Supplemental Documents

MACROPHAGE-DERIVED ONCOSTATIN M CONTRIBUTES TO HUMAN AND MOUSE NEUROGENIC HETEROTOPIC OSSIFICATIONS

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Supplemental "Material and Method" section

MSC transcriptomic analysis and bioinformatics

After extraction, RNA quality was checked via Agilent 2100 Bioanalyzer platform (Agilent technologies). Microarray RNA libraries were performed with T7-based amplification step and labeled with Cyanin 3 starting from 1 microgram of total RNA (Quick Amp Labeling Kit, Agilent technologies). Overnight hybridization was performed at 65°C on 4x44K V1 Agilent Human Oligo Microarray (Agilent technologies). Microarrays were scan with Microarray Scanner System (Agilent Technologies) and normalized with Agilent Feature Extraction Software.

A "Bone Marrow Niche" geneset curated from Pubmed database was built as already described (1). Briefly, by using keywords such as bone marrow, osteoblasts, cytokines, cell communication, macrophages, stromal cells and bone marrow stroma, we have been able to list genes related to the bone marrow niche(s). A supervised microarray analysis by using Significance Analysis of Microarray was performed between NHO-MSC and BM-MSC samples and classes them according to the previous selected genes. Differential expressed genes (DEG) were determined by using multi-testing correction with a threshold of 0.05 for the False Discovery Rate (2). Unsupervised classification using Euclidean distances was performed on the DEG list to validate discrimination between NHO-MSCs and BM-MSCs samples. Heatmap was drawn with heatplot function from made4 R-package (3) and using principal component analysis performed with FactoMineR R-package. Statistical analysis was performed in R software environment version 3.0.2. Functional enrichment was performed with Enrichr webtool on Wikipathway database (4). Normalized data are accessible on public database with the GSE94683 assigned GEO accession number.

Side population (SP) cell detection

Lin^- cells were stained with hoechst33342 (5µg/ml/10^6 cells) in pre-warmed DMEM, 2% FCS, 10mM HEPES (Life Technologies) at 37°C for 90min (5). After incubation, cells were placed on ice and stained with CD45, CD34, CD38 antibodies and 7AAD for viability (Supplemental Table 5). Hoechst33342 active efflux was confirmed by the addition of 50µM verapamil (Sigma-Aldrich) to control samples before Hoechst incubation as previously described (6).

To analyze the effect of stromal cells on the induction of SP cells from unmobilized peripheral blood, Lin^- cells were cultured alone or on a confluent layer of human NHO-MSCs in SynH medium (ABCell-Bio) supplemented with SCF, TPO and Flt3L (10ng/ml, Miltenyi Biotec). After 4 days, cells were flushed and stained with hoechst33342, CD45, CD34, CD38 antibodies and 7AAD for viability as previously described (5).

All flow cytometry analysis was carried out on BD Fortessa apparatus using FACSDiva software (BD Biosciences).

Long term culture-initiating cell (LTC-IC) assays

$CD34^+$ cells ($5x10^3$) isolated from the BM of HDs were seeded or not on $2x10^4$ NHO-MSCs (previously irradiated at 60 Gy) in a 96-well plate. Cells were cultured for 5 weeks at 37°C in 5% CO_2 atmosphere. Each week, 10^3 hematopoietic cells from co-cultures were seeded in methylcellulose medium with cytokines and cultured for 14 days using the same conditions that those described above. Colony forming units were then scored using an inverted microscope.

Xenografts of CD34+ cells from human NHOs into immunodeficient mice

NSG immunodeficient mice were conditioned by 2 intraperitoneal injections of 25 mg/kg of Busulfan (Busilvex®, Pierre Fabre) at 24 hours interval as previously described (7). On the

following day, NSG mice were anesthetized by intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine and retro-orbital injection of $CD34^+$ cells (0.5-6 10^5 per mouse) from human NHOs were performed. After 2 months, mouse BM cells were recovered, labelled with specific human and mouse antibodies (Supplemental Table 5) and analyzed with BD Fortessa flow cytometer (BD Biosciences).

Total BM cells from NSG mice engrafted with $CD34^+$ cells from human NHO were then injected intravenously in secondary NSG recipient mice. Two months post-transplantation, bone marrow cells from secondary NSG recipient mice were analyzed by flow cytometry for expression human hematopoietic CD45 and B lymphoid CD19 markers.

Matrigel Assay

Vascular network formation was performed by seeding human endothelial cells (10^5cells/well/500µL) on Matrigel^{TM} (BD Biosciences) into a 12-well plate and cultured for 24 h at 37°C in 5% CO_2 atmosphere.

Expression of ICAM-1 and VCAM-1 on human endothelial cells

Human endothelial cells were seeded (10^5 cells/well/2mL) into a 6-well plate and stimulated with 10 ng/ml of TNF-α for 16 h at 37°C h in 5% CO_2 atmosphere. Cells were then washed with PBS, trypsinized and incubated with an antibody against human VCAM-1-AF488 or ICAM-1-PE (Supplemental Table 5; Biolegend) for 30 min at 4 °C. Cells were then washed with PBS containing 0.5% bovine serum albumin and analyzed with BD Fortessa flow cytometer (BD Biosciences).

Quantification of mineralization by Alizarin Red S staining

For human cells, quantification of mineralization was performed after fixation in 4% paraformaldehyde for 10 minutes and incubation in a solution of 2% Alizarin Red S (Sigma-Aldrich) for 5 minutes. Cells were then washed and dried. A buffer composed of 0.5N hydrochloric acid and 5% SDS was added for extraction of Alizarin Red S staining and read at 405 nm.

For mouse cells, wells were washed with PBS, fixed for 30 minutes in 4% paraformaldehyde at room temperature, washed in PBS, water and then air dry overnight. Fixed cells were incubated in 1% Alizarin Red S solution at room temp on a rotating rocker for 5-10 minutes. After rinsed with PBS, water and dried, wells were destained in 100µl destain solution (10% Cetylpyridinium Chloride dissolved in water containing 10mM sodium phosphate) for 15 minutes at room temp with gentle rocking. Destain solution was aliquoted into a new 96 well plate and the absorbance measured at 562nm.

In vitro adipogenic differentiation

Human stromal cells were seeded at 21,000 per cm^2 in α-MEM medium supplemented with 10% FCS and 1% antibiotics. After cells reached confluence, medium was removed and DMEM high glucose supplemented with 10% FCS, 1% antibiotics, 0.52 µg/mL dexamethasone, 0.2 mM indomethacine, 0.01 mg/mL insulin and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) (All from Sigma-Aldrich) were added for 3 days. Medium was removed and DMEM high glucose supplemented with 10% FCS, 1% antibiotics and 0.01 mg/mL insulin were added for 1 day. Medium was removed for 2 additional cycles as previously and incubated in DMEM high glucose supplemented with 10% FCS, 1% antibiotics and 0.01 mg/mL insulin for the last week.

Cells were cultured for 3 weeks at 37°C in 5% CO_2 atmosphere. Differentiation into adipocytes was evaluated by Oil Red O staining.

In vitro chondrogenic differentiation

Human stromal cells were seeded at $2.5x10^5$ in a conical tube (volume 15 mL) in α-MEM supplemented with 10% FCS, 1% antibiotics. Cells were centrifuged and washed in 500µL of DMEM high glucose supplemented with 10% FCS, 1% antibiotics, 0.052 µg/mL dexamethasone, 1 mM sodium pyruvate, 0.35 mM L-Proline, 1X Insulin-Transferrin-Selenium (ITS), 5.33 µg/mL linoleic acid, 0.044 mg/mL ascorbic acid (All from Sigma-Aldrich), 1.14 mg/mL human albumin (LFB). After an additional centrifugation, medium was removed and cells were resuspended in the same medium but supplemented or not with 0.01 µg/mL human TGF-β3 (Sigma-Aldrich). A last centrifugation was performed and cell pellets were incubated at 37°C in 5% CO_2 atmosphere for 3 weeks. Medium was changed two times in a week. Differentiation into chondrocytes was evaluated by Alcian blue staining.

OSM level evaluation by ELISA

For evaluation of OSM levels in plasma, blood samples from HDs and from patients with NHOs collected on citrate solution from Percy and Garches hospitals, respectively with the informed consent of patients were centrifuged at 2,000 RPM for 10 minutes at 4°C. Plasma fractions were conserved and frozen at -80°C. The OSM plasma level was evaluated using Human Oncostatin M ELISA Kit (Abcam) according to the manufacturer procedure.

For evaluation of OSM levels in conditioned media, $CD14^+$ macrophages isolated from NHOs were seeded at the density of $1.25\ 10^6$ per mL of medium (α-MEM 10% FCS 1% antibiotics) with or without LPS (100ng/mL). After 3 days of culture, supernatant was recovered, centrifuged

at 1500 rpm for 10mn and frozen at -80°C. The level of OSM contained in supernatants was evaluated using the same kit as previously described.

IHC on human and mouse tissues

For human tissues, embedded paraffin sections were deparaffined in xylene and rehydrated. For immunohistochemical staining, pre-treatment of sections with antigen retrieval citrate-based solution (Vector Laboratories, Burlingame, CA, USA) for anti-OSM (ab198830, Abcam,) and anti-lamin A/C (clone EPR4100, Epitomics) staining or with antigen retrieval Tris-based solution (Vector Laboratories) for CD68 (clone PG-M1, Dako) staining at 95°C for 15mn, was realized. Sections were incubated in Bloxall blocking solution (Vector Laboratories) for 10 mn to inactivate endogenous peroxidase. For lamin A/C staining, FC receptor blocking reagent (Innovex biosciences) was then added for 45 mn. All sections were incubated in PBS1X-FCS 10% for 30 mn and primary antibodies diluted in PBS1X-FCS 5%-BSA 1.5% (1/200 for anti-OSM, 1/500 for anti-lamin A/C; anti-CD68 was ready to use) were then added on sections overnight at 4°C. After washing in PBS1X-Triton 0.1%, ImPRESS REAGENT anti-rabbit IgG or anti-mouse Ig (Vector Laboratories) was incubated on sections for 30 mn. After washing in PBS1X-Triton 0.1%, HistoGreen substrate solution (Linaris) was added on sections for 1 mn. Counter coloration was done with Fast Nuclear Red (Vector Laboratories). All sections were analyzed using a Lamina slide scanner (Perkin Elmer) and with Pannoramic Viewer software (version 1.15.4).

For mouse tissues, a 3 step procedure using biotinylated F(ab)2 secondary antibodies (sc-3840, sc3826, sc-3854, Santa Cruz Biotechnologies) and horse radish peroxidase conjugated streptavidin (Dako) was used to detect primary antibodies. Primary antibodies used were: rat anti-mouse F4/80 (clone CI:A3-1, Abcam), rabbit anti-mouse Osterix/Sp7 (ab22552, Abcam), rabbit

anti-mouse collagen type 1 (C7510-13, US Biological), goat anti-mouse OSM (AF-495NA, R&D Systems), or relevant isotype control antibodies (ratIgG2b, 400602, Biolegend), normal rabbit IgG or normal goat IgG (Santa Cruz Biotechnology). Diaminobenzidine (Dako, Glostrup, Denmark) was used as the chromogen and sections were counterstained with Mayer's haematoxylin (Sigma Aldrich) and mounted using permanent mounting media (Thermo Fisher Scientific). Tissue staining was viewed using an Olympus BX50 microscope with an attached DP26 camera and imaged using Olympus CellSens standard 1.7 imaging software (Olympus).

Mouse mRNA extraction and qRT-PCR analysis

Muscle samples (right hamstring muscles) were harvested from mice that underwent SCI or sham SCI followed by intramuscular injection of CDTX or PBS 2 and 4 days post-surgery (3-4 mice/group). Muscle samples were homogenized in Trizol (15596018, Thermo Fisher Scientific) following manufactor's instruction.

Reverse transcription was performed using iScript cDNA kit (BioRad) per manufacturer's instructions. mRNA for *Osm* and *Actb* (β-actin*)* was measured using predesigned real-time PCR assays from Applied Biosystems (Thermo Fisher Scientific) *Osm* (Mm01193966_m1) and *Actb* (Mm01205647_g1). The PCR setting consisted of a denaturation step at 50°C (2 min), followed by 10 mins at 95°C. Amplifications were performed with 50 cycles each of 95°C (10 sec) and 60°C (60 sec). Results were normalized relative to β-actin mRNA.

Supplemental References

1. Desterke C, Martinaud C, Ruzehaji N, and Le Bousse-Kerdiles MC. Inflammation as a Keystone of Bone Marrow Stroma Alterations in Primary Myelofibrosis. *Mediators of inflammation.* 2015;2015(415024.
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4. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic acids research.* 2016;44(W1):W90-7.
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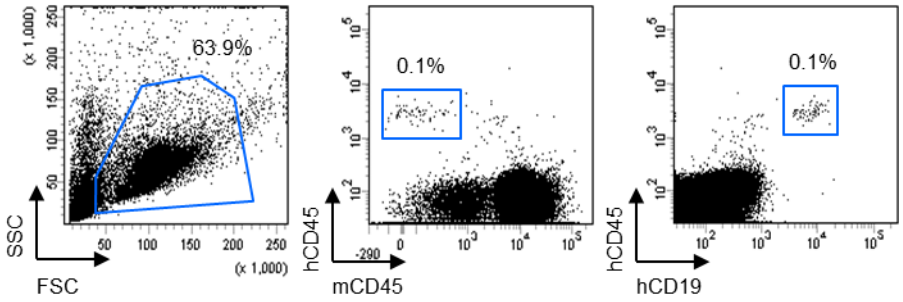
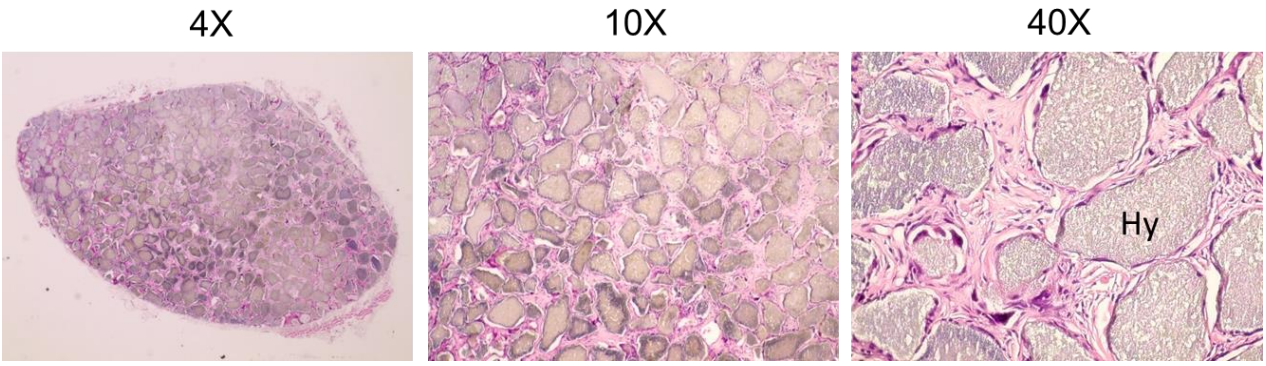
6. Goodell MA, Brose K, Paradis G, Conner AS, and Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *The Journal of experimental medicine.* 1996;183(4):1797-806.
7. Chevaleyre J, Duchez P, Rodriguez L, Vlaski M, Villacreces A, Conrad-Lapostolle V, Praloran V, Ivanovic Z, and Brunet de la Grange P. Busulfan administration flexibility increases the applicability of scid repopulating cell assay in NSG mouse model. *PloS one.* 2013;8(9):e74361.

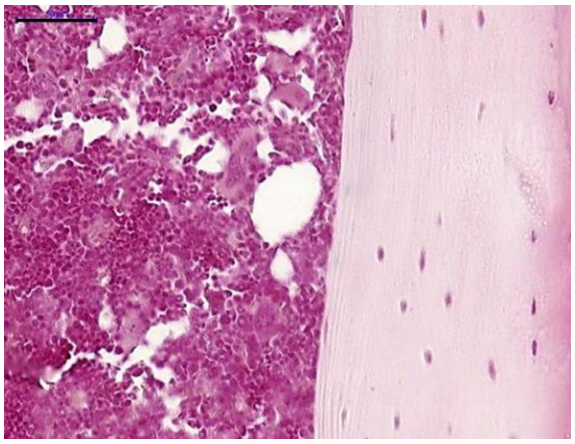
Supplemental Figures



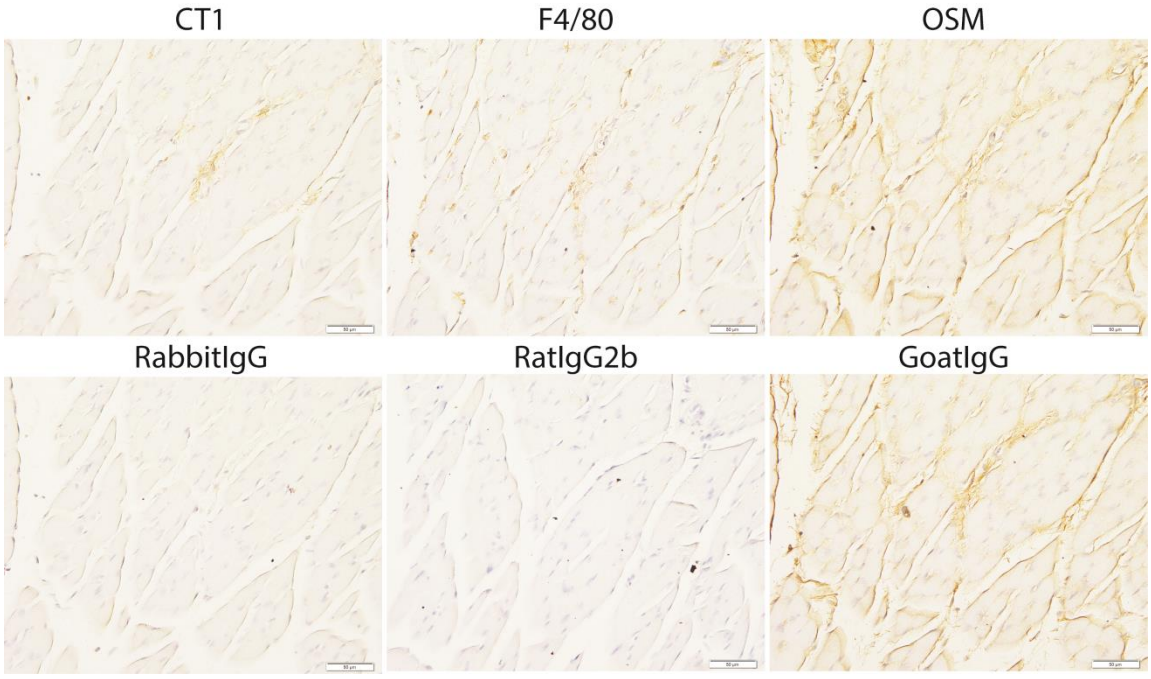
Supplemental Figure 1. Secondary graft of HSCs from human NHO in NSG mice. After a primary engraftment of $CD34^+$ cells from human NHO in NSG mice, total bone marrow cells from primary recipient mice were injected intravenously in secondary NSG recipient mice. Two months post-transplantation, bone marrow cells from secondary recipient mice were analyzed by flow cytometry for expression of human hematopoietic CD45 and B lymphoid CD19 markers. The figure shows a representative experiments on the two performed (n=2).



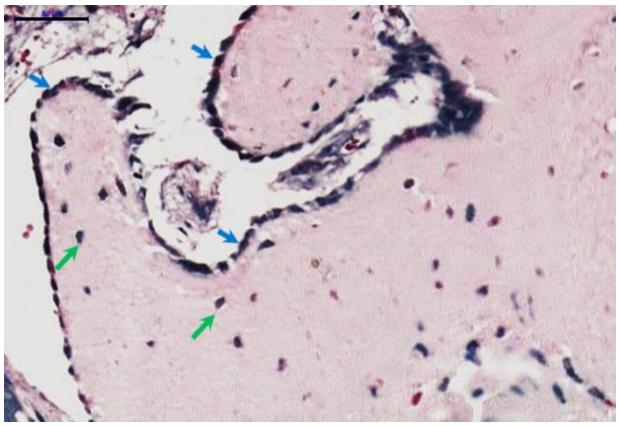
Supplemental Figure 2. Lack of bone and hematopoietic tissue formation in scaffolds implanted without NHO-MSCs into nude mice. Representative section 10 weeks after implantation in mice (n≥3) (H&E). Hy: Hydroxyapatite particles.



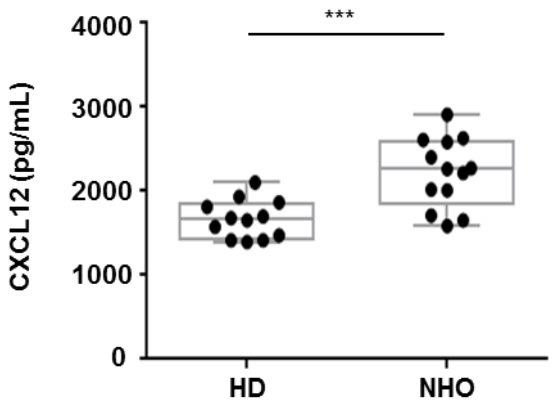
Supplemental Figure 3. Absence of human lamin A/C staining on murine bone marrow section. Human lamin A/C antibody was tested on C57BL/6 mouse bone marrow section as negative control. Scale bar: 50µm.



Supplemental Figure 4. Lack of NHO development in muscle of spinal cord injured (SCI) mice without cardiotoxin injection. Representative immunohistochemistry (IHC) images and matched isotype control antibodies in a SCI mouse (no CDTX injection) 21 days post-surgery. IHC for collagen type 1 (CT1) confirms the lack of CT1 positive bone foci and lack of F4/80^{+} macrophages and oncostatin M (OSM) in the muscle. Original magnification: x40, scale bar indicates 50μm.



Supplemental Figure 5. OSM staining on human NHO section. Arrows indicate the OSM staining in osteoblastic cells (blue arrows) and osteocytes (green arrows) from NHO biopsy of patient with a stroke. Scale bar: 50µm.



Supplemental Figure 6. CXCL12 concentration is elevated in plasma of NHO patients. CXCL12 level was measured by ELISA in plasma from healthy donors (HD) or patients with NHO (NHO). Each dot represents a different donor/patient, box and whisker show median, 25th and 75th percentile, minimum and maximum values (n=12-13). Asterisks indicate significance between human samples by non-parametric Mann-Whitney test (***, $p \leq 0.001$).

Supplemental Table 1 : Percentage of human CD45$^+$ subpopulations in bone marrow cells of primary NSG graft with CD34 cells from human NHO

Mice	% of human CD45$^+$ cells	% of cells in human CD45$^+$ population			
		CD45$^+$CD34$^+$	CD45$^+$CD19$^+$	CD45$^+$CD15$^+$	CD45$^+$CD11b$^+$
1	**6,2**	10,4	92,5	2,2	ND
2	**19,1**	12,6	91,7	2	ND
3	**0,9**	8,5	94,2	0,8	ND
4	**20,2**	15,9	91,4	2,2	ND
5	**77,9**	9,9	90,9	4,6	5,4
6	**73,5**	13,2	86	5	5,9
Mean		**11,75**	**91,12**	**2,80**	**5,65**
SD		**2,68**	**2,76**	**1,64**	**0,35**

Supplemental Table 2 : Geneset enrichment analysis table performed with transcriptome of NHO-MSCs as compared to BM-MSCs (Charbord et al. gene list for BM support)

RanKingGSEA	mean NHO	mean BM	ratio NHO/BM	GENE SYMBOL	GENE_TITLE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
1	792.979681285714	363.717775888889	2.18020601095933	MARCKS	myristoylated alanine-rich protein kinase C substrate	66	1.694	0.0085	Yes
2	4.93449314285714	1.97206655555556	2.50219402025559	MID2	midline 2	97	1.593	0.0183	Yes
3	3.28559185714286	0.459001333333333	7.15813140080099	EN1	engrailed homolog 1	166	1.460	0.0249	Yes
4	2.60542457142857	1.07800677777778	2.41689071454582	RAB3IL1	RAB3A interacting protein (rabin3)-like 1	194	1.426	0.0337	Yes
5	40.1270312857143	22.4755338888889	1.78536498772791	DHRS7	dehydrogenase/reductase (SDR family) member 7	201	1.410	0.0435	Yes
6	1.34582785714286	0.55494	2.42517723923822	MN1	meningioma (disrupted in balanced translocation) 1	209	1.395	0.0532	Yes
7	1428.33141214286	550.234623	2.59585884355165	PTX3	pentraxin-related gene, rapidly induced by IL-1 beta	250	1.343	0.0606	Yes
8	22.6286652857143	11.5486064444444	1.95942821279532	IFIT1	interferon-induced protein with tetratricopeptide repeats 1	266	1.326	0.0693	Yes
10	2.07200385714286	0.517237666666667	4.00590287729018	B4GALNT1	beta-1,4-N-acetyl-galactosaminyl transferase 1	501	1.177	0.0733	Yes
11	9.62785885714286	4.03027	2.38888681332587	NUDT14	nudix (nucleoside diphosphate linked moiety X)-type motif 14	511	1.173	0.0812	Yes
12	367.858466428571	66.4583915555556	5.53516956727823	SPON2	spondin 2, extracellular matrix protein	536	1.162	0.0882	Yes
13	37.5391634285714	21.6973336666667	1.7301279505252	TMEM47	transmembrane protein 47	564	1.148	0.0950	Yes
14	1.30554814285714	0.569606555555556	2.29201741118271	MMP23B	matrix metallopeptidase 23B	592	1.137	0.1016	Yes
15	28.4114334285714	13.7568288888889	2.06526036327447	PLEKHG4	pleckstrin homology domain containing, family G (with RhoGef domain) member 4	605	1.131	0.1091	Yes
16	0.166794857142857	0.0646425555555556	2.58026397176561	ZDHHC15	zinc finger, DHHC-type containing 15	654	1.111	0.1144	Yes
17	110.244709857143	30.6602015555556	3.59569423108266	POSTN	periostin, osteoblast specific factor	658	1.111	0.1222	Yes
18	79.6054762857143	35.7269138888889	2.22816548144316	ADAM12	ADAM metallopeptidase domain 12 (meltrin alpha)	698	1.094	0.1279	Yes
19	3.27454785714286	1.28672466666667	2.54487066423135	RTP4	receptor transporter protein 4	705	1.092	0.1355	Yes
20	6.52060471428571	2.92901133333333	2.2262135486055	HSPB8	heat shock 22kDa protein 8	718	1.087	0.1426	Yes
21	3.81979985714286	1.41479066666667	2.69990461991281	CTSH	cathepsin H	884	1.029	0.1407	Yes
22	221.763209857143	117.844464222222	1.88182967541826	PDGFRB	platelet-derived growth factor receptor, beta polypeptide	908	1.023	0.1467	Yes
23	0.427014857142857	0.105587555555556	4.04417788532078	WNT10B	wingless-type MMTV integration site family, member 10B	921	1.018	0.1534	Yes
24	8.70452657142857	1.27744211111111	6.81402820191784	ROR2	receptor tyrosine kinase-like orphan receptor 2	981	1.001	0.1573	Yes
26	568.805722714286	288.106537111111	1.97428954031307	CD248	CD248 molecule, endosialin	1084	0.970	0.1657	Yes
27	0.123590857142857	0.0252157777777778	4.90133035879526	NCAM1	neural cell adhesion molecule 1	1208	0.941	0.1655	Yes
28	0.935990428571429	0.449709222222222	2.0813236249554	EIF4E3	eukaryotic translation initiation factor 4E member 3	1257	0.928	0.1695	Yes
29	0.297794428571429	0.118025444444444	2.52313753168371	EDA	ectodysplasin A	1295	0.920	0.1740	Yes
30	237.749199857143	159.144617333333	1.49391920280389	PMP22	peripheral myelin protein 22	1499	0.876	0.1688	Yes
31	2.99601314285714	1.54099255555556	1.94421000416646	MMP15	matrix metallopeptidase 15 (membrane-inserted)	1688	0.845	0.1642	Yes
32	3.19439628571429	1.64774188888889	1.93865089384135	LPHN2	latrophilin 2	1756	0.833	0.1664	Yes
33	18.1545627142857	8.32158344444445	2.18162358588206	LGMN	legumain	1798	0.823	0.1701	Yes
34	20.7034831428571	9.96345877777778	2.07794136600772	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	1824	0.819	0.1745	Yes
35	0.523919857142857	0.299218666666667	1.75095980133656	CDYL2	chromodomain protein, Y-like 2	1842	0.816	0.1795	Yes
36	87.261296	53.8637545555556	1.62003738358042	PLXND1	plexin D1	1892	0.808	0.1825	Yes
38	4.10175014285714	2.255891	1.81823950840583	TRAM1L1	translocation associated membrane protein 1-like 1	2011	0.790	0.1873	Yes
39	2.32455271428571	1.15256166666667	2.01685756303918	RNF150	ring finger protein 150	2040	0.786	0.1914	Yes
40	5.75106085714286	4.20573055555556	1.36743445191609	PXMP4	peroxisomal membrane protein 4, 24kDa	2106	0.776	0.1933	Yes
41	155.038398571429	135.721252888889	1.14232955614073	COL6A2	collagen, type VI, alpha 2	2130	0.772	0.1975	Yes
42	8.25678285714286	5.04858211111111	1.63546569619439	POPDC3	popeye domain containing 3	2196	0.760	0.1993	Yes
43	0.378776	0.184182888888889	2.05652111488219	PCDHB15	protocadherin beta 15	2228	0.756	0.2030	Yes
44	5.43128714285714	3.69280177777778	1.47077678946676	ZDHHC2	zinc finger, DHHC-type containing 2	2300	0.743	0.2043	Yes
45	90.2521321428571	57.3444168888889	1.57386084015346	ATP10A	ATPase, Class V, type 10A	2316	0.741	0.2088	Yes
46	81.3020212857143	48.2859751111111	1.6837605764123	ADCY3	adenylate cyclase 3	2320	0.740	0.2140	Yes
47	2.07179557142857	1.39606944444444	1.48402042582705	ZNF14	zinc finger protein 14	2350	0.736	0.2177	Yes
48	3.730241	1.19789377777778	3.11399981300512	SPP1	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	2373	0.732	0.2217	Yes

Supplemental Table 3 : Fold changes of niche related genes in the MSC transcriptome from NHO vs BM

Gene	Ranking pubmed-gene association	False discovery rate q-values pubmed-gene association	Fold change NHO/BM
DKK1	109	0.012747757	4.70969521405796
ACP5	26	0.006388641	3.7209612739334
POSTN	153	0.014434171	3.59569415566333
CFD	172	0.016848968	3.32179715128482
SPP1	15	0.006388641	3.11399981300512
ACAN	166	0.016111989	3.01575020730045
ITGAM	24	0.006388641	2.48326424444568
ACTA2	246	0.031121177	2.45296433826824
SCARB1	152	0.014434171	2.31134838789903
HAS1	281	0.040242457	2.18218501945884
SDC2	241	0.030156874	2.17990946046593
CDH2	201	0.022201365	2.0284521221223
CSF1R	65	0.009066569	2.00475274914767
THBS1	176	0.017142711	1.98456086811457
CADM1	120	0.013082065	1.96872617852161
CD58	103	0.012262663	1.91446584070757
PDGFRB	181	0.017500643	1.88182964262106
AXL	222	0.026561882	1.79079502818849
JAG1	170	0.016482101	1.78923270660551
GDNF	274	0.038101566	1.7580475570864
TFRC	158	0.015482918	1.71238605481121
CDKN1B	304	0.047884197	1.71056106613062
TIMP2	167	0.016167028	1.66649968121827
SLC3A2	273	0.038101566	1.59981281338103
KITLG	9	0.006388641	1.56537682110009
CNOT6	204	0.023063738	1.55842638298243
RAC1	227	0.027301147	1.51996425539124
RHOA	233	0.028324362	1.48643633360217
CD164	230	0.027921656	1.46730080703459
SCARB2	154	0.014434171	1.43174168846694
HIF1A	206	0.023272937	1.41760362480651
IFIT3	306	0.048697644	1.40201919226435
GLB1	285	0.040568222	1.37617973655337
MET	157	0.015392947	1.3376247244245
MCL1	226	0.026839962	1.32544877534465
FAS	199	0.021342021	1.31018062121329
ROCK1	286	0.041066755	1.3011781633013
BMPR2	253	0.032330930	1.28209847508118
TCEAL1	256	0.033077876	1.26360942179887
ATF4	284	0.040568222	1.23991380455814
IGBP1	142	0.014163171	1.23649157640842
ANXA6	205	0.023122881	1.23051073605363
GALNS	299	0.046127024	1.22776481095018
SMAD2	229	0.027812968	1.22541849843411
NDUFA2	20	0.006388641	1.22055664464886
JAK1	265	0.034636352	-1.50954697724128
NT5E	96	0.012262663	-1.61403610252432
SDC4	249	0.032243694	-1.69293813070849
JAK2	195	0.020394463	-2.76063592646256

Supplemental Table 4 : NHO samples used in experiments

Samples	Injuries	Samples	Injuries
NHO4	Stroke	NHO101	TBI
NHO33	TBI	NHO102	SCI
NHO34	Neuropathy	NHO104	TBI
HO35	Burn	NHO105	Stroke
NHO36	TBI	NHO106	SCI
NHO37	TBI	NHO108	TBI
NHO40	SCI	NHO109	Stroke
NHO41	Stroke	NHO110	TBI
NHO42	Stroke	NHO112	SCI
NHO43	Neuropathy	NHO113	SCI
NHO44	TBI	NHO118	Stroke
NHO47	Neuropathy	NHO119	Stroke
NHO52	Neuropathy	NHO122	TBI
NHO59	TBI	NHO123	Neuropathy
NHO60	Neuropathy	NHO126	TBI
NHO77	TBI	NHO128	Cerebral Anoxia
NHO78	SCI	NHO132	Cerebral Anoxia
NHO79	TBI	NHO133	Stroke
NHO80	TBI	NHO134	TBI
NHO82	TBI	NHO135	TBI
NHO83	TBI	NHO138	TBI
NHO84	TBI	NHO139	TBI
NHO85	Stroke	NHO140	Stroke
NHO87	TBI	NHO141	TBI
NHO88	Stroke	NHO142	SCI
NHO90	Stroke	NHO143	SCI
NHO91	Stroke	NHO144	Stroke
NHO92	TBI	NHO145	Stroke
NHO93	Stroke	NHO146	TBI
NHO95	Stroke	NHO147	Stroke
NHO96	Stroke	NHO148	Stroke
NHO97	TBI	NHO149	TBI
NHO98	TBI	NHO152	Stroke
NHO99	TBI	NHO153	TBI
NHO100	SCI	NHO159	SCI

TBI: traumatic brain injury; SCI: spinal cord injury

Supplemental Table 5: List of antibodies used in flow cytometry studies

Antibody	Clone or catalog number	Manufacturer
CD3-FITC	UCHT1	BD Pharmingen
CD4-APC	RPA-T4	BD Pharmingen
CD8-PE	RPA-T8	BD Pharmingen
CD11b-APC/Cy7	ICRF44	BD Pharmingen
CD11b-FITC	IM0530	Beckman Coulter
CD14-AF700	M5E2	Sony
CD15-FITC	80H5	Beckman Coulter
CD19-PE	J3-119	Beckman Coulter
CD19-APC	HIB19	BD Pharmingen
CD31-FITC	WM59	BD Pharmingen
CD34-APC	581	Beckman Coulter
CD34-BV605	581	Sony
CD38-PE	HIT2	Sony
CD45-FITC	HI30	BD Pharmingen
CD45-BV421	HI30	Sony
mouse CD45	30F11	BD Pharmingen
CD73-PE/Cy7	AD2	Ebiosciences
CD90-BV605	5E10	Sony
CD130-Biotin	AN-G30	Ebiosciences
CD105-PE	166707	R&D
CD163-PE	215927	R&D
CD144-PE	TEA 1/31	Beckman Coulter
CD206-APC	19.2	BD Pharmingen
ICAM-1-AF488	HCD54	Biolegend
VCAM-1-PE	STA	Biolegend
OSMR	AN-V2	Ebiosciences
mIgG1-FITC	679.1Mc7	Beckman Coulter
mIgG1-PE	679.1Mc7	Beckman Coulter
mIgG1-Pe/Cy7	MOPC-21	Biolegend
mIgG1-APC	679.1Mc7	Beckman Coulter
mIgG1-APC/Cy7	MOPC-21	BD Pharmingen
mIgG1-BV421	MOPC-21	Sony
mIgG1-BV605	MOPC-21	Sony
mIgG2a-FITC	U7.27	Immunotech
mIgG2a-AF700	MOPC-173	Sony
mIgG2a-Biotin	eBM2a	Ebiosciences
mIgM-FITC	G155-228	BD Pharmingen
Streptavidin APC/Cy7	2626040	Sony