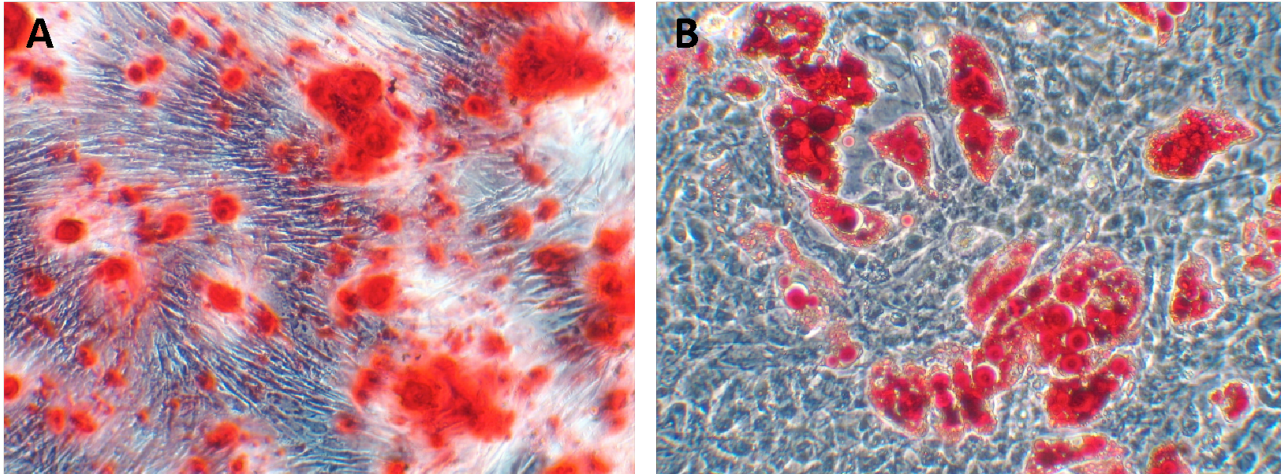


MSCs multipotency characterization

Figure S1. *MSCs multipotency characterization.* (A) Alizarin Red staining on MSCs exposed to osteogenic differentiation medium demonstrates the ability of isolated cell to differentiate into osteoblasts. (B) Oil Red O staining on MSCs exposed to adipogenic differentiation medium confirms that isolated cells are able to differentiate into adipocytes.



Surface expression of rat mesenchymal stem cell markers

The staining for cell surface proteins was performed by incubating the cells with pre-optimized concentrations of antibodies at 4°C for 30 minutes in FACS buffer (PBS with 2%FBS and 1mM EDTA). Flow cytometry data were acquired on LSRFortessa X-20 (BD) and analyzed by FlowJo software version 10. All antibodies were supplied by BD. Anti-CD45 APC-Cy7 (clone OX-1), CD11b/c PE-Cy7 (clone OX-42), CD44 BV421 (clone OX-49), CD54 BB700 (clone 1A29), CD90 BV510 (clone HIS51) antibodies were all mouse anti-rat antibodies; RT1A[a,b,l] FITC (clone B5) was a rat anti-rat antibody and anti-CD29 Alexa Fluor 647 (clone Ha2/5) was a hamster anti-rat antibody. Appropriate isotype antibodies were used as controls.

Results: Rat MSC are negative for CD45 and CD11b expression. Rat MSC expressed high levels of CD44, CD29, CD90, CD54 and low levels of RT1-A (MHC I)

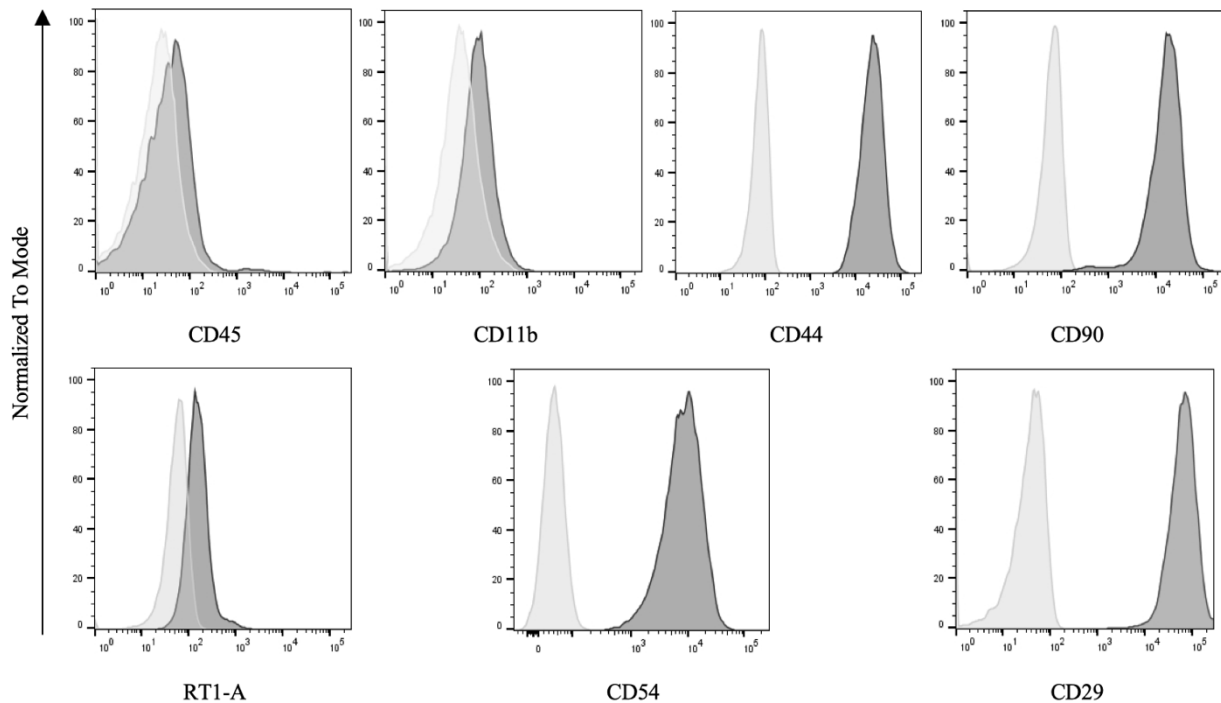


Figure S2. Representative flow cytometry histograms for rat MSC surface marker expression. Light gray histogram represents isotype expression (negative controls).

Live/Dead

In order to investigate if cells died during the long-term culture, before detaching, in flat controls and in 3D Nichoids, we cultured MSCs in the same conditions and performed a Live/Dead Viability/Cytotoxicity test (Invitrogen detection technologies) to detect live and dead cells. The results of these experiments showed that almost no cells were stained by EthD-1 in 2D control MSC from 1 to 14 days, only occasionally we observed some cells per microscopic field stained with EthD-1 in the Nichoid substrate. Representative images are reported in Figure S3. While this does not exclude that some cells could detach from the substrate before dying, it is a sign that in both conditions all cells are viable.

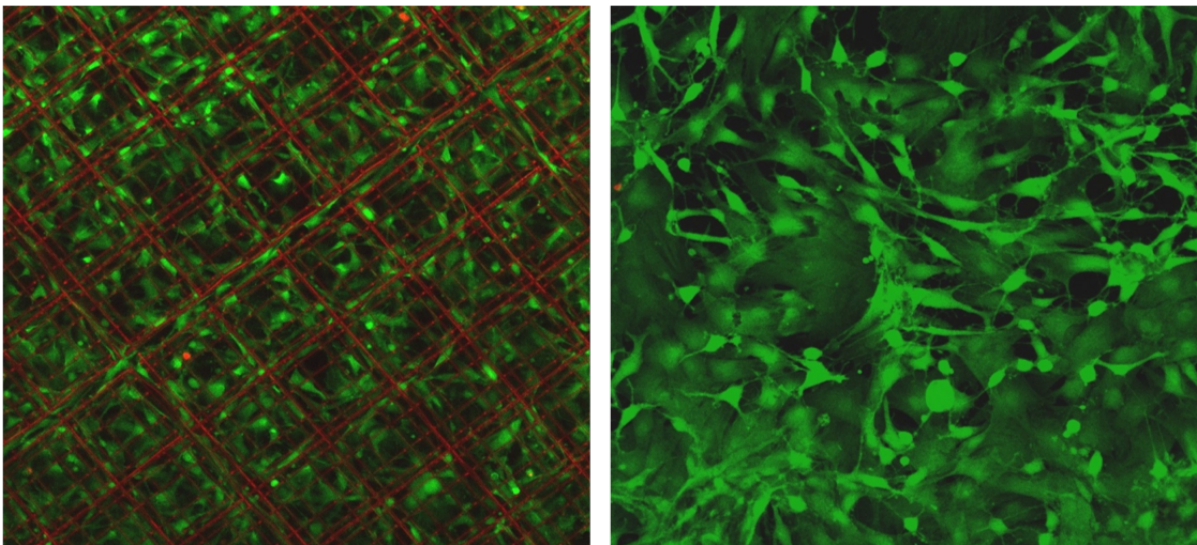


Figure S3. Live/Dead assay showing cells at 14 days of culture in 3D Nichoids (left) and flat controls (right).

Rat Mesenchymal Stem Cell RT2 profiler PCR array (PARN-082ZC-6 SABioscience) - Gene table

1	Abcb1a	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	Abcb1, Mdr1a
2	Acta2	Smooth muscle alpha-actin	-
3	Adipoq	Adiponectin, C1Q and collagen domain containing	Acadc, Acrp30
4	Alcam	Activated leukocyte cell adhesion molecule	-
5	Anpep	Alanyl (membrane) aminopeptidase	AP-M, AP-N, Apm, Apn, KZP, Lap1, rAPN
6	Anxa5	Annexin A5	Anx5, LC5
7	Bdnf	Brain-derived neurotrophic factor	-
8	Bglap	Bone gamma-carboxyglutamate (gla) protein	Bglap2, Bgp, Bgpr, Bgpra
9	Bmp2	Bone morphogenetic protein 2	-
10	Bmp4	Bone morphogenetic protein 4	BOMPR4A
11	Bmp6	Bone morphogenetic protein 6	VGR
12	Bmp7	Bone morphogenetic protein 7	BMP-7
13	Casp3	Caspase 3	Lice, Yama
14	Cd44	Cd44 molecule	CD44A, META, RHAMM
15	Col1a1	Collagen, type I, alpha 1	COLIA1
16	Csf2	Colony stimulating factor 2 (granulocyte-macrophage)	Gm-csf, Gmcsf
17	Csf3	Colony stimulating factor 3 (granulocyte)	Gcsf
18	Ctnnb1	Catenin (cadherin associated protein), beta 1	Catnb
19	Egf	Epidermal growth factor	-
20	Eng	Endoglin	-
21	ErbB2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	-
22	Fgf10	Fibroblast growth factor 10	FGF-10
23	Fgf2	Fibroblast growth factor 2	Fgf-2, bFGF
24	Fut1	Fucosyltransferase 1	Futa
25	Fut4	Fucosyltransferase 4 (alpha (1,3) fucosyltransferase, myeloid-specific)	Fuct
26	Fzd9	Frizzled homolog 9 (Drosophila)	-
27	Gdf15	Growth differentiation factor 15	-
28	Gdf5	Growth differentiation factor 5	Cdmp1
29	Gdf6	Growth differentiation factor 6	BMP-13, gdf16
30	Gdf7	Growth differentiation factor 7	-
31	Hat1	Histone acetyltransferase 1	-
32	Hdac1	Histone deacetylase 1	-
33	Hgf	Hepatocyte growth factor	HPTA
34	Hnf1a	HNF1 homeobox A	HNF1, LF-B1, Lfb1, Tcf1
35	Icam1	Intercellular adhesion molecule 1	CD54, ICAM
36	Ifng	Interferon gamma	IFNG2
37	Igf1	Insulin-like growth factor 1	-
38	Il10	Interleukin 10	IL10X
39	Il1b	Interleukin 1 beta	-
40	Il6	Interleukin 6	ILg6, Ifnb2
41	Ins2	Insulin 2	-
42	Itga6	Integrin, alpha 6	-
43	Itgav	Integrin, alpha V	Cd51
44	Itgax	Integrin, alpha X	RGD1561123
45	Itgb1	Integrin, beta 1	-

46	Jag1	Jagged 1	-
47	Kdr	Kinase insert domain receptor	Vegfr-2
48	Kitlg	KIT ligand	Kitl, Mgf, SCF
49	Lif	Leukemia inhibitory factor	-
50	Mcam	Melanoma cell adhesion molecule	CD146, Muc18
51	Mitf	Microphthalmia-associated transcription factor	-
52	Mmp2	Matrix metalloproteinase 2	-
53	Nes	Nestin	-
54	Ngfr	Nerve growth factor receptor (TNFR superfamily, member 16)	LNGFR, RNNGFRR, Tnfrsf16, p75, p75NTR
55	Notch1	Notch homolog 1, translocation-associated (Drosophila)	NOTCH, TAN1
56	Nt5e	5' nucleotidase, ecto	CD73, Nt5
57	Nudt6	Nudix (nucleoside diphosphate linked moiety X)-type motif 6	Gfg
58	Pdgfrb	Platelet derived growth factor receptor, beta polypeptide	PDGFR-1
59	Pigs	Phosphatidylinositol glycan anchor biosynthesis, class S	-
60	Pou5f1	POU class 5 homeobox 1	-
61	Pparg	Peroxisome proliferator-activated receptor gamma	-
62	Prom1	Prominin 1	CD133, Prom
63	Ptk2	PTK2 protein tyrosine kinase 2	FAK, FRNK, p125FAK
64	Ptprc	Protein tyrosine phosphatase, receptor type, C	CD45, L-CA, Lca, RT7, T200
65	Smurf1	Similar to RIKEN cDNA 4930431E10	RGD1309707
66	Rhoa	Ras homolog gene family, member A	Arha, Arha2
67	Runx2	Runt-related transcription factor 2	Cbfa1, OSF-2
68	Slc17a5	Solute carrier family 17 (anion/sugar transporter), member 5	-
69	Smad4	SMAD family member 4	Madh4
70	Smurf2	SMAD specific E3 ubiquitin protein ligase 2	RGD1310067
71	Sox2	SRY (sex determining region Y)-box 2	RGD1565646
72	Sox9	SRY-box containing gene 9	SRY
73	Tbx5	T-box 5	-
74	Tert	Telomerase reverse transcriptase	-
75	Tgfb1	Transforming growth factor, beta 1	Tgfb
76	Tgfb3	Transforming growth factor, beta 3	TGF-B3
77	Thy1	Thy-1 cell surface antigen	CD7
78	Tnf	Tumor necrosis factor (TNF superfamily, member 2)	RATTNF, TNF-alpha, Tnfa
79	Vcam1	Vascular cell adhesion molecule 1	VCAM1B
80	Vegfa	Vascular endothelial growth factor A	VEGF-A, VEGF164, VPF, Vegf
81	Vim	Vimentin	-
82	Vwf	Von Willebrand factor	-
83	Wnt3a	Wingless-type MMTV integration site family, member 3A	-
84	Zfp42	Zinc finger protein 42	Zfp971
85	Actb	Actin, beta	Actx
86	B2m	Beta-2 microglobulin	-
87	Hprt1	Hypoxanthine phosphoribosyltransferase 1	Hgpptase, Hprt
88	Ldha	Lactate dehydrogenase A	Ldh1
89	Rplp1	Ribosomal protein, large, P1	-