark for 2 h. Then the cells were washed twice with cold PBS and resuspended in pre-warmed EGM-2 during 1 h at 37ºC for the cellular esterases to hydrolyze the acetate groups and render the dye responsive to

oxidation. Finally, cells were harvested and characterized by FACS analysis (FACS Calibur, BD Biosciences, San Diego, CA).

The production of ROS in $CD34^+$ cells after the incubation with NPs was calculated using the freshly-isolated $CD34^+$ cells. 200,000 cells in 200 µL medium were used for each sample and triplicates were used for each condition. The cells were incubated with different concentrations of NPs (500 and 1000 µg/mL for $10^6$ cells) for 4 h at 37ºC. Then, the steps described above were followed for the ROS measurements. For both cell types, cells treated with $H_2O_2$ (1 mM) and incubated with probe were used as positive control while the cells incubated only with probe was used as negative control.

***Gene expression analyses.*** Freshly isolated $CD34^+$ cells or HUVECs were incubated with fluorescent-labeled NPs (500 µg NP for 1 million $CD34^+$ cells or $4\times10^5$ HUVECs) in M200 medium (Invitrogen, Carlsbad, USA) for 4 h at 37ºC. After incubation, the cells washed 3 times with M200 and the cells labeled with NPs were collected using a FACSAria II cell sorter. After cell sorting, HUVECs were incubated in EGM-2 and $CD34^+$ cells were incubated in QBSF (supplemented with 50 ng/mL Stem Cell Factor (SCF) and 50 ng/mL Flt-3) up to 1 week. At time points 24 h and 7 d, cells with or without NPs were homogenized in Trizol reagent (Invitrogen) and total RNA was extracted by a RNeasy Mini Kit (Qiagen, Valencia, USA), according to manufacturer's instructions. The RNA concentration was measured by a NanoDrop ND-1000 UV-VIS spectrophotometer. The quality of the RNA was assessed in the Agilent 2100 Bioanalyser (G2943CA) using the RNA 6000 Pico Kit (5067-1513). Before labeling, a mixture of ten *in vitro* synthesized RNAs were added to total RNA to allow for hybridization quality control and normalization of the microarrays. The RNA was amplified and labeled according to the One-Color Microarray-Based Gene Expression Analysis (Agilent). The efficiency of cRNA synthesis and dye incorporation was measured using NanoDrop spectrophotometer and samples with either a yield

below 1.65 µg or a specific activity (pmol Cy3 per µg cRNA) below 6 were not considered for hybridization.

Labeled cRNA was hybridized to the whole human genome (4×44K) microarray (G4112F from Agilent Technologies). For each sample, a solution of cyanine 3-labelled cRNA (1.65 µg in 41.8 µL with DNAse-free water) was mixed with Agilent 10× blocking agent (11 µL) and fragmentation buffer (2.2 µL) and incubated at 60ºC for exactly 30 min to fragment RNA. To stop the fragmentation reaction, 2x GEx hybridization buffer (55 µL) was added. After a short spin-down, the labeled cRNA mixture was applied to a microarray slide, assembled in a SureHyb hybridization chamber fitted with a gasket slide (Agilent), and incubated for 18 h at 65ºC in a hybridization oven (G2545A, SHEL LAB - Agilent), with 10 rpm rotation speed. Slides were washed as described in the Agilent one-color microarray-based gene expression analysis protocol. Afterwards, the microarray and gasket slide were briefly disassembled inside a staining dish containing 250 mL of GE wash buffer 1 and the slides (up to 4 slides) were washed in fresh 250 mL of GE Wash Buffer 1 solution at room temperature, during 1 min, with gentle agitation from a magnetic stirrer. A second wash step was carried out by immersing the slides in GE Wash Buffer 2 solution, previously warmed to 37ºC, during 1 min, also with gentle magnetic stirring. Finally, slides were dried by centrifugation at 800 rpm for 3 min.

Microarrays were scanned in the Agilent B scanner (G2565BA) using specific scanning protocols for gene expression microarrays and the format 4×44K. Agilent feature extraction image analysis software (Version 10.7.3.1) was used to obtain fluorescence intensities from raw microarray image files.

Analysis of raw data was performed using BRB-ArrayTools v3.4.0 developed by Dr. Richard Simon and BRB-ArrayTools Development Team[5]. BRB-Array Tools incorporates the Bioconductor R functions and the R programming language required for raw data normalization within arrays.

Each gene's measured intensity was median normalized to correct for differences in the labelling efficiency between samples. This analysis provided a median normalized dataset that was subjected to statistical analysis and clustering using MeV software[6]. Here, genes were identified as differentially expressed using the following criteria: (i) Test-design: between subjects (test vs control); (ii) Variance assumption: Welch approximation; (iii) P-value parameters: p-values based on t-distribution; alpha critical p-value =0.01; (iv) False discovery corrections: just alpha.

The previous step provided a differentially expressed genes (DEGs) list that we used to calculate the M-value. We considered as differentially expressed a variation equal or higher than 2-fold between test condition and the control. Only genes with significance level below an alpha corrected p-value of $10^{-3}$ were considered as differentially expressed. The down- and up-regulated genes were analyzed using DAVID 6.7 (Database for Annotation, Visualization and Integrated Discovery, http://david.abcc.ncifcrf.gov/) web-accessible program to identify the altered cellular processes and functions.

***TLR experiments.*** $CD34^+$ cells were treated with 500 µg/mL NPs for 4 h as described previously. Untreated cells were used as controls. After 4 h incubation with NPs, cells were washed several times and resuspended in fresh medium. To activate TLRs, cell culture medium was supplemented with human TLR1-9 agonist kit solutions from InvivoGen (1 µg/mL Pam3CSK4, $10^8$ cells/mL HKLM, 50 µg/mL Poly(I:C), 10 µg/mL LPS, 1 µg/mL *S. typhimurium* flagellin, 1 µg/mL FSL-1, 2.5 µg/mL Imiquiomod, 2.5 µg/mL ssRNA40, 10 µg/mL ODN2006 and endotoxin-free water). Cells were incubated with TLR agonists for 6 h. After 6 h, cells were collected separately and centrifuged at 300g for 10 min. Supernatants were collected, immediately frozen and kept at -80ºC until Bioplex measurements.

***Reverse transcription and quantitative real time polymerase chain reaction (qRT-PCR) analyses.*** Untreated and treated $CD34^+$ cells were homogenized in Trizol reagent (Life Technologies) and

total RNA was extracted using the RNeasy mini kit (Qiagen), according to manufacturer's instructions. In all cases, cDNA was prepared from 1 μg total RNA using Taqman Reverse transcription reagents (Applied Biosystems). Quantitative real time PCR (qRT-PCR) was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) and the detection was carried out in a 7500 Fast Real-Time PCR System (Applied Biosystems). Quantification of target genes was performed relatively to the reference GAPDH gene: relative expression = $2^{[-(Ct_{sample}-Ct_{GADPH})]}$. Primer sequences are given in **Supplementary Table 1**.

***Cytokine secretion analyses.*** Cell culture supernatants were evaluated for the presence and concentrations of cytokines using a Bio-Plex Pro Human Cytokine 17-Plex Panel Assay (Bio-Rad, Hercules, CA, USA), according to manufacturer's instructions, in a Bio-Plex 200 System (Bio-Rad). The human 17-plex panel evaluated the presence of the following analytes: interleukin-1β (IL-1β), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8; IL-10, IL-12(p70), IL-13, IL-17, granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), monocyte chemotactic protein (monocyte chemotactic activating factor [MCP-1 (MCAF)], macrophage inflammatory protein-β (MIP-1 β) and tumor necrosis factor-α (TNF-α). Supernatant media samples were collected, centrifuged to remove precipitates and the measurements were done immediately. A standard range of 0.2 to 3,200 pg/mL was used. Four replicates were used for the samples and the controls.

***Statistical analysis.*** An unpaired t test or one-way ANOVA analysis of variance with Bonferroni post-test was performed for statistical tests using GraphPad Prism software (San Diego, CA, USA, http://www.graphpad.com/). Results were considered significant when $P<0.05$. Data are shown as mean ± SEM.

**References**


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**Supplementary Table 1.** Primers used for qRT-PCR analyses.

| Gene | Forward sequence | Reverse sequence |
|---|---|---|
| GAPDH | AGCCACATCGCTCAGACACC | GTACTCAGCGCCAGCATCG |
| MMP-9 | CTTCCAGTACCGAGAGAAA | CACCTGGTTCAACTCACT |
| MME | ATATTCTGCCAGAGATCTTCAA | AGTTGCTGTTTCTGAGGTT |
| IL-8 | TTGGCAGCCTTCCTGATTTC | AACTTCTCCACAACCCTCTG |
| TNF-α | CCATGTTGTAGCAAACCC | GAGTAGATGAGGTACAGGC |
| S100A12 | GGGAATTGTCAATATCTTCCAC | CGACCTGTTCATCTTGATTAG |
| MT2A | GCTCCCAGATGTAAAGAAC | AAAGGAATATAGCAAACGGT |
| MT1X | TACAACCCTGACCCGTTTG | TTGCCATTCACATATTTCATAGA |
| PROK2 | GTCTTACTTGTGATTGTGCCAAAC | AACCAGTTCCATAATGCCTTACAC |
| TLR1 | CCCTACAAAAGGAATCTGTATC | TGCTAGTCATTTTGGAACAC |
| TLR2 | CTTTCAACTGGTAGTTGTGG | GGAATGGAGTTTAAAGATCCTG |
| TLR3 | AGATTCAAGGTACATCATGC | CAATTTATGACGAAAGGCAC |
| TLR4 | AGTTATTGCCTACTAAGTAATGA | CTTCCTTCCTGCCTCTAG |
| TLR5 | ATCTTTCACATGGGTTTGTC | TTCCCCCAGAAGGTTATATG |
| TLR6 | AGAGATCTTGAATTTGGACTC | TGTCTTTGGTCATGATGTTG |
| TLR7 | AGATATAGGATCACTCCATGC | CTTCCAAAATGGAATGTAGAGG |
| TLR8 | TGGAAAACATGTTCCTTCAG | TGCTTTTTCTCATCACAAGG |
| TLR9 | AAATCCCTCATATCCCTGTC | TTGTAATAACAGTTGCCGTC |

**Supplementary Table 2.** Genes that were down-regulated on $CD34^+$ cells exposed to NP210-PFCE for 4 h followed by 20 h of culture in the absence of NPs (total time: 24 h).

| Unique ID | Target ID | Gene Symbol | Gene Name | M Value |
|---|---|---|---|---|
| A_23_P373017 | D00044 | CCL3 | chemokine (C-C motif) ligand 3 | -5.56 |
| A_24_P228130 | NM_001001437 | CCL3L3 | chemokine (C-C motif) ligand 3-like 3 | -5.55 |
| A_23_P214079 | NM_003122 | SPINK1 | serine peptidase inhibitor, Kazal type 1 | -5.21 |
| A_23_P150609 | NM_000612 | IGF2 | insulin-like growth factor 2 (somatomedin A) | -5.09 |
| A_23_P17053 | NM_019618 | IL36G | interleukin 36, gamma | -5.05 |
| A_23_P89431 | NM_002982 | CCL2 | chemokine (C-C motif) ligand 2 | -4.83 |
| A_23_P321920 | NM_001001437 | CCL3L3 | chemokine (C-C motif) ligand 3-like 3 | -4.75 |
| A_23_P343398 | NM_001838 | CCR7 | chemokine (C-C motif) receptor 7 | -4.69 |
| A_23_P71037 | NM_000600 | IL6 | interleukin 6 (interferon, beta 2) | -4.36 |
| A_23_P121596 | NM_002704 | PPBP | pro-platelet basic protein (chemokine (C-X-C motif) ligand 7) | -4.09 |
| A_23_P153185 | NM_001143818 | SERPINB2 | serpin peptidase inhibitor, clade B (ovalbumin), member 2 | -3.99 |
| A_23_P15146 | NM_001012631 | IL32 | interleukin 32 | -3.98 |
| A_23_P207564 | NM_002984 | CCL4 | chemokine (C-C motif) ligand 4 | -3.97 |
| A_23_P1691 | NM_002421 | MMP1 | matrix metallopeptidase 1 (interstitial collagenase) | -3.96 |
| A_23_P393620 | NM_006528 | TFPI2 | tissue factor pathway inhibitor 2 | -3.95 |
| A_24_P245379 | NM_002575 | SERPINB2 | serpin peptidase inhibitor, clade B (ovalbumin), member 2 | -3.73 |
| A_23_P85503 | NM_020393 | PGLYRP4 | peptidoglycan recognition protein 4 | -3.72 |
| A_23_P94533 | NM_001912 | CTSL1 | cathepsin L1 | -3.66 |
| A_24_P85888 | NM_006922 | SCN3A | sodium channel, voltage-gated, type III, alpha subunit | -3.65 |
| A_23_P404494 | NM_002185 | IL7R | interleukin 7 receptor | -3.48 |
| A_32_P26092 | NM_003327 | TNFRSF4 | tumor necrosis factor receptor superfamily, member 4 | -3.47 |
| A_23_P256470 | NM_000905 | NPY | neuropeptide Y | -3.45 |
| A_24_P79403 | NM_002619 | PF4 | platelet factor 4 | -3.40 |
| A_23_P62652 | NM_003037 | SLAMF1 | signaling lymphocytic activation molecule family member 1 | -3.30 |

| | | | | |
|---|---|---|---|---|
| A_24_P936145 | NM_138736 | GNAO1 | guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O | -3.25 |
| A_23_P23048 | NM_002965 | S100A9 | S100 calcium binding protein A9 | -3.25 |
| A_32_P65616 | NM_000948 | PRL | prolactin | -3.21 |
| A_23_P83184 | NM_198946 | LCN6 | lipocalin 6 | -3.14 |
| A_23_P434809 | NM_002964 | S100A8 | S100 calcium binding protein A8 | -3.01 |
| A_23_P8640 | NM_001039966 | GPER | G protein-coupled estrogen receptor 1 | -3.01 |
| A_23_P125278 | NM_005409 | CXCL11 | chemokine (C-X-C motif) ligand 11 | -2.98 |
| A_23_P51936 | NM_001561 | TNFRSF9 | tumor necrosis factor receptor superfamily, member 9 | -2.98 |
| A_23_P398566 | NM_173200 | NR4A3 | nuclear receptor subfamily 4, group A, member 3 | -2.98 |
| A_23_P129665 | NM_005353 | ITGAD | integrin, alpha D | -2.97 |
| A_23_P133445 | NM_006144 | GZMA | granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3) | -2.91 |
| A_23_P209129 | NM_002288 | LAIR2 | leukocyte-associated immunoglobulin-like receptor 2 | -2.89 |
| A_24_P252945 | NM_032966 | CXCR5 | chemokine (C-X-C motif) receptor 5 | -2.88 |
| A_23_P7582 | NM_003202 | TCF7 | transcription factor 7 (T-cell specific, HMG-box) | -2.83 |
| A_23_P93348 | NM_002341 | LTB | lymphotoxin beta (TNF superfamily, member 3) | -2.82 |
| A_24_P303091 | NM_001565 | CXCL10 | chemokine (C-X-C motif) ligand 10 | -2.81 |
| A_23_P404481 | NM_001400 | S1PR1 | sphingosine-1-phosphate receptor 1 | -2.77 |
| A_23_P312920 | NM_006235 | POU2AF1 | POU class 2 associating factor 1 | -2.76 |
| A_23_P51767 | NM_001765 | CD1C | CD1c molecule | -2.76 |
| A_23_P212061 | NM_007289 | MME | membrane metallo-endopeptidase | -2.75 |
| A_24_P290751 | NM_004416 | DTX1 | deltex homolog 1 (Drosophila) | -2.71 |
| A_23_P111126 | L06175 | HCP5 | HLA complex P5 (non-protein coding) | -2.71 |
| A_23_P29152 | NM_007128 | VPREB1 | pre-B lymphocyte 1 | -2.69 |
| A_23_P203882 | NM_002429 | MMP19 | matrix metallopeptidase 19 | -2.69 |
| A_24_P260101 | NM_007289 | MME | membrane metallo-endopeptidase | -2.68 |
| A_23_P359245 | NM_000245 | MET | met proto-oncogene (hepatocyte growth factor receptor) | -2.66 |
| A_23_P31816 | NM_005217 | DEFA3 | defensin, alpha 3, neutrophil-specific | -2.64 |
| A_23_P212360 | NM_006641 | CCR9 | chemokine (C-C motif) receptor 9 | -2.62 |
| A_23_P315964 | NM_173568 | UMODL1 | uromodulin-like 1 | -2.59 |

| | | | | |
|---|---|---|---|---|
| A_23_P38630 | NM_001050 | SSTR2 | somatostatin receptor 2 | -2.57 |
| A_23_P82651 | NM_002523 | NPTX2 | neuronal pentraxin II | -2.54 |
| A_23_P211233 | NM_001849 | COL6A2 | collagen, type VI, alpha 2 | -2.52 |
| A_32_P87013 | NM_000584 | IL8 | interleukin 8 | -2.49 |
| A_24_P17870 | NR_040662 | HCP5 | HLA complex P5 (non-protein coding) | -2.46 |
| A_23_P38795 | NM_002029 | FPR1 | formyl peptide receptor 1 | -2.45 |
| A_23_P207058 | NM_003955 | SOCS3 | suppressor of cytokine signaling 3 | -2.43 |
| A_23_P97141 | NM_002922 | RGS1 | regulator of G-protein signaling 1 | -2.43 |
| A_23_P4662 | NM_005178 | BCL3 | B-cell CLL/lymphoma 3 | -2.42 |
| A_23_P116371 | NM_152866 | MS4A1 | membrane-spanning 4-domains, subfamily A, member 1 | -2.39 |
| A_23_P40174 | NM_004994 | MMP9 | matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) | -2.38 |
| A_23_P72096 | NM_000575 | IL1A | interleukin 1, alpha | -2.35 |
| A_23_P133408 | NM_000758 | CSF2 | colony stimulating factor 2 (granulocyte-macrophage) | -2.35 |
| A_24_P20607 | NM_005409 | CXCL11 | chemokine (C-X-C motif) ligand 11 | -2.35 |
| A_23_P209055 | NM_001771 | CD22 | CD22 molecule | -2.33 |
| A_23_P77612 | NM_172229 | KREMEN2 | kringle containing transmembrane protein 2 | -2.32 |
| A_24_P254106 | AK026467 | CD22 | CD22 molecule | -2.32 |
| A_23_P119202 | NM_001252 | CD70 | CD70 molecule | -2.31 |
| A_32_P32254 | NM_001848 | COL6A1 | collagen, type VI, alpha 1 | -2.30 |
| A_23_P74001 | NM_005621 | S100A12 | S100 calcium binding protein A12 | -2.29 |
| A_23_P98410 | NM_000073 | CD3G | CD3g molecule, gamma (CD3-TCR complex) | -2.27 |
| A_24_P318656 | NM_000212 | ITGB3 | integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61) | -2.26 |
| A_23_P163402 | NM_000499 | CYP1A1 | cytochrome P450, family 1, subfamily A, polypeptide 1 | -2.26 |
| A_23_P119478 | NM_005755 | EBI3 | Epstein-Barr virus induced 3 | -2.23 |
| A_23_P45871 | NM_006820 | IFI44L | interferon-induced protein 44-like | -2.22 |
| A_32_P85500 | NR_040662 | HCP5 | HLA complex P5 (non-protein coding) | -2.22 |
| A_23_P121695 | NM_006419 | CXCL13 | chemokine (C-X-C motif) ligand 13 | -2.20 |
| A_24_P411121 | NM_148901 | TNFRSF18 | tumor necrosis factor receptor superfamily, member 18 | -2.20 |
| A_23_P310274 | NM_002770 | PRSS2 | protease, serine, 2 (trypsin 2) | -2.18 |
| A_32_P94444 | NM_002770 | PRSS2 | protease, serine, 2 (trypsin 2) | -2.16 |

| | | | | |
|---|---|---|---|---|
| A_24_P165864 | NM_014879 | P2RY14 | purinergic receptor P2Y, G-protein coupled, 14 | -2.16 |
| A_23_P214360 | NM_002460 | IRF4 | interferon regulatory factor 4 | -2.16 |
| A_23_P345118 | NM_002648 | PIM1 | pim-1 oncogene | -2.15 |
| A_23_P50508 | NM_003706 | PLA2G4C | phospholipase A2, group IVC (cytosolic, calcium-independent) | -2.12 |
| A_23_P49759 | NM_002981 | CCL1 | chemokine (C-C motif) ligand 1 | -2.11 |
| A_24_P185854 | NM_004010 | DMD | dystrophin | -2.07 |
| A_23_P360744 | NM_000448 | RAG1 | recombination activating gene 1 | -2.07 |
| A_23_P114185 | NM_004615 | TSPAN7 | tetraspanin 7 | -2.05 |
| A_23_P7144 | NM_001511 | CXCL1 | chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) | -2.01 |
| A_23_P55706 | NM_006509 | RELB | v-rel reticuloendotheliosis viral oncogene homolog B | -2.01 |

**Supplementary Table 3**. Genes that were up-regulated on $CD34^+$ cells exposed to NP210-PFCE for 4 h followed by 20 h of culture in the absence of NPs (total time: 24 h).

| Unique ID | Target ID | Gene Symbol | Gene Name | M Value |
|---|---|---|---|---|
| A_23_P15174 | NM_005949 | MT1F | metallothionein 1F | 3.86 |
| A_24_P97342 | NM_021935 | PROK2 | prokineticin 2 | 3.77 |
| A_23_P110531 | NM_013409 | FST | follistatin | 3.63 |
| A_23_P66241 | NM_176870 | MT1M | metallothionein 1M | 3.54 |
| A_23_P201538 | NM_002228 | JUN | jun proto-oncogene | 3.46 |
| A_23_P106194 | NM_005252 | FOS | FBJ murine osteosarcoma viral oncogene homolog | 3.39 |
| A_24_P42264 | NM_000239 | LYZ | lysozyme | 3.28 |
| A_23_P130961 | NM_001972 | ELANE | elastase, neutrophil expressed | 3.08 |
| A_24_P125096 | NM_005952 | MT1X | metallothionein 1X | 2.99 |
| A_23_P206724 | NM_175617 | MT1E | metallothionein 1E | 2.98 |
| A_23_P63343 | NM_021995 | UTS2 | urotensin 2 | 2.92 |
| A_23_P303242 | NM_005952 | MT1X | metallothionein 1X | 2.92 |
| A_23_P414343 | NM_005951 | MT1H | metallothionein 1H | 2.83 |
| A_23_P89192 | NM_000502 | EPX | eosinophil peroxidase | 2.81 |
| A_23_P156327 | NM_000358 | TGFBI | transforming growth factor, beta-induced, 68kDa | 2.81 |
| A_23_P151637 | NM_002934 | RNASE2 | ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin) | 2.76 |
| A_23_P427703 | NR_001447 | MT1L | metallothionein 1L (gene/pseudogene) | 2.75 |
| A_23_P60933 | NM_005950 | MT1G | metallothionein 1G | 2.73 |
| A_23_P163025 | NM_002935 | RNASE3 | ribonuclease, RNase A family, 3 | 2.72 |
| A_23_P404536 | NM_005021 | ENPP3 | ectonucleotide pyrophosphatase/phosphodiesterase 3 | 2.70 |
| A_32_P1712 | NM_002934 | RNASE2 | ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin) | 2.69 |
| A_23_P141173 | NM_000250 | MPO | myeloperoxidase | 2.62 |
| A_23_P500464 | NM_001844 | COL2A1 | collagen, type II, alpha 1 | 2.47 |
| A_23_P116898 | NM_000014 | A2M | alpha-2-macroglobulin | 2.47 |
| A_24_P236251 | NM_003836 | DLK1 | delta-like 1 homolog (Drosophila) | 2.47 |
| A_23_P37983 | NM_005947 | MT1B | metallothionein 1B | 2.45 |
| A_23_P43337 | NM_144966 | FREM1 | FRAS1 related extracellular matrix 1 | 2.43 |
| | | | proteoglycan 2, bone marrow (natural killer cell | |

| | | | | |
|---|---|---|---|---|
| | | | activator, eosinophil granule major basic protein) | |
| A_23_P163782 | NM_005951 | MT1H | metallothionein 1H | 2.36 |
| A_23_P137935 | NM_002432 | MNDA | myeloid cell nuclear differentiation antigen | 2.36 |
| A_23_P8981 | NM_000349 | STAR | steroidogenic acute regulatory protein | 2.34 |
| A_24_P11825 | NM_001123041 | CCR2 | chemokine (C-C motif) receptor 2 | 2.29 |
| A_24_P453970 | AV691872 | ENPP3 | Ectonucleotide pyrophosphatase/phosphodiesterase 3 | 2.22 |
| A_23_P157879 | NM_002003 | FCN1 | ficolin (collagen/fibrinogen domain containing) 1 | 2.19 |
| A_23_P215913 | NM_001831 | CLU | clusterin | 2.15 |
| | | | hepatocyte growth factor (hepapoietin A; scatter factor) | |
| A_24_P944788 | ENST00000222390 | HGF-001 | [Source:HGNC Symbol;Acc:4893] | 2.12 |
| A_23_P212354 | NM_001123041 | CCR2 | chemokine (C-C motif) receptor 2 | 2.08 |
| A_24_P45620 | NM_021995 | UTS2 | urotensin 2 | 2.06 |

**Supplementary Table 4.** Genes that were down-regulated on $CD34^{+}$ cells exposed to NP210-PFCE for 4 h followed by 7 days of culture in the absence of NPs.

| Unique ID | Target ID | Gene Symbol | Gene Name | M Value |
|---|---|---|---|---|
| A_23_P26965 | NM_005408 | CCL13 | chemokine (C-C motif) ligand 13 | -4.33 |
| A_23_P15146 | NM_001012631 | IL32 | interleukin 32 | -3.08 |
| A_23_P256425 | NM_014479 | ADAMDEC1 | ADAM-like, decysin 1 | -2.57 |
| A_23_P137366 | NM_000491 | C1QB | complement component 1, q subcomponent, B chain | -2.56 |
| A_23_P340698 | NM_002426 | MMP12 | matrix metallopeptidase 12 (macrophage elastase) | -2.55 |
| A_23_P149545 | NM_003528 | HIST2H2BE | histone cluster 2, H2be | -2.47 |
| A_24_P260101 | NM_007289 | MME | membrane metallo-endopeptidase | -2.44 |
| A_23_P101407 | NM_000064 | C3 | complement component 3 | -2.35 |
| A_23_P106194 | NM_005252 | FOS | FBJ murine osteosarcoma viral oncogene homolog | -2.17 |
| A_23_P40174 | NM_004994 | MMP9 | matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase) | -2.11 |
| A_23_P74001 | NM_005621 | S100A12 | S100 calcium binding protein A12 | -2.04 |

**Supplementary Table 5.** Genes that were up-regulated on $CD34^+$ cells exposed to NP210-PFCE for 4 h followed by 7 days of culture in the absence of NPs.

| Unique ID | Target ID | Gene Symbol | Gene Name | M Value |
|---|---|---|---|---|
| A_23_P17663 | NM_002462 | MX1 | myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse) | 4.58 |
| A_23_P121596 | NM_002704 | PPBP | pro-platelet basic protein (chemokine (C-X-C motif) ligand 7) | 4.09 |
| A_23_P66241 | NM_176870 | MT1M | metallothionein 1M | 3.43 |
| A_23_P131785 | NM_001725 | BPI | bactericidal/permeability-increasing protein | 3.25 |
| A_23_P48212 | NM_016509 | CLEC1B | C-type lectin domain family 1, member B | 3.21 |
| A_23_P53137 | NM_000559 | HBG1 | hemoglobin, gamma A | 3.12 |
| A_23_P64539 | NM_000559 | HBG1 | hemoglobin, gamma A | 2.88 |
| A_23_P17481 | NM_023068 | SIGLEC1 | sialic acid binding Ig-like lectin 1, sialoadhesin | 2.84 |
| A_23_P326080 | NM_001925 | DEFA4 | defensin, alpha 4, corticostatin | 2.71 |
| A_23_P304897 | NM_000623 | BDKRB2 | bradykinin receptor B2 | 2.60 |
| A_23_P15174 | NM_005949 | MT1F | metallothionein 1F | 2.44 |
| A_24_P97342 | NM_021935 | PROK2 | prokineticin 2 | 2.38 |
| A_23_P206724 | NM_175617 | MT1E | metallothionein 1E | 2.33 |
| A_23_P137697 | NM_003005 | SELP | selectin P (granule membrane protein 140kDa, antigen CD62) | 2.27 |
| A_24_P125096 | NM_005952 | MT1X | metallothionein 1X | 2.23 |
| A_23_P135486 | NM_016633 | AHSP | alpha hemoglobin stabilizing protein | 2.22 |
| A_23_P37856 | NM_000558 | HBA1 | hemoglobin, alpha 1 | 2.20 |
| A_23_P87346 | NM_000519 | HBD | hemoglobin, delta | 2.18 |
| A_23_P60933 | NM_005950 | MT1G | metallothionein 1G | 2.18 |
| A_23_P303242 | NM_005952 | MT1X | metallothionein 1X | 2.15 |
| A_23_P6263 | NM_002463 | MX2 | myxovirus (influenza virus) resistance 2 (mouse) | 2.14 |
| A_23_P427703 | NR_001447 | MT1L | metallothionein 1L (gene/pseudogene) | 2.14 |
| A_23_P414343 | NM_005951 | MT1H | metallothionein 1H | 2.11 |
| A_23_P354146 | NM_005546 | ITK | IL2-inducible T-cell kinase | 2.04 |
| A_23_P26457 | NM_000517 | HBA2 | hemoglobin, alpha 2 | 2.01 |

**Supplementary Table 6.** Significantly affected biological processes after 24 h and 7 d.

| Significantly affected biological processes for CD34$^+$ cells after 24 hours | | | |
|---|---|---|---|
| | **DAVID category** | **Nº of genes** | **List of genes** |
| **Down-regulated** | Immune response | 41 | CXCL1, CCL1, CSF2, IL1F9, CCL3, CCL2, VPREB1, RAG1, CD70, IL32, PF4, IFI44L, CXCL11, IL7R, TNFRSF4, CCL4, CXCL10, LOC100133678, PGLYRP4, CCL3L1, CCL3L3, MS4A1, BCL3, IGKC, LTB, EBI3, IL1A, POU2AF1, IL6, TCF7, IL18RAP, IL8, GZMA, RELB, CD1C, LOC652493, HLA-DQA1, CCR9, CCR7, RGS1, PPBP, CXCL13, P2RY14, ITGAD, LOC650405, LOC100130100 |
| | Chemotaxis | 17 | CCL1, CXCL1, IL6, CCL3, CCL2, IL8, S100A9, FPR1, PF4, CXCL11, CCL4, CXCL10, CCR9, CCR7, PPBP, CXCL13, CCL3L1, CCL3L3 |
| | Defense response | 26 | CXCL1, CCL3, CCL2, S100A8, INS-IGF2, S100A9, IL32, CXCL11, TNFRSF4, CCL4, CXCL10, PGLYRP4, CCL3L1, INS, CCL3L3, BCL3, HCP5, IL1A, IL6, IL18RAP, IL8, IGF2, S100A12, CCR9, CCR7, PPBP, CXCL13, DEFA3, PLA2G4C |
| | Response to wounding | 24 | CXCL1, CCL3, CCL2, S100A8, INS-IGF2, S100A9, PF4, ITGB3, CXCL11, CCL4, TNFRSF4, CXCL10, CCL3L1, INS, CCL3L3, TFPI2, IL1A, IL6, IL18RAP, IL8, CYP1A1, IGF2, S100A12, CCR7, CXCL13, SERPINB2, PLA2G4C |
| | Locomotory behavior | 18 | CCL1, CXCL1, IL6, CCL3, GNAO1, CCL2, IL8, S100A9, FPR1, PF4, CXCL11, CCL4, CXCL10, CCR9, CCR7, PPBP, CXCL13, CCL3L1, CCL3L3 |
| | Inflammatory response | 19 | CXCL1, IL6, CCL3, IL18RAP, CCL2, IL8, S100A8, INS-IGF2, S100A9, IGF2, CXCL11, TNFRSF4, CCL4, S100A12, CXCL10, CCR7, INS, CXCL13, CCL3L1, CCL3L3, PLA2G4C, IL1A |
| | Behavior | 21 | CCL1, CXCL1, IL6, CCL3, GNAO1, CCL2, IL8, S100A9, MET, FPR1, PF4, NR4A3, CXCL11, CCL4, CXCL10, CCR9, CCR7, PPBP, NPY, CXCL13, CCL3L1, CCL3L3 |
| | Cell activation | 16 | CSF2, IL6, CD3G, IL8, INS-IGF2, RELB, RAG1, IGF2, PF4, IL7R, TNFRSF4, SLAMF1, CXCR5, INS, MS4A1, BCL3, IRF4, LTB |
| | Leukocyte activation | 13 | CSF2, CD3G, IL8, INS-IGF2, RELB, RAG1, IGF2, IL7R, SLAMF1, TNFRSF4, CXCR5, INS, MS4A1, BCL3, IRF4 |
| | Lymphocyte activation | 11 | CD3G, INS-IGF2, RELB, RAG1, IGF2, IL7R, SLAMF1, TNFRSF4, CXCR5, INS, MS4A1, BCL3, IRF4 |
| | Regulation of apoptosis | 19 | CSF2, IL6, CD3G, CCL2, INS-IGF2, SOCS3, MMP9, GPR109B, GPR109A, PIM1, RAG1, CD70, IGF2, PF4, TNFRSF4, TNFRSF9, INS, TNFRSF18, SERPINB2, BCL3, LTB, IL1A |
| | Lymph node development | 4 | CXCR5, CXCL13, IL7R, LTB |
| | T cell activation | 8 | CD3G, INS-IGF2, INS, RELB, RAG1, BCL3, IGF2, IRF4, IL7R, TNFRSF4 |
| | Cell-cell signaling | 16 | IL1F9, CCL3, IL6, INS-IGF2, S100A9, MME, CD70, IGF2, CXCL11, CCL4, CXCL10, SSTR2, NPY, INS, CXCL13, NPTX2, DMD, LTB |
| | Cell surface receptor linked signal transduction | 31 | CXCL1, CCL3, CCL2, INS-IGF2, GPR109B, GPR109A, FPR1, PF4, ITGB3, CXCL11, IL7R, CXCL10, S1PR1, CXCR5, INS, PRL, TCF7, GNAO1, CD3G, IL18RAP, IL8, GPER, DTX1, MET, IGF2, CCR9, CCR7, SSTR2, RGS1, PPBP, NPY, KREMEN2, P2RY14, ITGAD |
| | Regulation of cytokine production | 9 | IL6, INS-IGF2, INS, BCL3, IGF2, PF4, IRF4, TNFRSF4, LTB, EBI3, IL1A |
| | Negative regulation of apoptosis | 12 | CSF2, IL6, CCL2, INS-IGF2, SOCS3, RAG1, PIM1, PF4, IGF2, INS, TNFRSF18, SERPINB2, BCL3, IL1A |
| | Response to bacterium | 9 | IL6, CCL2, CYP1A1, PPBP, PGLYRP4, SOCS3, DEFA3, BCL3, S100A12 |
| | Cell chemotaxis | 5 | IL6, CCL2, IL8, S100A9, PF4 |
| | Regulation of cytokine biosynthetic process | 6 | IL6, BCL3, IRF4, LTB, EBI3, IL1A |
| | Immune system development | 10 | CSF2, CXCR5, CXCL13, MMP9, RELB, RAG1, BCL3, IRF4, IL7R, LTB |
| | Regulation of cell proliferation | 17 | CXCL1, CSF2, IL6, CCL2, IL8, INS-IGF2, IGF2, TNFRSF4, SLAMF1, CXCL10, TNFRSF9, SSTR2, S1PR1, INS, CCL3L1, CCL3L3, LTB, PRL, EBI3, IL1A |
| | G-protein coupled receptor protein signaling pathway | 21 | CXCL1, CCL3, GNAO1, CCL2, IL8, INS-IGF2, GPER, GPR109B, GPR109A, FPR1, IGF2, PF4, CXCL11, CXCL10, CCR9, SSTR2, CCR7, S1PR1, RGS1, PPBP, CXCR5, NPY, INS, P2RY14 |
| | Collagen catabolic process | 4 | MMP9, PRSS2, MMP19, MMP1 |
| | Positive regulation of cytokine biosynthetic process | 5 | BCL3, IRF4, LTB, EBI3, IL1A |
| | Leukocyte differentiation | 7 | CSF2, MMP9, RELB, RAG1, BCL3, IRF4, IL7R |
| **Up-regulated** | Response to inorganic substance | 8 | FOS, A2M, STAR, EPX, JUN, MPO, MT1H, MT1X |
| | Defense response | 10 | FOS, PROK2, A2M, RNASE3, PRG2, CCR2, CLU, MNDA, LYZ, MPO |
| | Response to oxidative stress | 6 | FOS, STAR, EPX, JUN, CLU, MPO |

| Significantly affected biological processes for $CD34^+$ cells after 7 days | | | |
|---|---|---|---|
| | **DAVID category** | **Nº of genes** | **List of genes** |
| **Down-regulated** | Defense response | 9 | C1QB, FOS, CCL13, LOC100133511, LOC653879, C3, HIST2H2BE, IL32, S100A12 |
| **Up-regulated** | Defense response | 12 | SELP, ITK, SIGLEC1, PROK2, BPI, PPBP, IL8, DEFA4, BDKRB2, MX1, MX2, CLEC1B |

**Supplementary Table 7.** Significantly affected cellular compartments after 24 h and 7 d.

| **Significantly affected cellular compartments for CD34$^+$ cells after 24 hours** | | | |
|---|---|---|---|
| | **DAVID category** | **Nº of genes** | **List of genes** |
| **Down-regulated** | Extracellular region part | 34 | CXCL1, CCL1, CSF2, IL1F9, CCL3, CCL2, INS-IGF2, MMP9, CD70, PF4, IL32, CXCL11, CCL4, MMP1, CXCL10, INS, CCL3L1, PRSS2, CCL3L3, COL6A2, COL6A1, TFPI2, LTB, IL1A, EBI3, IL6, IL8, MMP19, IGF2, PPBP, NPY, CXCL13, DEFA3, SERPINB2 |
| | Extracellular region | 50 | INS-IGF2, MMP9, SPINK1, CXCL11, MMP1, CXCL10, PGLYRP4, CCL3L1, CCL3L3, LTB, PRL, TFPI2, IL1A, EBI3, GZMA, LAIR2, MMP19, LOC652493, UMODL1, PPBP, SERPINB2, DEFA3, CXCL1, CCL1, IL1F9, CSF2, CCL3, CCL2, VPREB1, CD70, PF4, IL32, IL7R, CCL4, CTSL1, NPTX2, INS, PRSS2, COL6A2, TNFRSF18, COL6A1, IGKC, IL6, IL8, IGF2, NPY, LCN6, CXCL13, LOC650405, LOC100130100 |
| | Extracellular space | 29 | CXCL1, CCL1, CSF2, IL1F9, CCL3, CCL2, INS-IGF2, MMP9, CD70, PF4, IL32, CXCL11, CCL4, CXCL10, CCL3L1, INS, PRSS2, CCL3L3, LTB, IL1A, EBI3, IL6, IL8, IGF2, PPBP, NPY, CXCL13, SERPINB2, DEFA3 |
| | Intergral to plasma membrane/ intrinsic to plasma membrane | 24 | FXYD2, IL6, CD3G, SCN3A, GPER, GPR109B, GPR109A, MET, CD1C, TSPAN7, MME, CD70, ITGB3, TNFRSF4, HLA-DQA1, CCR9, LOC100133678, TNFRSF9, SSTR2, CCR7, CXCR5, MS4A1, CD22, ITGAD |
| **Up-regulated** | Extracellular region | 18 | A2M, UTS2, RNASE2, RNASE3, ENPP3, FST, PRG2, ELANE, CLU, LYZ, COL2A1, DLK1, HGF, PROK2, FREM1, TGFB1, FCN1, MPO |

| **Significantly affected cellular compartments for CD34$^+$ cells after 7 days** | | | |
|---|---|---|---|
| **Up-regulated** | **DAVID category** | **Nº of genes** | **List of genes** |
| | Hemoglobin complex | 5 | AHSP, HBG1, HBA2, HBA1, HBD |

**Supplementary Table 8.** Significantly affected molecular functions after 24 h and 7 d.

| **Significantly affected molecular functions for CD34$^+$ cells after 24 hours** | | | |
|---|---|---|---|
| | **DAVID category** | **Nº of genes** | **List of genes** |
| **Down-regulated** | Cytokine activity | 21 | CCL1, CXCL1, CSF2, IL1F9, CCL3, IL6, CCL2, IL8, CD70, IL32, PF4, CXCL11, CCL4, CXCL10, PPBP, CXCL13, CCL3L1, CCL3L3, LTB, EBI3, IL1A |
| | Chemokine activity/ chemokine receptor binding | 13 | CXCL1, CCL1, CCL3, CCL2, IL8, PF4, CXCL11, CCL4, CXCL10, PPBP, CXCL13, CCL3L1, CCL3L3 |
| | Cytokine binding | 8 | CCR9, CCR7, CXCR5, IL10RA, TNFRSF18, IL7R, TNFRSF4, EBI3 |
| **Up-regulated** | Ion binding | 10 | MT1L, MT1M, MT1E, MT1B, MT1JP, MT1H, MT1G, MT1P3, MT1X, MT1F |

| **Significantly affected molecular functions for CD34$^+$ cells after 7 days** | | | |
|---|---|---|---|
| | **DAVID category** | **Nº of genes** | **List of genes** |
| **Down-regulated** | Metalloendopeptidase activity | 4 | MMP9, MME, ADAMDEC1, MMP12 |
| **Up-regulated** | Ion binding | 9 | MT1L, MT1M, MT1E, MT1JP, MT1H, MT1G, MT1P3, MT1X, MT1F |